Universidad de Oviedo

Programa de Doctorado en Análisis Químico, Bioquímico y
Estructural y Modelización Computacional

# AVANCES EN LA DETECCIÓN ELEMENTAL, SELECTIVA Y UNIVERSAL, EMPLEANDO CROMATOGRAFÍA DE GASES Y ESPECTROMETRÍA DE MASAS

TESIS DOCTORAL

**JAVIER GARCÍA BELLIDO**

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PhD Programme in Chemical, Biochemical and Structural Analysis
and Computational Modelling

# ADVANCES IN SELECTIVE AND UNIVERSAL ELEMENTAL DETECTION USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

PhD THESIS

**JAVIER GARCÍA BELLIDO**

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## RESUMEN (en español)

El uso de nuevas fuentes de energía requiere la caracterización de materias primas, procesos y productos. Esta tarea con cierta dificultad debido a la complejidad de las matrices, proponen un reto analítico hacia la determinación a bajas concentraciones de compuestos en cuya estructura se incluyen heteroátomos como S, N, O y Cl. Debido al carácter dependiente de la especie, la técnica más ampliamente usada para el análisis de estos compuestos, la cromatografía de gases-espectrometría de masas, se encuentra limitada al uso de patrones específicos para cuantificar. Diferentes detectores selectivos se han desarrollado para abordar el análisis de los elementos mencionados, sin embargo, tienen algunas desventajas como *quenching* en matrices complejas o límites de detección altos. La presente tesis doctoral se presenta como un paso adelante en el desarrollo de metodologías basadas en la GC y la MS, para llevar a cabo la detección selectiva y posterior cuantificación en muestras de interés energético usando patrones genéricos.

En el primer capítulo, se presenta una metodología para el análisis de Cl en muestras reales de aceite de pirolisis. Mediante el uso de patrones genéricos que contienen Cl, se llevó a cabo el análisis de especiación y contenido total tras el desarrollo y optimización de una metodología para una cuantificación independiente de la especie. En el caso del contenido total, el análisis se realizó intercambiando una columna por una línea de transferencia inerte y mediante calibración externa con patrón genérico. Para la validación de la metodología se utilizó un material certificado y se analizaron varias muestras reales de aceites obtenidos de pirólisis de plásticos.

El detector de GC-combustión-MS, previamente desarrollado en el grupo de investigación para la cuantificación universal de carbono, se extendió para su uso como detector selectivo multielemental, para S, H, y N simultáneamente junto con el C. Se desarrolló una interfase de combustión (formada por un tubo de alúmina con dos tubos de platino como catalizadores) y fue instalada entre el cromatógrafo de gases y el detector de MS consiguiendo una transformación cuantitativa de cada compuesto que eluye de la columna en las correspondientes especies. Así el carbono se detecta como $CO_2$, el hidrógeno como $H_2O$, el nitrógeno como NO y el azufre como $SO_2$. De esta forma, podemos obtener un perfil selectivo y simultáneo para hasta cuatro elementos importantes en diferentes campos como es, por ejemplo, el de los combustibles. Tras evaluar la combustión cuantitativa de una mezcla de patrones genéricos, se llevó a cabo la cuantificación de varias muestras de diésel cuyo contenido en S y N era conocido. Se obtuvieron resultados exitosos en la cuantificación, así como unos límites de detección más bajo incluso que los detectores selectivos específicos de S y N.

El prototipo desarrollado en el anterior capítulo se usó en este para evaluarlo críticamente como detector selectivo de N. Para ello, se llevó a cabo un estudio más extendido para el cálculo de la equimolaridad del detector usando hasta 16 compuestos con distintas estructuras y funciones. También se evaluó el límite de detección en un nuevo prototipo desarrollado conjuntamente con la empresa Shimadzu. Con este prototipo se consiguieron los límites de detección más bajos hasta el momento, mejorando la sensibilidad de la técnica más de un orden de magnitud. Se utilizó un detector GC-NCD para la comparación analítica con un detector selectivo y ampliamente establecido en la industria, obteniendo mejores prestaciones que este en cuanto a sensibilidad. La metodología se certificó con un CRM en concentración total de N y se usaron diferentes muestras de aceites de biomasa para su cuantificación mediante cuantificación independiente de la especie usando patrones genéricos.

El detector GC-combustión-MS permite la detección universal (C) mediante la combustión de los compuestos. A su vez, permite una detección selectiva y simultánea de elementos tales como N, S y H. En este capítulo de la tesis doctoral se extendió esta detección selectiva a un nuevo elemento, el oxígeno, para el que en la actualidad no existen detectores con los niveles de detección requeridos por la industria. Mediante la adición de un flujo de $He:O_2$ enriquecido en $^{18}O$ se conseguiría diferenciar el oxígeno de la estructura de los compuestos, del oxígeno del sistema. Se ha trabajado en el desarrollo matemático para la obtención de una metodología que permita la detección de los compuestos de oxígeno, obteniendo resultados aceptables para la equimolaridad de una mezcla de compuestos de oxígeno. La metodología fue validada mediante el análisis de un SRM y una muestra real de diésel.

**RESUMEN (en Inglés)**

The use of new energy sources requires the characterization of feedstocks, processes and products. This task with certain difficulty due to the complexity of the matrices and proposes an analytical challenge towards the determination at low concentrations compounds whose structure includes heteroatoms such as S, N, O and Cl. Due to the species-dependent character, the most widely used technique for the analysis of these compounds, gas chromatography-mass spectrometry, is limited to the use of specific standards to quantify. Different selective detectors have been developed to approach the analysis of the mentioned elements, however, they have some disadvantages such as quenching in complex matrices or high detection limits. The Doctoral tTesis is presented as a step forward in the development of new methodologies based on GC and MS, to perform selective detection and subsequent quantification in samples of energetic interest using generic standards.

In the first chapter, a GC-ICP-MS/MS based method for the analysis of chlorine in real pyrolysis plastics oil samples is presented. The speciation and total quantification analysis of real samples was accomplished using generic Cl-containing standards after development and optimization of a compound-independent quantification methodology. In the case of total chloride content, the analysis was performed after changing the column by an inert transfer line and resorting to external calibration with a chlorine-containing generic standard. Detection limit as low as 1 ng/g was obtained under optimal conditions. For validation of both total and speciation methodologies a certified reference material was used. Several real pyrolysis oils obtained from diverse plastic sources were successfully analyzed.

In the following chapter the GC-combustion-MS concept, initially developed in the GEAB Research Group for universal carbon quantification exclusively, was improved and extended to the multi-element selective detection of S, H, and N as well. Notably, the system developed maintained the structural elucidation capabilities of MS by simply actuating a switching valve.
A combustion interface (consisting of an alumina tube with two platinum wires acting as catalysts for the reaction) was developed and installed in a GC−MS instrument for the quantitative conversion of each and every organic compound eluting from the column into their corresponding volatile species before the detection by MS, making their quantification truly compound-independent. In that way, carbon could be detected as CO2, hydrogen as H2O, nitrogen as NO and sulphur as SO2. After evaluating the quantitative combustion of a mixture of generic standards, the approach was validated by the analysis of two diesel samples with known S and N content.

The prototype developed in the previous chapter was used in this chapter to critically evaluate it as a selective detector for N. For this purpose, a more extensive study was carried out to calculate the equimolarity of the detector using up to 16 compounds with different structures and functions. The detection limit was also evaluated on a new prototype developed jointly with the company Shimadzu. This prototype achieved the lowest detection limits so far, improving the sensitivity of the technique by more than an order of magnitude. A GC-NCD detector was used for analytical comparison with a selective and widely established detector in the industry, obtaining better performance than the latter in terms of sensitivity. The methodology was certified with a CRM at total N concentration and different biomass oil samples were used for quantification by species-independent quantification using generic standards.

The GC-combustion-MS detector allows universal detection (C) by combustion of the compounds. Likewise, it allows a selective and simultaneous detection of elements such as N, S and H. In this chapter of the doctoral thesis, this selective detection was extended to a new element, oxygen, for which there are currently no detectors with the detection levels required by the industry. By adding a flow of He:$O_2$ enriched in $^{18}O$, it would be possible to differentiate the oxygen in the structure of the compounds from the oxygen in the system. It has been developed a mathematical discrimination methodology for the selective detection of oxygen compounds obtaining good results for the equimolarity of a mixture of oxygen compounds. The methodology was validated by analysis of an SRM and a real diesel samples spiked with O-containing standards.

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*"El tiempo es el mayor artífice de la realidad"*

Diego Pablo Simeone

# AGRADECIMIENTOS

*BLABLABLA*

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# INTRODUCCIÓN

# 1. Combustibles, desarrollo e importancia a lo largo de la historia

El descubrimiento y uso de los diferentes combustibles a lo largo de la historia de la humanidad ha estado directamente ligado al desarrollo de esta, siendo, de hecho, el factor clave que ha permitido el impulso de las distintas sociedades hasta llegar a nuestros días. Partiendo del descubrimiento del fuego como primera fuente de energía, a lo largo de siglos el ser humano ha sido capaz de descubrir y manipular diferentes fuentes de energía cada vez más complejas. Como se puede observar en la Figura 1, hay una relación directa entre las necesidades del ser humano y el consumo de energía, lo que ha impulsado la búsqueda de fuentes de energía nuevas cada vez más eficientes.

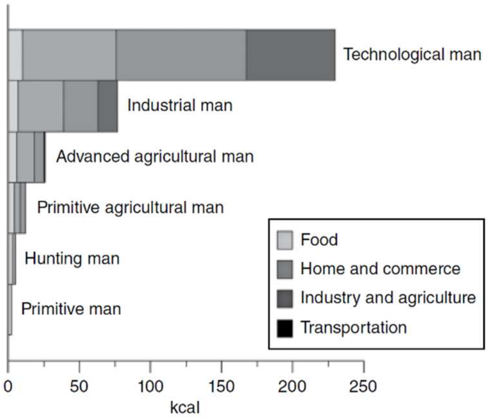

**Figura 1. Estimación del consumo de energía por persona y día a lo largo de la historia.**[1]

Indudablemente, el crecimiento económico y el aumento de la industrialización ha permitido una mayor eficiencia en el uso de la energía a

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[1] Ngô, C. and Natowitz, J. B.; Our Energy Future: Resources, Alternatives, and the Environment, **2016**, John Wiley & Sons.

la vez que ha conllevado un aumento en la necesidad del consumo de esta, principalmente, de combustibles derivados de materia orgánica, conocidos como combustibles fósiles, tales como carbón, petróleo, gas natural, etc.

Aproximadamente el 81% de la energía primaria producida mundialmente se obtiene de los combustibles fósiles, habiéndose mantenido este porcentaje a lo largo de las últimas décadas relativamente estable y, pudiendo aumentar, según algunas fuentes, hasta el 90% en el año 2030.[2]

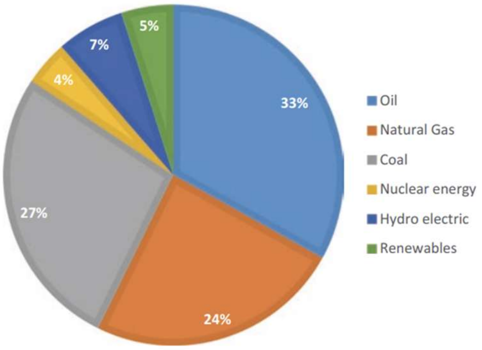

**Figura 2. Contribución de las distintas fuentes de energía al consumo total de energía en el año 2019.**[3]

## 1.1. Combustibles fósiles: perspectivas

El petróleo o crudo se define como una mezcla de hidrocarburos formados a partir de la descomposición de materia orgánica bajo la presión de grandes capas de sedimentos.

El petróleo es sometido a un proceso conocido como refinado como se puede apreciar en la Figura 3. Durante este proceso, el petróleo se separa y transforma en distintos productos tales como la gasolina, fuel para aviación,

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2 Ishaq, M.; Ghouse, G.; Fernandez-Gonzalez, R.; Puime-Guillen, F.; Tandir, N.; Santos de Oliveira, H. M. "From Fossil Energy to Renewable Energy: Why is Circular Economy Needed in the Energy Transition?". Frontiers in Environmental Science; **2022**; 10; 941791.

[3] Cronshaw, Mark. Energy in Perspective. Springer, **2021**.

diésel, etc. cuyas propiedades tanto macroscópicas como microscópicas a nivel de composición, difieren, lo que hace obligatorio el control de estas para diferenciar los productos y optimizar procesos.

Actualmente, la política de descarbonización y reducción de emisión de gases invernadero plasmada en el Acuerdo de París del año 2015,[4] que pretende evitar el aumento de la temperatura global promedio por encima de los 2ºC respecto a los niveles preindustriales con el objetivo de reducir significativamente los riesgos y efectos de un cambio climático, apunta a un cambio en el modelo de obtención de energía donde las fuentes renovables toman cada vez más protagonismo.

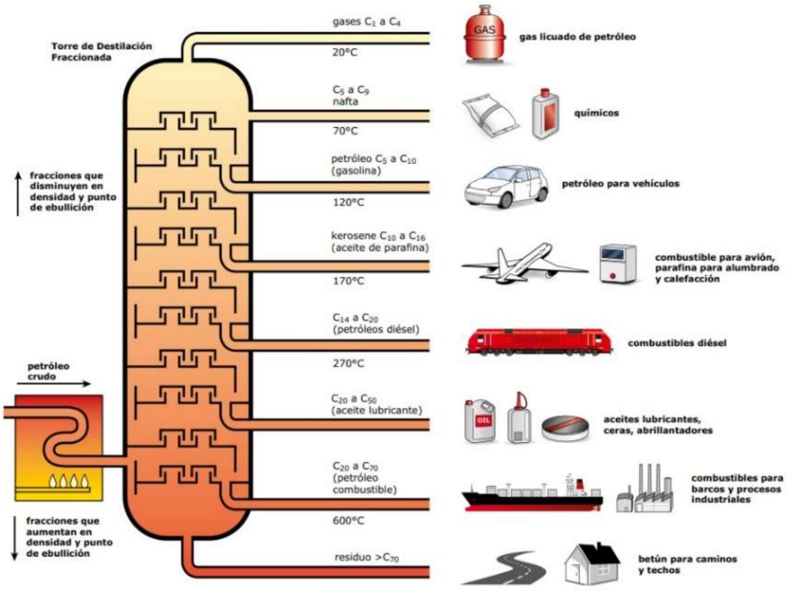

**Figura 3. Esquema genérico del proceso de refinado de un crudo de petróleo.**[5]

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[4] Naciones Unidas (ONU), *Acuerdo de París de la Convención Marco de las Naciones Unidas sobre el Cambio Climático (UNFCCC)*, 12 diciembre **2015**.

[5] https://www.oiltanking.com/en/news-info/glossary/the-petroleum-refining-process.html

## 1.2. Nuevas alternativas a los combustibles fósiles

Los combustibles fósiles, por su propia condición, son combustibles limitados y disponibles en cantidades finitas y se espera que en las próximas décadas se llegue a un agotamiento de estos recursos. La necesidad de nuevas fuentes de combustibles alternativas es urgente, siendo no solo una cuestión de futuro, sino una cuestión del presente.

Las fuentes adicionales y alternativas de productos intermedios y finales, ya sean combustibles o productos petroquímicos, contribuyen directamente a la conservación de los recursos petrolíferos del mundo, al ofrecer opciones adicionales de materias primas para generar productos similares a los actuales para los consumidores. Encontramos algunos ejemplos como el biogás para metanol, el etanol obtenido de grano, biodiesel obtenido de algas, BTX (benceno, tolueno y xilenos) obtenidos de carbón, biogás obtenido de residuos de la agricultura.

Durante las últimas décadas, se han hecho esfuerzos para reducir las emisiones de contaminantes del aire que están directamente relacionados con el proceso de combustión. Esto incluye SOx, NOx, COx, VOCs o el material particulado (PMs) entre otros.[6] El aumento de la emisión de gases de efecto invernadero (GEI) y su efecto directo en el aumento del calentamiento global ha puesto el foco en la reducción de estos, siendo el $CO_2$ generado por la combustión de los combustibles fósiles uno de los principales GEI. Además de esto, se ha demostrado el efecto negativo sobre la salud humana del aumento de estos gases. [7] Así se ha puesto el foco en la reducción de la emisión de estos gases, bien desarrollando nuevos combustibles alternativos de origen no fósil o, mitigando y fijando el $CO_2$ emitido cuando se usan combustibles fósiles.

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[6] Lee, S; Speight, J.G; Loyalka, S.K; *Handbook of Alternative Fuel Technologies, 2nd Edition*; Taylor & Francis Group, Boca Raton, **2014**.

[7] Deshmukh, M. K. G.; Sameeroddin, M.; Abdul, D.; & Sattar, M. A. "Renewable energy in the 21st century: A review" Materials Today: Proceedings; **2023**; 80; 1756-1759.

Algunas de las energías renovables que se han desarrollado incluyen la energía solar, eólica, hidroeléctrica, mareomotriz, todas ellas caracterizadas por una sostenibilidad a largo plazo. Si bien, como se comentaba anteriormente, lo que se busca hoy en día es intentar llegar a un estado de no dependencia de combustibles fósiles, es importante en este transcurso el desarrollo de combustibles que sustituyan, al menos, parcialmente, parte de las necesidades que cubren los combustibles fósiles.

Por ejemplo, el uso de biomasa y biofueles ha ido creciendo en importancia, existiendo multitud de procesos e investigaciones para mejorar estos productos,[8] estando incluso regulada la producción de biodiesel de acuerdo a diferentes normativas europeas y estadounidenses (EN14214 y ASTM6751).

El biodiesel es un combustible que se genera a partir de distintas materias primas, desde vegetales hasta grasas animales, en el que el material se somete a una reacción de transesterificación con metanol, lo que produce una mezcla de ésteres metílicos de ácidos grasos (FAMEs; *fatty acid methyl esthers*). Una de las principales desventajas de este combustible es la generación durante el proceso de glicerol. Este compuesto, además de reducir la eficacia del proceso, es altamente polimerizable formando a altas temperaturas polímeros de glicerol o acroleína. Diferentes alternativas en los procesos de generación del biofuel han sido estudiadas exitosamente para eliminar esta desventaja.[9] Aunque la capacidad calorífica del biodiesel tiene un valor similar a la del diésel, el problema con este combustible viene debido a su viscosidad y densidad y los problemas derivados en la inyección en motores. Es por ello por lo que normalmente, este combustible es mezclado con diésel en pequeños porcentajes para facilitar su combustión. Si bien, su capacidad como

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[8] Stančin, H.; Mikulčić, H.; Wang, X.; Duić, N. "A review on alternative fuels in future energy system". Renewable and sustainable energy reviews,; **2020**; 128; 109927.

[9] Estevez, R.; Aguado-Deblas, L.; López-Tenllado, F. J.; Luna, C.; Calero, J.; Romero, A. A.; Luna, D. "Biodiesel is dead: Long life to advanced biofuels—A comprehensive critical review". Energies; **2022**; 15(9); 3173.

combustible es limitada, el rendimiento en motores de combustión convencionales es considerable y, además, se han reportado una reducción en las emisiones de PM y CO, aunque con una tendencia a aumentar la formación de NOx y $CO_2$.

Una alternativa al proceso de transesterificación para generar nuevos combustibles, es la reacción conocida como pirólisis. En este tipo de procesos, la biomasa es introducida en un reactor a relativamente alta temperaturas (300-1500 ºC) en ausencia de oxígeno. De esta forma, una serie de reacciones tiene lugar generando un cambio en la estructura química de los compuestos, rompiendo sus enlaces y generando moléculas más pequeñas. Las propiedades del aceite generado dependen en gran medida del tipo de reactor usado, la temperatura y las condiciones operacionales. [9,10] Este tipo de proceso puede aplicarse de forma similar a muchos otros productos más allá de la biomasa.

Otro de los problemas con los que nos encontramos hoy en día es la generación de residuos en cantidades inabarcables para nuestro planeta. Uno de estos residuos, el plástico, cuyo origen es el petróleo, es una de las mayores amenazas en términos de contaminación. Se espera que su consumo se doble en las próximas dos décadas,[11] por lo tanto, una posible solución para reducir estos residuos estaría en el reaprovechamiento de estos plásticos, cuyo principal final en estos momentos es su combustión, con la consecuente emisión de gases que esto supone. De esta forma se cumpliría el objetivo de economía circular, aprovechando el reciclaje del plástico post-consumo.

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[10] Pontes, N. S.; Silva, R. V.; Ximenes, V. L.; Pinho, A. R.; Azevedo, D. A. "Chemical speciation of petroleum and bio-oil coprocessing products: Investigating the introduction of renewable molecules in refining processes". Fuel; **2021**; 288; 119654.

[11] Padmanabhan, S.; Giridharan, K.; Stalin, B.; Kumaran, S.; Kavimani, V.; Nagaprasad, N.; Krishnaraj, R. "Energy recovery of waste plastics into diesel fuel with ethanol and ethoxy ethyl acetate additives on circular economy strategy". Scientific Report; **2022**; 12(1); 5330.

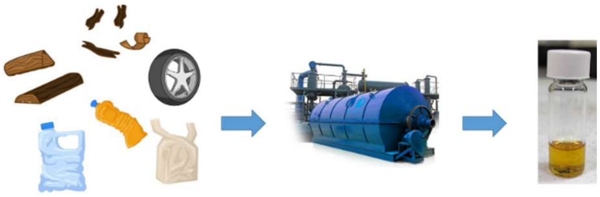

**Figura 4. Esquema general del proceso de pirólisis. Diferentes residuos tales como plásticos, neumáticos o biomasa, entre otros, son pirolizados a altas temperaturas para generar aceites de pirólisis para su uso posterior como combustibles alternativos.**

Así, los residuos plásticos, formados por polímeros de hidrocarburos, pueden utilizarse en un proceso similar al comentado anteriormente para la biomasa. Cuando son pirolizados en las condiciones adecuadas, generan aceites formados por cadenas más cortas de estos hidrocarburos que podrían sustituir parcialmente el uso de petróleo. Se puede decir que la pirólisis, además de ser ecológica y rentable es una tecnología que permite la reutilización de residuos de plástico para generar energía a través de la transformación de estos en combustibles.[12]

## 1.3. Importancia de las metodologías analíticas en la caracterización de combustibles

La caracterización analítica de muestras como combustibles es sin lugar a dudas, uno reto importante dada la inherente complejidad de estas debido a su origen. Este tipo de muestras se encuentran constituidas principalmente por hidrocarburos con infinidad de ramificaciones, grupos funcionales e isómeros, incluyendo derivados con átomos metálicos en su interior. Por lo tanto, su composición a nivel molecular es prácticamente imposible de determinar por técnicas analíticas convencionales. Un conocimiento exhaustivo de este tipo de muestras es de suma importancia para la comprensión, no solo de su rendimiento en motores, sino para procesos

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[12] Kunwar, B.; Cheng, H. N.; Chandrashekaran, S. R.; Sharma, B. K. "Plastics to fuel: a review". Renewable and Sustainable Energy Reviews; **2016**; 54; 421-428.

previos tales como el refinado, la extracción o incluso el efecto sobre los ecosistemas o la salud humana.[13] A parte de estos hidrocarburos mayoritarios que conforman un alto porcentaje de la composición de los combustibles, existe un gran número de compuestos cuyas estructuras contienen heteroátomos como oxígeno, azufre, nitrógeno, halógenos, etc. Todos estos compuestos, pueden ser considerados como impurezas debido a los efectos adversos que tienen sobre las propiedades finales de los combustibles, así como en el medioambiente cuando son combustionados. Es por ello que existe una necesidad de conocer, no solo la composición mayoritaria de este tipo de muestras, sino también, las fracciones de compuestos que se encuentran a nivel de traza o ultratraza.

El estudio de los combustibles pasa por una caracterización integral de estos, desde el átomo hasta la molécula, pasando por las propiedades macroscópicas tales como densidad, viscosidad, contenido de ceniza y un largo etcétera de propiedades físico-químicas hasta su composición molecular. Para ello, multitud de metodologías han sido desarrolladas durante las pasadas décadas y, han sido recogidas, evaluadas y normalizadas por distintas oficinas de estandarización tales como la Sociedad Estadounidense para Pruebas y Materiales (ASTM; *American Society for Testing and Materials*) o la Organización Internacional de Estandarización (ISO; *International Organization for Standarization*).

Es en este punto es donde la química analítica juega un papel fundamental, siendo parte fundamental de todo el proceso. Desde el análisis del crudo sin procesar, hasta el producto final, pasando por todos los procesos intermedios, distintas metodologías analíticas son aplicadas con el fin de regular estos procesos, ayudando no sólo a que el combustible en cuestión

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[13] Palacio Lozano, D. C.; Thomas, M. J.; Jones, H. E.; Barrow, M. P. "Petroleomics: tools, challenges, and developments". Annual Review of Analytical Chemistry; **2020**; 13; 405-430.

cumpla los requisitos legales, si no también, a "guiar" todo el proceso intermedio hasta llegar al producto final.

La química analítica debe observarse como una herramienta integral en prácticamente cualquier proceso industrial. Con las herramientas adecuadas se busca superar retos que surgen en el día a día de la industria, bien por nuevas legislaciones, bien por necesidades intrínsecas a la producción. También debe usarse, no solamente como una herramienta de control, en el más estricto significado de la palabra, sino que la técnica adecuada puede proporcionar una información hasta el momento desconocida. Es por ello fundamental, que las industrias se nutran de un departamento de Desarrollo e Investigación, en el que, de la mano de la química analítica, se consiga una mejora en los distintos procesos que la conforman.

Con las herramientas adecuadas, se pueden caracterizar muestras de una forma bastante exhaustiva.[14] Conociendo sus propiedades físico-químicas tales como el número cetano, densidad o punto de ebullición. Mediante diferentes técnicas espectroscópicas podemos también determinar la composición detallada de las muestras, pudiendo además conocer la concentración de compuestos muy concretos.[15] Por ejemplo, en el caso de la industria petrolera, es fundamental la evaluación de los productos obtenidos durante una destilación del crudo de petróleo determinando el punto final e inicial de ebullición de un determinado producto.[16]

El uso de la química analítica nos permite conocer cualitativamente y cuantitativamente la composición de un cierto material. Existen técnicas, como la difracción de rayos X, la cromatografía de gases, la espectrometría de

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[14] Staš, M.; Kubička, D.; Chudoba, J.; Pospíšil, M. "Overview of analytical methods used for chemical characterization of pyrolysis bio-oil". *Energy & Fuels*; **2014**; *28*(1); 385-402.

[15] Vempatapu, B. P.; Kanaujia, P. K. "Monitoring petroleum fuel adulteration: A review of analytical methods". Trends in Analytical Chemistry; **2018**; 92; 1-11.

[16] Lee, D. M.; Lee, D. H.; Hwang, I. H. "Gasoline quality assessment using fast gas chromatography and partial least-squares regression for the detection of adulterated gasolina". Energy & Fuels; **2017**; 32(10); 10556-10562.

masas o la espectroscopía de absorción atómica, que nos proporcionarán una información, tanto cualitativa como cuantitativa de la composición de la muestra de estudio.[17]

## 2. Cromatografía de gases como técnica para la caracterización de muestras complejas

La cromatografía de gases (GC) es una técnica desarrollada a partir de los años 50 del siglo pasado que se ha consolidado como una poderosa herramienta para el análisis de mezclas complejas en diferentes campos científicos.[18,19] Esta técnica basa la separación de los distintos componentes de una mezcla en función de su punto de ebullición e interacción con una fase estacionaria. De esta forma, se pueden analizar una amplia gama de compuestos que van desde moléculas pequeñas muy volátiles (e.g. $O_2$, $H_2O$, $CO_2$, acetona, etc.) hasta compuestos de alto peso molecular (pesticidas, hidrocarburos, PAHs, etc.). Haciendo uso de distintas configuraciones del sistema, podemos seleccionar columnas cromatográficas, el núcleo de cualquier cromatógrafo de gases, para llevar a cabo el análisis de unas matrices u otras.[20] El tipo de información que podemos conseguir a través del análisis por GC depende, en gran parte, del tipo de detector usado, pudiendo obtener información tanto cualitativa como cuantitativa, según la metodología utilizada.

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[17] Bojkovic, A.; Vermeire, F. H.; Kuzmanovic, M.; Dao Thi, H.; Van Geem, K. M. "Analytics driving kinetics: advanced mass spectrometric characterization of petroleum products". Energy & Fuels; **2021**; 36(1); 6-59.

[18] Gruber, B.; David, F.; Sandra, P. "Capillary gas chromatography-mass spectrometry: Current trends and perspectives". Trends in Analytical Chemistry; **2020**; 124; 115475.

[19] Sudol, P. E.; Pierce, K. M.; Prebihalo, S. E.; Skogerboe, K. J.; Wright, B. W.; Synovec, R. E. "Development of gas chromatographic pattern recognition and classification tools for compliance and forensic analyses of fuels: A review". *Analytica Chimica Acta*; **2020**; *1132*; 157-186.

[20] Dettmer-Wilde, K., & Engewald, W. "Practical gas chromatography. A Comprehensive Reference". Springer; **2014**; 902.

La cromatografía, no solo de gases, al ser una técnica de separación requiere posteriormente un detector que detecte los analitos que eluyen de la columna. De esta forma, los sistemas cromatográficos pueden resumirse como un tándem de separación cromatográfica más detector. Por lo tanto, cuando se habla de selectividad en cromatografía, es preciso acotar los dos términos.

Un concepto importante en cromatografía de gases es el de selectividad. La IUPAC la define como "la capacidad de una metodología para determinar un analito en particular en mezclas o matrices sin interferencias de otros componentes de comportamiento similar".[21]

La selectividad cromatográfica, aunque viene determinada principalmente por la columna cromatográfica, puede controlarse con otros parámetros para mejorarla. La selectividad cromatográfica va directamente relacionada con la capacidad de resolver (separar) dos analitos. Esta capacidad puede cuantificarse, para dos analitos que eluyen consecutivamente, mediante el coeficiente de resolución (Rs):

$$Rs = \frac{t_{R(2)} - t_{R(1)}}{(w_{b(2)} + w_{b(1)})/2}$$

Siendo $t_R$ el tiempo de retención de cada uno de los dos compuestos y $w_b$ el ancho en la base de pico. La siguiente ecuación refleja qué términos afectan a la resolución, pudiendo mejorarse bien, aumentando la distancia entre los picos, asociado a la selectividad de la columna o, reduciendo el ancho de pico de ambos mejorando la eficiencia de la columna (número de platos teóricos):

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

En esta ecuación aparecen recogidos los distintos términos que pueden afectar a la separación en la columna. Por un lado, el término N, numero de platos teóricos, está relacionado con la eficiencia de la columna, y puede

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[21] Vessman, J., Stefan, R. I., Van Staden, J. F., Danzer, K., Lindner, W., Burns, D. T., Fajgelj, A. & Müller, H. (**2001**). Selectivity in analytical chemistry (IUPAC Recommendations 2001). Pure and Applied Chemistry, 73(8), 1381-1386.

aumentarse con la longitud de la columna. El segundo término, relacionado con la separación, puede modificarse con cambios en el gradiente cromatográfico o cambio en la fase estacionaria usada. Por último, k, se relaciona con el tiempo que un analito pasa entre la fase estacionaria y la fase móvil, e indica cuánto más un analito es retenido en la fase estacionaria comparado con el tiempo que se necesita para eluirlo de la columna. En definitiva, sin querer entrar en profundidad en el tema explicando los diferentes factores, los indicados son los diferentes términos que pueden afectar a la selectividad de una separación cromatográfica debido a la columna.

Como se ha indicado, la forma de pico de cada compuesto es importante en la separación cromatográfica. Esta no solo está determinada por la interacción analito-columna, si no que existe otro parámetro a tener en cuenta, la inyección. Mediante una inyección adecuada en la columna, podremos obtener compuestos con un ancho de pico correcto y una forma gaussiana. Así, algunos de los parámetros a tener en cuenta en la inyección es la temperatura del inyector, el tipo de *liner* usado, el volumen inyectado o, incluso el propio tipo de inyector usado. El campo de la inyección en cromatografía de gases, aunque amplio, está ya bien documentado, por lo que no se entrará en profundidad a explicar las distintas alternativas que existen.

## 2.1. Cromatografía de gases multidimensional

Hasta ahora, se ha descrito la cromatografía de gases como un sistema conformado, a grandes rasgos, por un inyector, una columna cromatográfica y un detector. Si bien, esta combinación de elementos, proporciona en multitud de análisis los resultados adecuados en términos de resolución, selectividad, etc., existen ciertas carencias en algunas de las matrices más complejas que existen, como es el caso de matrices medioambientales, combustibles con alto contenido de carbono, o simplemente, muestras donde el número de analitos de interés está por encima de los 100 compuestos, valor que se estima que una única columna puede resolver satisfactoriamente.[20]

Así, existen diferentes aproximaciones para acometer el análisis de este tipo de muestras. Por ejemplo, una alternativa, es la del uso de extracciones que permiten separar selectivamente los analitos de interés de las matrices complejas. Estas metodologías están bastante extendidas en análisis de rutina y en investigación, sin embargo, tienen la desventaja de que resultan laboriosas, pues requieren de tiempos de pretratamiento de la muestra y material de laboratorio, como filtros o jeringas especiales que suponen un coste extra al laboratorio.

Existe, sin embargo, otra alternativa para aumentar la selectividad de la metodología aplicada. Esto se puede realizar mediante el acoplamiento de dos, o más, columnas cromatográficas, y es lo que se conoce como cromatografía de gases multidimensional. En la cromatografía de gases multidimensional (*multidimensional* GC*,* MDGC*)*, fracciones seleccionadas del eluyente de la primera columna, son transferidas online, a una segunda columna, con una fase estacionaria diferente de forma que permite una segunda separación.[22] Esta técnica, está actualmente en desuso, y hoy en día, la técnica que más extendida está, es la cromatografía de gases bidimensional integral (*comprehensive two-dimensional GC,* GC×GC),[23] en la que, en vez de una pequeña fracción, en la segunda columna entra toda la muestra que eluye de la primera. La separación en la segunda columna suele ser muy rápida, en el orden de los pocos segundos, para no perder la resolución adquirida durante la primera dimensión. De esta forma, con prácticamente el mismo tiempo de análisis, se obtendrá un análisis bidimensional.

La GC×GC permite separar analitos, que en un principio coeluyen y no pueden separarse por cromatografía, ni puede usarse un detector selectivo

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[22] Marriott, P., & Shellie, R. (**2002**). Principles and applications of comprehensive two-dimensional gas chromatography. TrAC Trends in Analytical Chemistry, 21(9-10), 573-583.

[23] Tranchida, P. Q.; Purcaro, G.; Dugo, P.; Mondello, L. “Modulators for comprehensive two-dimensional gas chromatography”. Trends in Analytical Chemistry,; **2011**; 30(9), 1437-1461.

para distinguir entre uno y otro.[24] Así, usando columnas con fases estacionarias con mecanismos de separación distintos, suele usarse el término ortogonales, podremos separar analíticos que con la GC unidimensional estándar no se podría.[24]

Una de las partes más críticas en la GC×GC es la unión entre ambas columnas de forma que se permita “enviar” solutos de una columna a otra sin modificar significativamente los tiempos de elución, de forma cuantitativa y sin perder la resolución obtenida en la primera columna. La parte encargada de esto se llama modulador,[25] existiendo en la actualidad numerosos tipos, pero todos, tienen las tres mismas funciones:

- Recoger cada fracción que eluye de la primera dimensión.
- Reinyectar estas fracciones en la segunda dimensión
- Atrapar la siguiente fracción que eluye de la primera dimensión al mismo tiempo que manda la anterior a la segunda dimensión.

Existen moduladores basados en distintos mecanismos que pueden agruparse a grandes rasgos en aquellos basados en la aplicación de distintos flujos[26,27] o aquellos que basan la modulación en cambios de temperatura.[28]

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[24] Tranchida, P. Q.; Purcaro, G.; Visco, A.; Conte, L.; Dugo, P.; Dawes, P.; Mondello, L. “A flexible loop-type flow modulator for comprehensive two-dimensional gas chromatography”. Journal of Chromatography A; 2011; 1218(21); 3140-3145.

[25] Bahaghighat, H. D.; Freye, C. E.; Synovec, R. E. “Recent advances in modulator technology for comprehensive two dimensional gas chromatography. TrAC Trends in Analytical Chemistry; **2019**; 113; 379-391.

[26] Ferracane, A.; Zoccali, M.; Cacciola, F.; Salerno, T. M. G.; Tranchida, P. Q.; Mondello, L. “Determination of multi-pesticide residues in vegetable products using a “reduced-scale” Quechers method and flow-modulated comprehensive two-dimensional gas chromatography-triple quadrupole mass spectrometry”. Journal of Chromatography A; **2021**; 1645; 462126.

[27] Franchina, F. A.; Machado, M. E.; Tranchida, P. Q.; Zini, C. A.; Caramão, E. B.; Mondello, L. “Determination of aromatic sulphur compounds in heavy gas oil by using (low-) flow modulated comprehensive two-dimensional gas chromatography–triple quadrupole mass spectrometry”. *Journal of Chromatography A;* **2015**; *1387*; 86-94.

[28] Flego, C.; Zannoni, C. “N-containing species in crude oil fractions: An identification and quantification method by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry”. *Fuel*; **2011**; *90*(9); 2863-2869.

En cuanto a la combinación de columnas usadas, la mayoría de los estudios usan combinaciones de fases estacionarias diferentes siguiendo configuraciones tipo apolar × polar o polar × apolar, obteniendo así separaciones por punto de ebullición en una y, por polaridad u otro mecanismo de separación en la segunda, resultando una separación ortogonal.[29] Para aumentar todavía más la selectividad de las metodologías, suelen usarse en combinación no solo de detectores universales, sino también de detectores selectivos, como pueden ser el SCD y NCD.

Así, hasta ahora se ha reflejado la importancia que tiene la parte cromatográfica en un análisis. La segunda acepción del término selectividad viene dada por el tipo de selector usado. Este campo, se ampliará más en detalle en el siguiente punto.

## 2.2. Detectores en cromatografía de gases

En un análisis por cromatografía de gases, si bien la inyección y la separación en la columna tienen una importancia relevante de cara a conseguir una transmisión cuantitativa y una separación adecuada para todos los compuestos de la mezcla, el factor más determinante, y probablemente, el que más limita los análisis en una muestra es, el detector.

El detector es el encargado, una vez han eluído los compuestos de la columna, de recolectarlos y obtener la información adecuada sobre ellos. Muy simplificadamente, el proceso de detección en un cromatógrafo de gases viene dado por el cambio en alguna de las propiedades del detector con el paso de los compuestos, de forma que este es capaz de transformarlo en una corriente eléctrica a través de multitud de maneras y procesarlo adecuadamente, transmitiendo la información que buscamos en nuestro análisis.[20]

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[29] Pollo, B. J.; Alexandrino, G. L.; Augusto, F.; Hantao, L. W. "The impact of comprehensive two-dimensional gas chromatography on oil & gas analysis: Recent advances and applications in petroleum industry". Trends in Analytical Chemistry; 2018; 105; 202-217.

Asumimos generalmente que, la información obtenida del detector, representa fidedignamente la composición de nuestra mezcla y, es ahí donde reside la importancia de obtener una información correcta y ser conscientes de las limitaciones del detector usado, para, posteriormente, obtener las conclusiones cualitativas y/o cuantitativas pertinentes a nuestro análisis y, no obtener una interpretación errónea. Por lo tanto, es importante conocer los principios operacionales de nuestro detector de cara a obtener resultados analíticos de calidad.

Algunos de las características más importantes que definen a un detector son la sensibilidad, selectividad, reproducibilidad, rango lineal o el límite de detección. Así, unos detectores podrán proporcionarnos ciertas características que otros no, y viceversa y es en este punto donde reside la importancia en la selección de uno u otro detector en función del análisis requerido.

La clasificación de detectores cromatográficos puede hacerse con respecto a diferentes criterios. Por ejemplo, una clasificación general, es aquella que tiene en cuenta el tratamiento de la muestra, de forma que existen detectores destructivos que, tras algún tipo de reacción, consumen el analito y de ahí se genera una señal y, por otro lado, existen detectores no destructivos, cuya interacción con el analito no implica un consumo de este. Otra clasificación de cierta importancia es la que se hace en función de si la respuesta obtenida es dependiente del flujo másico o de la concentración. Si bien, esta clasificación está directamente relacionada con la primera, correspondiendo generalmente a los detectores destructivos y no destructivos, respectivamente, es importante de cara a entender el funcionamiento del detector y cómo la señal puede variar en función del flujo de fase móvil.[30] En un detector de masa, la respuesta será independiente del flujo de columna usado, obteniendo una señal en unidades de g/unidad de tiempo, mientras que en los detectores de concentración, la señal está

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[30] Poole, C. F.; Gas Chromatography, Elsevier, **2021**.

directamente relacionada con la cantidad de analito que eluye en un flujo determinado de fase móvil, lo que hace la señal dependiente de este último valor.

La principal clasificación que suele hacerse de los detectores de cromatografía de gases, y la que se realizará en la presente introducción, es con respecto al tipo de señal que obtenemos cuando los compuestos son detectados, si obtenemos una señal para prácticamente todos los compuestos que pueden analizarse por cromatografía de gases, estaremos hablando de detectores universales, mientras que, si la detección tiene lugar solo de aquellos compuestos que contienen un cierto átomo o grupo funcional, lo haremos de los detectores selectivos.

### *2.2.1. Detectores universales*

Los detectores universales se definen como aquellos detectores que son capaces de proporcionar una señal para prácticamente cualquier analito que puede ser analizado por cromatografía de gases. Así, cualquier compuesto capaz de ser inyectado y eluir en una columna cromatográfica, generará una señal en este tipo de detectores.[20] Los detectores universales se caracterizan por su versatilidad, siendo de gran utilidad para multitud de análisis en campos tan dispares como el análisis de combustibles,[31,32] la farmacéutica,[33] o medioambientales[34] entre muchos otros. En este apartado, se introducirán

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[31] Staš, M.; Kubička, D.; Chudoba, J.; Pospíšil, M. "Overview of analytical methods used for chemical characterization of pyrolysis bio-oil". Energy & Fuels; **2014**; 28(1); 385-402.

[32] Chua, C. C.; Brunswick, P.; Kwok, H.; Yan, J.; Cuthbertson, D.; van Aggelen, G.; Shang, D. "Tiered approach to long-term weathered lubricating oil analysis: GC/FID, GC/MS diagnostic ratios, and multivariate statistics". Analytical Methods; **2020**; 12(43); 5236-5246.

[33] Kay, J.; Thomas, R.; Gruenhagen, J.; Venkatramani, C. J. "Simultaneous quantitation of water and residual solvents in pharmaceuticals by rapid headspace gas chromatography with thermal conductivity detection (GC-TCD)". Journal of Pharmaceutical and Biomedical Analysis; **2021**; 194; 113796.

[34] Hrouzková, S.; Brišová, M.; Szarka, A. "Development of fast, efficient and ecological method employing vortex-assisted dispersive liquid–liquid microextraction combined

algunos de los detectores universales más usados como son el detector de ionización de llama (*flame ionization detector*, FID), el detector de conductividad térmica (*thermal conductivity detector*, TCD) o el detector de espectrometría de masas (mass spectrometer, MS) entre otros.

*Detector de ionización de llama (FID)*

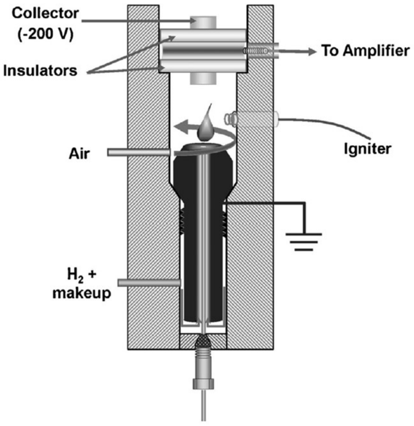

**Figura 5. Esquema de un detector de ionización de llama.**[30]

El detector FID es probablemente el detector más extendido en la cromatografía de gases junto con el MS desde su introducción en la década de 1960. Debido a su robustez, el escaso mantenimiento necesario y el gran rango lineal, su uso se ha extendido a números aplicaciones.[35] Si bien, la detección es selectiva respecto al carbono, suele considerarse un detector universal dado que la gran mayoría de compuestos analizados por cromatografía de gases contienen carbono en su estructura.[30] Basa esta detección universal en la combustión de los compuestos orgánicos bajo una llama de hidrógeno/aire. El eluyente de la columna es mezclado con una corriente de hidrógeno (gas de combustión) generando una serie de reacciones complejas que conllevan a la

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with fast gas chromatography–mass spectrometry for pesticide residues analysis in alcohol-content simples". Journal of Chromatography A; **2017**; 1506; 18-26.

35 Aparicio-Ruiz, R.; García-González, D. L.; Morales, M. T.; Lobo-Prieto, A.; Romero, I. "Comparison of two analytical methods validated for the determination of volatile compounds in virgin olive oil: GC-FID vs GC-MS". Talanta; **2018**; 187; 133-141.

formación de metano. Este es posteriormente oxidado en una zona más energética de la llama. Durante este proceso, un pequeño porcentaje de los carbonos genera iones y, el movimiento de estos se mide como una corriente eléctrica entre dos electrodos. Si bien, hay diferentes teorías para explicar los distintos procesos que tienen lugar en la llama,[36,37] algunos concluyen que existe una respuesta uniforme con respecto a compuestos que contienen carbono en su estructura, mientras que en otros estudios se han observado desviaciones dependientes de la estructura.[38]

Existe, sin embargo, un aspecto a tener en cuenta con respecto a compuestos con heteroátomos en su estructura. La presencia de elementos como halógenos, O, N o S conllevan una bajada en la sensibilidad respecto a estos compuestos en el detector, haciendo necesario el uso del concepto *Effective Carbon Number* (ECN), mediante el cual, la señal de un patrón interno cuya transformación en la llama sea cuantitativa, se utiliza para normalizar la señal del compuesto en la que no lo es, de forma que distintos grupos funcionales pueden ser cuantificados con un patrón interno con este tratamiento matemático. Por lo tanto, esta ligera dependencia de la señal supone una desventaja para el detector FID.[39]

El amplio rango dinámico ($10^7$), así como el bajo límite de detección y la prácticamente universal respuesta respecto a compuestos que contienen

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[36] Holm, T. "Aspects of the mechanism of the flame ionization detector". Journal of Chromatography A; **1999**; 842(1-2); 221-227.

[37] Schofield, K. "The enigmatic mechanism of the flame ionization detector: Its overlooked implications for fossil fuel combustion modeling". Progress in energy and combustion science; **2008**; 34(3); 330-350.

[38] Hua, Y.; Luong, J.; Yang, X.; Sieben, L.; Yang, P.; Gras, R. "Post-column reaction gas chromatography with a two-stage microreactor for the determination of volatile oxygenated compounds in high-pressure liquefied hydrocarbons". Analytical Methods; **2019**; 11(3); 276-281.

[39] Sobrado, L. A.; Freije-Carrelo, L.; Moldovan, M.; Encinar, J. R.; Alonso, J. I. G. "Comparison of gas chromatography-combustion-mass spectrometry and gas chromatography-flame ionization detector for the determination of fatty acid methyl esters in biodiesel without specific standards. Journal of Chromatography A; **2016**; 1457; 134-143.

carbono hacen del FID un detector excelente para multitud de aplicaciones tales como detección de hidrocarburos en aguas.[40]

*Detector de conductividad térmica (TCD)*

Existen en el mercado de la cromatografía de gases, muchos otros detectores que pueden ser considerados como universales. Así, por ejemplo, el detector de conductividad térmica (TCD; *thermal conductivity detector)* cuya señal se debe a una variación en la conductividad térmica de la corriente de gas continua cuando los analitos la atraviesan. Este detector, dependiente de la concentración, tiene alguna ventaja respecto al FID. Por ejemplo, se puede usar para moléculas que no dan señal en este, como son pequeñas moléculas inorgánicas como Ar, $H_2O$ o $H_2$.[30]

El funcionamiento específico de este detector consiste en la comparación de dos corrientes de gas. En una, usado como referencia, solo el gas portador es introducido en la celda, en la otra, lo haceb el gas portador junto con el analito. En esta celda, un filamento es calentado con una corriente eléctrica a una temperatura constante, que por supuesto debe ser controlada con alta precisión. El circuito electrónico ajusta la corriente del filamento para mantener esta temperatura constante, de forma que, cuando existe una perturbación en esta temperatura, la corriente cambia. Así, cuando el analito alcanza la celda, las propiedades de conductividad térmica y calor específico del gas son modificadas, de forma que el hilo del filamento ve alterado su entorno, y se enfría debido a la diferencia en las propiedades especificadas antes. El componente electrónico del detector ajusta la corriente para compensar esta diferencia de temperatura y esta corriente, es la señal que se obtiene cuando el analito eluye de la columna.

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[40] Tankiewicz, M.; Morrison, C.; Biziuk, M. "Application and optimization of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–flame-ionization detector (GC–FID) to determine products of the petroleum industry in aqueous simples". Microchemical Journal; **2013**; 108; 117-123.

Si bien, este detector es útil para pequeñas moléculas que, como se decía antes, pueden no ser detectables en FID, tiene ciertas desventajas tales como un límite de detección más alto, en torno a 1 a 10 ppm y también un rango lineal menor ($10^4$-$10^5$). Además, otra de las desventajas es la necesidad de adaptar el gas portador en función de los analitos de interés, con el objetivo de maximizar la diferencia en la conductividad térmica entre ambos.[20]

Si bien este detector es útil para ciertas aplicaciones cualitativas, la cuantificación ha de hacerse usando patrones específicos de cada analito, lo que dificulta este proceso, al requerir estándares concretos para ello, al depender también del disolvente en el que se encuentren.[41,42]

*Otros detectores universales*

Hasta ahora se han expuesto algunos de los detectores universales más usados en la cromatografía de gases, pero existen muchos otros que no serán explicados en profundidad en esta introducción. Por ejemplo, los detectores Ultravioleta de vacío (GC-VUV) o de infrarrojo con transformada de furier (GC-FTIR) permiten análisis en un amplio rango de campos. Son considerados detectores de espectroscopía molecular y permiten una detección universal de diferentes compuestos. Para ello hacen uso de una radiación ultravioleta o infrarroja, respectivamente, para estudiar la interacción de los compuestos que eluyen de la columna con ellas. Tras una interpretación matemática de los datos, se pueden obtener conclusiones respecto al tipo de molécula que se está analizando e, incluso, pueden proporcionar información que otros detectores no pueden, como es por ejemplo la diferenciación entre isómeros sirviendo

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[41] Freire, R.; Mego, M.; Oliveira, L. F.; Mas, S., Azpiroz, F.; Marco, S.; Pardo, A. "Quantitative GC–TCD Measurements of Major Flatus Components: A Preliminary Analysis of the Diet Effect". Sensors; **2022**; 22(3); 838.

[42] Eckhard, T.; Wütscher, A.,; Muhler, M. "Simultaneous analysis of light gases and heavy pyrolyzates evolved from lignite and hard coal by pyrolysis–GC/MS–GC/TCD". Journal of Analytical and Applied Pyrolysis; **2020**; 149; 104833.

pues, como complemento para otros detectores más extendidos..[43] Otra ventaja de estos detectores es la posibilidad de acoplamiento con otros detectores ya que son no destructivos.

Por último, el detector universal por excelencia junto con el FID, la espectrometría de masas (MS) será abordado posteriormente en un capítulo dedicado concretamente a esta técnica tan importante en el mundo de la cromatografía de gases.

### *2.2.2. Detectores selectivos*

En el mundo de la cromatografía de gases, multitud de veces, los análisis requeridos no necesitan de un detector sensible a todos los compuestos de la mezcla, bien porque estamos interesados solamente en un cierto tipo, diferenciado por algún elemento o grupo funcional en su estructura o porque necesitamos de límites de detección por debajo de lo que nos suelen proporcionar los detectores universales. Así, existen detectores selectivos para distintos elementos como el S, N, los halógenos o el O, que pueden ser útiles para aplicaciones en distintos campos tales como la industria de los combustibles, farmacéutica, alimentación, biología y un largo etcétera.[20,30]

Un punto importante a tener en cuenta en estos detectores es la capacidad de proporcionar una señal libre de interferencias. Así, si se busca analizar únicamente el contenido de azufre de cierta muestra, debemos asegurar que no existe ningún tipo de interferencia en la matriz que dé señal en el detector, de forma que se pueda asociar indudablemente, que la señal medida pertenece al analito en cuestión. Esta propiedad es la que se conoce como selectividad, y expresa la capacidad de diferenciar una señal para un analito sin interferencia de ningún otro.

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[43] Zavahir, J. S.; Smith, J. S.; Blundell, S.; Waktola, H. D.,; Nolvachai, Y.; Wood, B. R.; Marriott, P. J. "Relationships in gas chromatography—Fourier transform infrared spectroscopy—comprehensive and multilinear análisis". Separations; **2020**; 7(2); 27.

Así, al igual que para los detectores universales, en los detectores selectivos se buscan propiedades analíticas tales como amplitud de rango lineal, reproducibilidad, linealidad, buenos límites de detección, que, por lo general, por la propia definición del detector selectivo, suelen ser más bajos que para un detector universal, entre otras cualidades.

Un punto importante a aclarar respecto a la terminología es el hecho de la distinción entre selectividad y especificidad. Es bastante común el uso indistinto de estos dos términos para referirse a lo mismo, sin embargo, la IUPAC recomienda el uso de selectividad cuando se hace referencia a la capacidad de distinguir un analito de otro sin interferencias. La selectividad, como se indicó anteriormente, puede cuantificarse, sin embargo, la especificidad, es más bien un término cualitativo que hace referencia a una detección selectiva sin interferencias, una detección infinita en la que el analito puede distinguirse indistintamente de la matriz donde esté analizado sin interferencias. Esta cualidad es, ciertamente, difícil de conseguir y es por ello por lo que se recomienda el uso de selectividad, que podemos cuantificar y numerar y resulta más útil para comparar un detector u otro.

A continuación, se expondrán algunos de los detectores selectivos más usados en el mundo de la cromatografía de gases, así como algunas de sus propiedades analíticas.

*Detector de emisión atómica (AED)*

El detector de emisión atómica es un detector selectivo multielemental ampliamente utilizado en cromatografía de gases capilar. Su respuesta se basa en las transiciones electrónicas de los distintos átomos e iones cuando son excitados y la diferenciación entre estas en función del elemento de estudio, marca la selectividad entre estos.

Los compuestos que eluyen de la columna son atomizados en un plasma de helio generado por microondas (MIP), posteriormente excitados y finalmente detectados como radiación de emisión cuando vuelven a su estado fundamental. Esta radiación es la que el detector capta y transforma en una señal analítica.

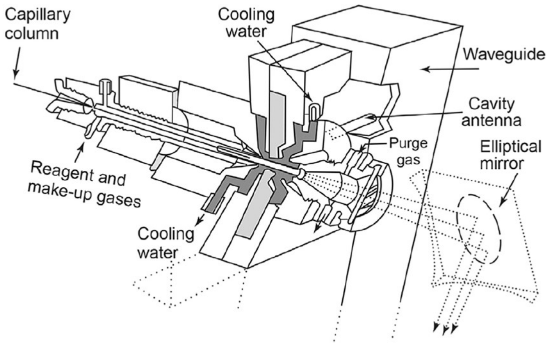

**Figura 6. Esquema del interior de un detector de emisión atómica.**[30]

Sin embargo, se considera un detector con relativa complejidad y alto coste, lo que limita su aplicabilidad en laboratorios de rutina. Si bien se considera al AED un detector multielemental, solo un número limitado de líneas de emisión (correspondientes a uno o varios elementos) se pueden adquirir. Durante un único análisis, solamente se puede detectar de 1 a 4 elementos dentro de una ventana de longitudes de onda de entre 20 a 25 nm, siendo el rango total del detector de 170 a 780 nm. Se pueden añadir diferentes gases al plasma en función del analito de interés con el objetivo de potenciar su ionización. Algunos de los elementos que pueden detectarse por espectroscopía de emisión atómica son diferentes heteroátomos como el oxígeno,[44] halógenos,[45] azufre,[45] nitrógeno, también carbono[44,46] e

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[44] Bartle, K. D.; Hall, S. R.; Holden, K.; Mitchell, S. C.; Ross, A. B. "Analysis of oxygen-containing polycyclic aromatic compounds by gas chromatography with atomic emission detection". Fuel; **2009**; 88(2); 348-353.

[45] Mazurek, M.; Witkiewicz, Z.; Popiel, S.; Śliwakowski, M. "Capillary gas chromatography–atomic emission spectroscopy–mass spectrometry analysis of sulphur mustard and transformation products in a block recovered from the Baltic Sea". Journal of Chromatography A; **2001**; 919(1); 133-145.

[46] Kim, T.; Alhooshani, K.; Ali, S. A.; Park, J. I.; Al-Yami, M.; Yoon, S. H.; Mochida, I. "Identification and quantification of (alkyl) benzenes in hydrocracked products of light cycle oil by GC–AED". Fuel; **2013**; 111; 883-886.

hidrógeno[46] así como diferentes elementos metálicos que pueden encontrarse formando parte de ciertos compuestos orgánicos.

En cuanto a las características analíticas de este detector, tiene un rango lineal de entre $10^3$-$10^4$ unidades, con límites de detección en un rango de 3 a 300 pg en función del elemento a medir.

Teóricamente, la completa atomización de las especies que llegan al plasma asegura una respuesta uniforme e independiente de la especie,[44,20] sin embargo la capacidad de este detector de proporcionar una respuesta equimolar está a veces en entredicho.[47] Existen diversos factores que pueden alterar la respuesta, entre ellos, la presencia en exceso de la matriz en la que se encuentra el analito o, las variaciones en los flujos de gas del sistema.[30] Es por ello que esta técnica, pese a la capacidad de detección multielemental está en desuso durante las últimas décadas, habiendo tenido su máximo auge en los años 90 y 2000, dejando paso a técnicas más sensitivas como el ICP-MS.

*Detectores de quimioluminiscencia: Azufre y Nitrógeno (SCD y NCD)*

Los detectores de quimioluminiscencia de azufre y nitrógeno se basan en el mismo principio ambos. Los compuestos con un átomo de azufre o nitrógeno son atomizados en una llama de hidrógeno y oxígeno, de forma que como producto de diferentes reacciones se forman las especies SO y NO respectivamente.[48]

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[47] Li, G.; Wu, D.; Xie, W.; Zhang, X.; Liu, B. "Evaluation of compound-independent calibration using gas chromatography with atomic emission detection". *Talanta*; **2012**; *95*; 36-41.

[48] Yan, X. "Unique selective detectors for gas chromatography: Nitrogen and sulfur chemiluminescence detectors". Journal of separation science; **2006**; 29(12); 1931-1945.

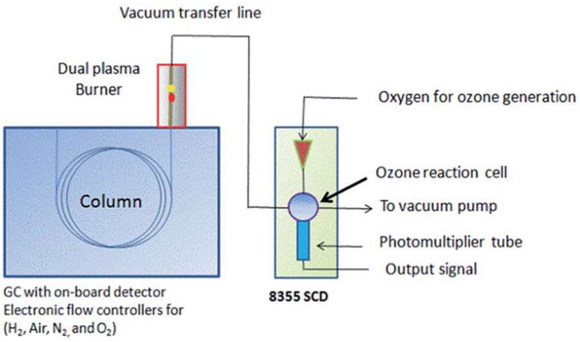

**Figura 7. Esquema general de un detector de quimioluminiscencia.**[49]

Posteriormente, se introducen en una cámara de reacción con ozono, y forman las especies electrónicamente excitadas, $SO_2^*$ y $NO_2^*$. Cuando estas especies vuelven a su estado natural, emiten una radiación cerca de 360 nm en el caso del S y de 1200 nm en el caso del N. Esta radiación es la señal analítica que relacionaremos con el analito en cuestión.

$$R - S \rightarrow SO \tag{1}$$

$$SO + O_3 \rightarrow SO_2^* + O_2 \tag{2}$$

$$SO_2^* \rightarrow SO_2 + hn \tag{3}$$

**Figura 8. Serie de reacciones que tienen lugar en un detector SCD. En el caso de un detector NCD, las reacciones son similares, pero generando las especies NO y $NO_2^*$.**

Pese a la aparente similitud entre el funcionamiento de ambos detectores, se puede hablar de dos detectores distintos, dada las diferencias instrumentales y de operatividad de cada uno de ellos. Los detectores de quimioluminiscencia se caracterizan por sus bajos límites de detección, en las bajas ppb para ambos elementos. También el amplio rango lineal (hasta $10^4$) y la capacidad para analizar matrices muy complejas, como combustibles,

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[49] Luong, J.; Gras, R.; Hawryluk, M.; Shearer, R. “A brief history and recent advances in ozone induced chemiluminescence detection for the determination of sulfur compounds by gas chromatography”. Analytical Methods; **2016**; 8(39); 7014-7024.

hacen de ellos detectores sumamente importantes en diferentes campos de la ciencia, aunque pueden existir casos de apantallamiento de la señal.

Una propiedad que también es importante en el caso de los detectores selectivos, es la capacidad de proporcionar una respuesta independiente de la especie (equimolaridad). Este concepto se estudiará en profundidad en un apartado posterior. En este punto hay que indicar, que existen ciertos estudios, que ponen en contradicho la equimolaridad de estos detectores para ciertos grupos funcionales como pueden ser los enlaces N – N. Es común también el uso de estos detectores unidos en línea con otros detectores universales (FID) con el fin de obtener simultáneamente un perfil universal y selectivo que ayude a comprender mejor determinadas muestras. [50]

*Detector de fotometría de llama (FPD)*

El detector de fotometría de llama ha sido uno de los detectores más usados para la detección selectiva de azufre, aunque también se puede usar con otro elemento importante como es el fósforo. Se basa, al igual que el SCD, en la medida de la quimioluminiscencia emitida como resultado de una serie de reacciones que generan productos en estado excitado.

A diferencia del SCD, en este caso se usa una llama enriquecida en hidrógeno lo que convierte la llama en una bastante fría. Tras varias versiones, es la versión de doble llama la que más extendida se encuentra actualmente. En este detector los analitos son combustionados en una primera llama, y posteriormente reducidos para generar la especie excitada $S_2^*$. En el caso del fósforo, la especie que se cree que se encuentra en un estado de excitación y que es la responsable de la quimioluminiscencia es el HPO*.

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[50] Prasantongkolmol, T.; Thongkorn, H.; Sunipasa, A.; Do, H. A.; Saeung, C.; Jongpatiwut, S. "Analysis of sulfur compounds for crude oil fingerprinting using gas chromatography with sulfur chemiluminescence detector". Marine Pollution Bulletin; **2023**; 186; 114344.

Una de las grandes desventajas de la detección por FPD es su respuesta no lineal[51] y la tendencia a sufrir el efecto conocido como quenching, una alteración de la señal debida a la coelución con el analito de interés de la matriz. Si bien esto ha sido en gran parte reducido por el uso del detector pulsado FPD (PFPD) y el uso de las dobles llamas, aún se puede llegar a observar ciertos defectos en la señal debida a la matriz. Esto es principalmente observado en el azufre, no tanto en la detección de fósforo.30 además, se pueden observar diferencias en la respuesta en función de la estructura del compuesto, siendo necesario el uso de diferentes patrones para llevar a cabo la cuantificación.[52]

### *Detector selectivo de Oxígeno-FID (O-FID)*

Uno de los compuestos de mayor interés en la industria energética son aquellos que contienen un átomo de oxígeno en su estructura. Aunque la detección de oxígeno puede realizarse selectivamente con el detector de GC-AED,[44,45] las desventajas comentadas anteriormente hacen necesaria la búsqueda de un detector que permita su detección con niveles de detección más bajos.

El detector selectivo de oxígeno de ionización de llama basa su funcionamiento en el detector universal FID.[53] Este detector incluye un reactor que tras la atomización completa de los compuestos, cada átomo de oxígeno reacciona con uno de carbono formando monóxido de carbono, que posteriormente es reducido con hidrógeno en un microreactor para su transformación en metano. Este metano es detectado de forma tradicional por

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[51] Kim, K. H. "Performance characterization of the GC/PFPD for H2S, CH3SH, DMS, and DMDS in air". Atmospheric Environment; **2005**; 39(12); 2235-2242.

[52] Fang, Y.; Qian, M. C. "Sensitive quantification of sulfur compounds in wine by headspace solid-phase microextraction technique". Journal of Chromatography A,; **2005**; 1080(2); 177-185.

[53] Makoś, P.; Przyjazny, A.; Boczkaj, G. "Methods of assaying volatile oxygenated organic compounds in effluent samples by gas chromatography—A review". Journal of Chromatography A; **2019**; 1592; 143-160.

el detector FID.[54] De esta forma, cada compuesto es estequiométricamente transformado en una molécula de metano y la respuesta estará directamente relacionada con el contenido de oxígeno.

Pese a la novedad del detector, este detector carece de límites de detección bajos, permitiendo el análisis en un rango de concentraciones solo por encima de las ppm.53 Además, la capacidad equimolar del detector no es aceptable, con recuperaciones dispares entre distintos compuestos, lo que impide el uso de un único patrón interno para la cuantificación de las especies, haciendo necesario el uso de patrones específicos para cada analito.[54]

*Detector nitrógeno-fósforo (NPD)*

Al igual que sucede con el O-FID, el detector de Nitrógeno-fósforo (NPD) se basa en el comportamiento del FID. A pesar del nombre, no solo es un detector selectivo de estos dos elementos, sino que también es selectivo para los halógenos, aunque su comportamiento no está del todo clarificado.

Sobre la llama de un detector FID modificado, se coloca una perla cerámica que contiene sales alcalinas de algún metal como cesio o rubidio. Cuando esta perla se calienta, en este caso con un gas enriquecido en hidrógeno y una corriente eléctrica, se forma una especie de plasma alrededor de ella. De esta forma, se obtiene una fuente energética de menor energía que el FID, por lo que disminuye la señal respecto a los hidrocarburos, mientras que los compuestos con átomos de fósforo o nitrógeno forman especies electronegativas tales como PO, $PO_2$ y CN, que posteriormente se ionizaran formando aniones. Teóricamente, estos reaccionan con el hidrógeno formando moléculas neutras y electrones que son recogidos posteriormente por un colector formando la corriente de electrones que se asocia a la señal.[20]

A pesar de los teóricamente buenos límites de detección, llegando hasta los fg en el caso del P, selectividad y rango lineal, el detector no proporciona

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[54] Sironi, A.; Verga, G. R. "The O-FID and its applications in petroleum product analisis". Journal of Chromatography Library; (Vol. 56, pp. 143-158). Elsevier. **1995**

una respuesta independiente de la especie, incluso con especies similares.[55] Esto implica, como en otros detectores selectivos, el uso de patrones específicos para cada compuesto cuando se pretende cuantificar.[56]

Este detector ha sido de especial utilidad en el caso de muestras de tipo ambiental, en la que pesticidas, muchos de ellos con nitrógeno y fósforo en su estructura pueden detectarse.

*Detector de captura de electrones (ECD)*

Por último, el detector de captura de electrones es un detector selectivo de grupos electróforos tales como halógenos y otros grupos electronegativos como pueden ser los grupos nitro. Se trata de un detector ionizante, en el que se generan electrones a través de una radiación $\beta$ proporcionada por $^{63}$Ni.[30]

Las moléculas del gas portador, tras la colisión con los electrones altamente energéticos emitidos por la fuente, se ionizan liberando electrones de baja energía. Estos electrones son atrapados por un ánodo, generando de esta forma una corriente base. Cuando un compuesto con un grupo detectable entra en la cámara de ionización, captura estos electrones y se genera una disminución en la corriente generada que se mide como la señal del detector para el compuesto. Este es el funcionamiento básico de los primeros detectores ECD, en la actualidad se han acometido mejoras en la estabilidad de la corriente de electrones. En la actualidad, los ECDs trabajan con una frecuencia modulada de forma que la mayor parte del tiempo no hay voltaje en la celda y la concentración de electrones de baja energía puede acumularse, reaccionando de esta forma más eficientemente con las moléculas que cuando son acelerados en un campo eléctrico.[20,30] De esta forma se permiten

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[55] Tian, F.; Liu, W.; Fang, H.; An, M.; Duan, S. "Determination of six organophosphorus pesticides in water by single-drop microextraction coupled with GC-NPD". Chromatographia; **2014**; 77; 487-492.

[56] Park, J. H.; Iqbal Rouf Mamun, M.; Abd El-Aty, A. M.; Na, T. W.; Choi, J. H.; Ghafar, M. W.; Shim, J. H. "Development and validation of a multiresidue method for determination of 37 pesticides in soil using GC-NPD". Biomedical Chromatography; **2011**; 25(9); 1003-1009.

concentraciones constantes de electrones, que también favorecen una ionización de las moléculas más equitativa.

Al igual que en otros detectores, la señal es específica de la especie, o del grupo detectado, además de que aumenta con el número de sustituyentes de una molécula orgánica con el mismo número de carbonos, siendo imposible de calcular. La ionización de las especies también es dependiente de la temperatura, siendo necesario mantener la temperatura del detector constante o realizar un calibrado para cada sustancia en las condiciones exactas del análisis. Aunque el ECD proporciona límites de detección en el rango de los fg, la dependencia de la señal con la especie hace su aplicación en análisis cuantitativos limitada. A pesar de ello, es uno de los detectores selectivos cuyo uso está más extendido.[57]

Con este detector se llega al final de esta breve introducción de detectores universales y selectivos, en el que se han querido abordar algunos de los más importantes. Queda patente en los distintos ejemplos mostrados, que la mayoría de los detectores muestran alguna carencia, siendo la principal de ellas, la dependencia de la señal con respecto a la estructura de los compuestos analizados. Esta falta de equimolaridad en la mayoría de los casos impide el uso de patrones genéricos para la cuantificación de muestras con un alto número de analitos. También, los límites de detección en ciertos detectores se encuentran muy por encima de lo requerido hoy en día.

A continuación, se muestra una tabla resumen en la que se recogen las principales características de los detectores comentados durante la introducción.

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[57] Ramezani, S.; Mahdavi, V.; Gordan, H.; Rezadoost, H.; Conti, G. O.; Khaneghah, A. M. "Determination of multi-class pesticides residues of cow and human milk samples from Iran using UHPLC-MS/MS and GC-ECD: A probabilistic health risk assessment". *Environmental Research*; **2022**; *208;* 112730.

**Tabla 1. Resumen de detectores y sus características usados en la cromatografía de gases.**

| **Detector** | **Respuesta** | **Especie detectada** | **Rango lineal** | **Equimolaridad** | **LOD** |
|---|---|---|---|---|---|
| **ICP-MS** | Selectiva | Átomos ionizados | $10^5$ | Sí | <1pg (depende del |
| **MS (SIM)** | Selectiva | Fragmentos de moléculas ionizados | $10^6$ | No | ~10 pg |
| **MS (Scan)** | Universal | Moléculas ionizadas | $10^6$ | No | ~10 pg |
| **O-FID** | Selectiva | Oxígeno | $10^3$ | Relativa | 300 pg (volátiles) |
| **NPD** | Selectiva | Nitrógeno/Fósforo | $10^5$ | No | ~10 pg |
| **SCD/NCD** | Selectiva | Azufre/Nitrógeno | $10^4$ | Sí | ~1 pg |
| **ECD** | Selectiva | Grupos y elementos electronegativos | $10^4$ | No | ~1 fg/µL |
| **FPD** | Selectiva | Fósforo/Azufre | No lineal | No | ~10 pg |
| **AED** | Selectiva | Emisión atómica | $10^3$-$10^4$ | Relativa | 3-300 pg |
| **TCD** | Universal | Conductividad | $10^5$ | No | <1 ng/µL |
| **FID** | Universal | Carbono | $10^7$ | Relativa | ~10 pg |

# 3. La espectrometría de masas como herramienta de caracterización en cromatografía de gases

Hasta ahora, se han detallado algunos de los detectores selectivos y universales más utilizados en la cromatografía de gases, dejando de lado el que es probablemente, el detector más usado en la cromatografía de gases, la espectrometría de masas (*mass spectrometry,* MS).

La espectrometría de masas, nace del estudio en el siglo XIX de la composición elemental de la materia con la obtención de lo que se puede considerar el primer espectro de masas.[58] Si bien, estos estudios iniciales se orientaban desde una óptica física con el fin de comprender la composición fundamental de la materia, no fue hasta entradas unas décadas en el siglo XX y el desarrollo de espectrómetros con mayor resolución y sensibilidad cuando comenzaron a suponer una diferencia real en la investigación en química analítica.

Las características excepcionales en términos de sensibilidad, límites de detección, velocidad de adquisición, robustez sumado a la multitud de aplicaciones, hacen de la espectrometría de masas la herramienta más completa en el campo de la química analítica.

La espectrometría de masas es una técnica capaz de separar analitos en función de su relación masa/carga (m/z) y posteriormente detectarlos. Este fundamento, a priori, sencillo, requiere de multitud de condiciones específicas para ser satisfecho, y es por ello que desde su inicio se han desarrollado numerosos sistemas que son capaces de llevar a cabo estas separaciones de modos completamente distintos. Así, dentro de los detectores que engloba la espectrometría de masas se pueden encontrar desde el sistema más básico

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[58] Griffiths, J. "A brief history of mass spectrometry". *Anal. Chem*; 2008; *80*(15); 5678-5683.

como es el cuadrupolo simple, hasta aquellos con capacidades de resolución mucho mayores como son los tiempo de vuelo o el doble enfoque.[59]

El desarrollo de la espectrometría de masas fue acompañado del de la cromatografía de gases, hasta que hacia mediados de siglo surgió el primer cromatógrafo de gases acoplado a un MS. El acoplamiento entre ambas técnicas fue un hecho lógico dada la capacidad del GC de separar moléculas orgánicas y eluir prácticamente puras en una fase gaseosa por un lado y, la del MS de recibirlas e ionizarlas para una posterior caracterización. Dadas las características similares en cuanto a condiciones generales de trabajo de ambas, fase gaseosa, rangos de temperatura similares, concentraciones bajas, el acoplamiento entre los dos fue un desarrollo lógico y relativamente sencillo de conseguir.

Para describir los distintos espectrómetros de masas disponibles en la actualidad, primero, habría que hacer una clasificación de las distintas partes que todos ellos comparten. El funcionamiento básico de un MS consiste en primer lugar, en una ionización de las moléculas de interés en la fuente de ionización. Una vez los analitos han sido ionizados son redirigidos mediante una serie de lentes hacia la zona del analizar, el corazón del espectrómetro de masas. El analizador permite separar los iones producidos en la fuente mediante su ratio m/z para posteriormente pasar al detector donde son recolectados y transformados en señales eléctricas que finalmente se transmiten a un ordenador como señales identificables por el operador. Las combinaciones entre los distintos tipos de fuentes de ionización y analizadores proporcionan numerosos sistemas de espectrometría de masas que permiten adaptarse a las distintas necesidades, ya sea por el tipo de analito, de matriz o de análisis requerido.[60] Algunos de los retos a los que se enfrenta la espectrometría de masas en la caracterización de muestras

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[59] Sparkman, O. D.; Penton, Z.; Kitson, F. G. "Gas chromatography and mass spectrometry: a practical guide". Academic press. **2011**

[60] Hoggmann, E.; Stroobant, V.; Mass Spectrometry. Principles and Applications, Third Edition. Wiley, **2001**.

complejas son la ionización, la capacidad de resolución y exactitud y, como se comentaba anteriormente, la versatilidad de la espectrometría de masas para elegir diferentes configuraciones permite resolver estos problemas.[13]

La técnica de GC-MS tiene aplicaciones tanto cualitativas, con la capacidad de obtener espectros que permiten identificar los compuestos que eluyen de la columna, como cuantitativas, siendo una herramienta fundamental en campos tales como la ciencia forense, medio ambiente, salud, medicina y un largo etcétera. En las últimas décadas el desarrollo de multitud de equipos de cromatografía de gases y de espectrómetros de masas ha dado lugar a un amplio rango de técnicas que permiten una caracterización sin igual de distintas matrices y analitos. Esto se debe principalmente a la capacidad de detección de la MS. Así, la MS es considerada a la vez un detector universal y selectivo ya que permite trabajar en ambos modos. Cuando el analizador, que se explicarán posteriormente, es utilizado en modo SCAN, permite hacer un barrido de distintas masas en un intervalo concreto. A la vez, la mayoría de los espectrómetros de masas, permiten realizar un análisis en SIM (*single ion monitoring*) en que el analizador filtrará determinadas relaciones m/z que se especifiquen. Estas relaciones concretas, normalmente están relacionadas con el tipo de molécula analizada, en función de su composición elemental, estructura molecular, etc. De esta forma, se considera al MS un detector selectivo, al permitir filtrar analitos en función de su relación m/z concreta.

En teoría, cualquier espectrómetro de masas puede ser acoplado a un cromatógrafo de gases siempre y cuando la interfase entre ambos tenga el diseño adecuado de forma que los requerimientos de ambos instrumentos satisfagan las necesidades analíticas del problema en cuestión.

## 3.1. Espectrometría de masas elemental y molecular: fuentes de ionización en espectrometría de masas

Los distintos sistemas de espectrometría de masas aceptan clasificaciones en función de multitud de parámetros. Uno de ellos es en función del ion generado que se detecta, pudiendo diferenciar a grandes rasgos la espectrometría de masas molecular, cuando es el ion molecular, o parte de él, el que se detecta, o elemental, cuando detectamos elementos que forman parte de la estructura del analito. Esta división viene principalmente dada por la capacidad de las distintas fuentes de ionización de proporcionar una fragmentación mayor o menor de la molécula, lo que se conoce como “dureza” de la fuente.

Una de las fuentes de ionización más usada en la espectrometría de masas, principalmente por la simpleza con la que trabaja y su versatilidad, es la ionización electrónica (*electron ionization*, EI). En esta fuente de ionización, considerada como dura, los analitos son bombardeados con un haz de electrones de alta energía que la transmiten a las moléculas. Estas, buscan un estado de energía más favorable de forma que liberan un electrón quedando ionizadas, tanto el ion molecular como diferentes fragmentos procedentes de este, dando lugar a patrones de fragmentación muy reconocibles en función de la estructura y composición elemental del compuesto. Estos patrones de fragmentación, están recogidos en bibliotecas que posteriormente pueden ser usadas para comparar y llevar a cabo un reconocimiento del compuesto objetivo. En esta fuente de ionización, debido a la alta fragmentación a la que son sometidos los analitos puede incluso llegarse a perder el ion molecular.[60]

Otra fuente de ionización con un creciente interés en las últimas décadas, es la ionización química (*chemical ionization, CI*). Esta fuente de ionización, basada en la EI, carece de una fragmentación tan fuerte como esta. En la CI, un gas reactivo, como pueden ser el metano o el amoniaco, son ionizados en una fuente EI. Posteriormente colisiones entre este gas reactivo y los analitos generan nuevas especies ionizadas del último. Como la energía de los iones reactivos es mucho menor que en EI, la fragmentación del ion molecular está mucho menos favorecida. El espectro generado depende del tipo de gas reactivo y del tipo de iones producidos, pudiendo usarse CI positiva o negativa, siendo esta última útil en el caso de análisis de compuestos halogenados.

Dentro de las fuentes de ionización que se usan en GC-MS, podemos incorporar la espectroscopía de plasma inductivo (ICP). Esta fuente de ionización es la más dura de todas, y permite la atomización completa de los analitos y posteriormente ionizar los átomos generados, de forma que la detección tiene lugar básicamente por el valor de la masa atómica de cada elemento de interés que forma parte de la estructura del compuesto. Pese a las buenas prestaciones de esta técnica, se encuentra algo limitada en cuanto a su aplicación, ya que trabaja a presión atmosférica, lo que impide detectar elementos que podemos encontrar en el aire tales como N, H y O y con ciertas limitaciones para C, entre otros.

En esta fuente de ionización los analitos son atomizados a través de la formación de un plasma de alta energía formado por la ionización de átomos de Ar con una radiofrecuencia. El plasma generado alcanza temperaturas por encima de los 8000 K lo que permite la completa desolvatación (no necesaria en el caso de la GC), atomización e ionización de las especies.[61]

Existen otras técnicas de ionización, como la ionización supersónica o la ionización química a presión atmosférica (APCI) pero dada su escasa popularidad en la cromatografía de gases de rutina no se comentarán en esta tesis doctoral.

## 3.2. Analizadores en espectrometría de masas

Una vez los aniones han sido generados en la fuente, el siguiente paso es la distinción entre estos en función de su relación m/Z. Si bien, en el inicio de la cromatografía de gases los analizadores de sector magnético, en los que los aniones son separados en función de su respuesta a un campo magnético, eran los más usados, hoy en día, los analizadores basados en el cuadrupolo (simple o en tándem) son los más extendidos en laboratorios de rutina dada su gran versatilidad, sus buenas características analíticas que permiten separaciones

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[61] Houk, R. S.; Fassel, V. A.; Flesch, G. D.; Svec, H. J.; Gray, A. L.; Taylor, C. E. "Inductively coupled argon plasma as an ion source for mass spectrometric determination of trace elements". *Analytical Chemistry*; **1980**; *52*(14); 2283-2289.

con límites de detección lo suficientemente bajos para cumplir los cada vez más bajos requisitos y, sobre todo su relativa simplicidad a la hora de trabajar con ellos. Si bien, este analizador es para la mayoría de las aplicaciones suficiente, existen muchos otros que permiten separaciones con resoluciones mucho mayores como son los analizadores de tiempo de vuelo[62] o el orbitrap.[63]

Los analizadores de tipo cuadrupolo están formados por cuatro barras en paralelo dispuestas a lo largo del haz de iones y emparejadas con una serie de voltajes dos a dos. Estos voltajes hacen que los iones que entren en el analizador, comiencen a oscilar de forma que solo aquellos con el ratio correcto tendrán una trayectoria estable y serán capaces de atravesar el filtro y ser detectados. Simplemente cambiando el voltaje aplicado se podrá modificar las masas que se permiten hacer pasar por el analizar. Si bien estos detectores tienen una resolución menor que otros, la velocidad de escaneo relativamente alta los hace muy versátiles sobre todo en cromatografía de gases, también debido a su bajo coste y simpleza de uso.[39] Los analizadores de tipo cuadrupolo son ampliamente usados también en tándem con otros analizadores como pueden ser otros cuadrupolos26 (tándem MS) o el detector de tiempo de vuelo (*time of flight*, TOF).[64]

En el caso de los sectores magnéticos, los iones se hacen pasare a través de un campo magnético que interacciona con estos. Este campo magnético afecta a los iones en mayor o menor medida en función de su masa, suponiendo igual carga para todos, haciéndoles modificar la trayectoria que

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[62] Gómez-Ramos, M. M., Ucles, S., Ferrer, C., Fernández-Alba, A. R., & Hernando, M. D. Exploration of environmental contaminants in honeybees using GC-TOF-MS and GC-Orbitrap-MS. Science of The Total Environment, **2019,** 647, 232-244.

[63] Ding, L., Wang, L., Nian, L., Tang, M., Yuan, R., Shi, A., Shi M., Han, Y., Liu, M., Zhang, Y. & Xu, Y. Non-targeted screening of volatile organic compounds in a museum in China Using GC-Orbitrap mass spectrometry. Science of The Total Environment, **2022,** 835, 155277.

[64] Ojanperä, I.; Mesihää, S.; Rasanen, I.; Pelander, A.; & Ketola, R. A. "Simultaneous identification and quantification of new psychoactive substances in blood by GC-APCI-QTOFMS coupled to nitrogen chemiluminescence detection without authentic reference standards". Analytical and bioanalytical chemistry; **2016**; 408; 3395-3400.

siguen hasta el detector. Solo aquellos con una relación m/z adecuada podrán atravesar el analizador y ser detectados. Algunas de las desventajas de estos analizadores son su gran tamaño y las relativas bajas velocidades de escaneo.

Con el detector de doble cuadrupolo se consigue un doble filtro a las masas que se están adquiriendo, permitiendo seleccionar en el primer cuadrupolo ciertas masas, que, tras colisionar en la celda existente entre ambos cuadrupolos, rellena de un gas de colisión, con este, fragmentan generando nuevos iones. Estos iones serán filtrados de nuevo, a distintas masas, de forma que se eliminan interferencias que en un principio compartirían masa con nuestro analito.

Los analizadores de tiempo de vuelo, aportan una mayor resolución que los de cuadrupolo. En este caso los iones son sometidos a un campo eléctrico de fuerza conocida, de forma que cualquier ion con la misma carga tendrá la misma energía cinética, dependiendo esta, por tanto, solamente de la masa del ion. Los iones más pesados alcanzarán velocidades más lentas, y por tanto llegarán más tarde al detector, y lo opuesto para los más ligeros. De esta forma, el tiempo que transcurrirá en el analizador será dependiente de la masa y con este valor se podrá caracterizar la molécula.

Otro de los espectrómetros de masa con una resolución alta es el orbitrap. En este analizador, los iones generados son retenidos en torno a dos electrodos, uno con forma de barril y otro coaxial en su interior, de forma que se mueven en órbita alrededor de este último. El campo eléctrico creado entre ambos electrodos, repele los iones haciéndolos oscilar en una trayectoria lineal dependiente del ratio m/z a lo largo del eje z. este movimiento induce una corriente en el electrodo exterior que es medida y transformada posteriormente mediante una transformada de Fourier en frecuencias e intensidades individuales, generando por tanto el espectro de masas.

Queda constancia con este breve repaso de la variedad de analizadores existentes en el mercado que son capaces de proporcionar unas características u otras, en función de las necesidades del análisis. Se puede recurrir a analizadores de baja resolución, como es el tipo cuadrupolo, cuando lo que se requiere es un análisis de bajo coste, robusto y con sencillez de uso. Sin embargo, cuando la aplicación va orientada al análisis de ratios isotópicos o la elucidación exacta de la estructura de compuestos desconocidos, es preciso hacer uso de analizadores con resoluciones mayores como son el TOF, el sector magnético o el orbitrap.

**Tabla 2. Resumen de algunos de los analizadores de masa más utilizados en cromatografía de gases.**

| GC-MS técnica | Resolución masa | Características |
|---|---|---|
| **GC-cuadrupolo simple** | Baja (Masa nominal) | Análisis dirigido o tipo *screening* |
| **GC-ICP (triple cuadrupolo)** | Baja | .Alta sensibilidad para análisis de especiación. |
| **GC-triple cuadrupolo** | Baja | Alta sensibilidad para análisis dirigido.<br>Elucidación estructural. |
| **GC-tiempo de vuelo** | Media-Alta (R ≈ 50000) | Análisis dirigido.<br>Elucidación estructural |
| **GC-orbitrap** | Alta (R ≈ 100000) | Análisis de screening.<br>Sensibilidad media alta para análisis dirigido. |

## 3.3. Cromatografía de gases-combustión-espectrometría de masas

Se han presentado a lo largo de la presente introducción los detectores más extendidos en la cromatografía de gases, haciendo referencia a aquellos universales, como el FID o la espectrometría de masas usada en modo *scan*. Sin embargo, ambos tienen ciertas desventajas en su aplicación, bien por la la imposibilidad de identificar compuestos desconocidos, en el caso del FID, o por la respuesta claramente dependiente de la especie que supone la espectrometría de masas con ionización de impacto electrónico.

Desde el año 2009 hasta la actualidad, en el Grupo de Investigación se ha desarrollado un nuevo detector en cromatografía de gases basado en la espectrometría de masas.[39,65] Este detector, conocido como Combustión-espectrometría de masas, se basa en la modificación instrumental de un GC-MS comercial. En este equipo se instaló una interfase de combustión entre el horno cromatográfico y el espectrómetro de masas, de forma que todos los compuestos que eluyen de la columna, son cuantitativamente transformados, mediante una reacción de combustión en la misma especie. Dado que la mayoría de los compuestos analizables por cromatografía de gases contienen C en su estructura, la formación de $CO_2$ en la reacción permite detectarlos todos a la relación m/z 44 correspondiente a la masa de este. La ionización en la fuente será por tanto independiente de la especie, de forma que la respuesta en el detector también y, por tanto, se obtiene la detección universal buscada.

Instrumentalmente hablando, la modificación se realizó sobre un cromatógrafo de gases comercial (Konik-Tech, Barcelona, España) con un espectrómetro de masas de tipo cuadrupolo sobre el que se instaló una interfase de combustión consistente en un tubo cerámico de 60 cm, con 3 mm de diámetro externo y 0.5 mm de interno, dentro del cual, se introdujeron hilos de cobre y platino, que actúan como catalizadores de la reacción. Este tubo era calentado mediante una resistencia y posteriormente aislado mediante lana de vidrio pudiendo trabajar a temperaturas de hasta 850 ºC, lo que puede suponer ciertas limitaciones de cara a combustionar compuestos con heteroátomos como N o S. Este primer prototipo tenía ciertas desventajas como el hecho de tener que realizar una oxidación previa de los catalizadores para la posterior reacción de combustión, requiriendo repetir este proceso

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[65] Cueto Díaz, S.; Ruiz Encinar, J.; Sanz-Medel, A.; García Alonso, J. I. "A quantitative universal detection system for organic compounds in gas chromatography with isotopically enriched 13CO2". Angewandte Chemie International Edition; **2009**; 48(14); 2561-2564.

semanalmente para mantener la cantidad de oxígeno disponible en el sistema relativamente constante.

A la capacidad de detección universal se le sumó otra ventaja más, y es que, parte de la modificación instrumental del equipo fue la instalación de una válvula manual de seis vías con dos posiciones, de forma que, en una posición el sistema trabajaba en modo GC-combustión-MS, y, en el otro, en modo GC-MS, sin afectar, por tanto, a la capacidad de elucidación estructural del equipo GC-MS estándar.

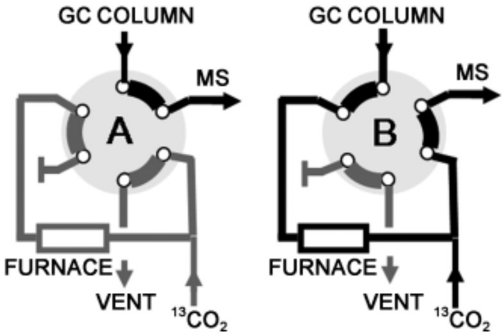

**Figura 9. Esquema del prototipo de GC-combustión-MS desarrollado por Sergio Cueto et al. En el que se pueden apreciar los dos modos de medida en función de la posición de la válvula de seis vías. [65]**

Este detector fue posteriormente extendido al análisis de los compuestos BTEX así como otros aromáticos,[66] quedando patente la posibilidad de usarlo como un detector verdaderamente universal manteniendo las características metodológicas de un GC-MS tradicional. Si bien, hay que tener en cuenta en este detector las dificultades instrumentales a la hora de desarrollarlo e implantarlo en un laboratorio, teniendo que recurrir a periodos de acondicionamiento continuo, así como multitud de conexiones, que si bien, no

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[66] Cueto Díaz, S. ; Encinar, J. R.; Sanz-Medel, A.; Garcia Alonso, J. I. "Gas chromatography-combustion-mass spectrometry with postcolumn isotope dilution for compound-independent quantification: its potential to assess HS-SPME procedures". Analytical chemistry, **2010**; 82(16); 6862-6869.

se han comentado en este apartado, suponen un verdadero lastre a la hora de obtener resultados reproducibles.

### 3.4. Selectividad en cromatografía de gases y espectrometría de masas.

Se ha comentado hasta ahora, la extrema capacidad que tiene la cromatografía de gases para separar muestras complejas, siendo capaz de resolver mezclas de hasta 100 compuestos. Esta primera selectividad, dada principalmente por la columna cromatográfica, acompañada de la inyección y el gradiente de temperatura adecuada, como se ha comentado anteriormente, permite una selectividad limitada en mezclas complejas, siendo necesario recurrir en muchas ocasiones a la cromatografía bidimensional para resolver mezclas más complejas. Existen, sin embargo, ciertas limitaciones incluso con la cromatografía bidimensional, donde familias de compuestos como los que se han mencionado anteriormente en la presente introducción, no son capaces de detectarse a las concentraciones presentes en la muestra.

Es aquí, donde los detectores selectivos, aportan una dimensión más a la selectividad del análisis cromatográfico. Esta combinación de separación cromatográfica-detector para evaluar la selectividad de una metodología se ve favorecida todavía más, cuando el detector selectivo, es un espectrómetro de masas, el detector capaz de proporcionar la selectividad según el significado más estricto de la palabra. En la MS, los analitos son directamente separados por la propiedad más característica de una molécula, su masa molecular. Si bien, la posibilidad de compuestos con la misma masa molecular es una realidad, la capacidad de unir dos MS permite obtener una selectividad doble a la hora de caracterizar los analitos y resolver estas posibles interferencias. Esta capacidad de doble filtro, permite reducir también, los fondos lo que aumenta en consecuencia la relación señal/ruido disminuyendo también, el límite de detección, llegando en sistemas de triple cuadrupolo a la detección a niveles de femtogramos.

Así, matrices muy complejas, dónde la capacidad cromatográfica de separación no sea suficiente, la espectrometría de masas nos permitiría diferenciar en una dimensión adicional. En cromatografía de gases, la selectividad, normalmente se asocia únicamente a la columna, sin embargo, queda presente que, a lo largo de todo el proceso analítico en un análisis por cromatografía de gases, se puede aplicar este concepto, desde el proceso de toma de muestra, preparación de esta, la introducción y sobre todo la separación y posterior detección, hasta incluso el procesado de la señal podría afectar a la selectividad del proceso.

## 4. Análisis cuantitativo en cromatografía de gases

Hasta este momento se ha introducido la cromatografía de gases como una técnica orientada hacia el análisis cualitativo, hablando principalmente en términos de selectividad y capacidad de detección, sin embargo, el cálculo de las concentraciones de todos estos analitos es parte fundamental del análisis cromatográfico.

El análisis cuantitativo es aquel en el que se obtiene la concentración o cantidad de un analito en una mezcla y se expresa con un valor numérico con un error asociado en unas unidades determinadas. Generalmente, los métodos analíticos proporcionan una señal instrumental que, en función de las propiedades del detector, pueden ser proporcionales a la concentración o al número de moles, es decir, son señales relativas o absolutas.

En la cromatografía de gases, el parámetro analítico que se escoge para relacionar con la concentración, normalmente es el área del pico, tras una integración con el software, de ahí la importancia de obtener separaciones cromatográficas adecuadas, de cara a distinguir y evaluar analitos que eluyen a tiempos de retención muy cercanos.

La relación entre la masa o concentración de analito y, la señal generada viene determinado por un coeficiente que representa la sensibilidad de la técnica. Cuanta mayor señal es generada como respuesta a nuestro analito, mayor será la sensibilidad. Este valor de sensibilidad a su vez, está intrínsecamente ligado al límite de detección de una metodología. A mayor sensibilidad de un método, mayor será la capacidad de distinguir entre concentraciones muy parecidas, y a la vez, menor límite de detección se obtendrá, siempre y cuando el ruido del fondo permanezca constante.

La sensibilidad del método ha de establecerse previamente para llevar a cabo un análisis cuantitativo, y existen diferentes procesos para ello. Este proceso conocido como calibración, es definido por la IUPAC como “el conjunto de operaciones que establecen, bajo unas condiciones concretas la relación entre los valores indicados por el instrumento analítico y los correspondientes valores conocidos de cantidad de analito”.

Esta correlación suele representarse como una curva de calibración, en la que se refleja la sensibilidad del método en todo el rango lineal de este cuando se analizan una serie de patrones de concentraciones conocidas. Esta calibración, permitirá posteriormente obtener un valor de concentración del analito de interés en función de la señal obtenida, siempre y cuando, tanto la muestra como los patrones hayan sido analizados en las mismas condiciones analíticas. En otras ocasiones, una calibración puede realizarse usando únicamente un patrón con el que se calcula el factor de correlación, en este caso conocido como factor de respuesta.

Existen, sin embargo, algunas metodologías en las que no es posible usar una recta de calibrado con patrones externos y se requiere de metodologías cuantificadoras más complejas. En el siguiente punto, se hará una explicación de todas estas metodologías, el porqué de su uso y su aplicabilidad a distintas muestras.

## 4.1. Metodologías para la cuantificación en cromatografía de gases

La cuantificación en cromatografía de gases puede llevarse a cabo por distintas metodologías, que se escogen en función del tipo de análisis, del tipo de analito, de la complejidad de la matriz en la que mediremos el analito de interés o de la metodología que se vaya a usar.

### *Método del estándar externo*

El método del estándar externo es el más común aplicado en toda la química analítica. En este método, previo al análisis del analito en la matriz de interés, se lleva a cabo un calibrado con varios patrones externos de concentraciones conocidas y variables del analito de interés, generalmente con un mínimo de 5 concentraciones dentro del rango lineal de la metodología. Estos patrones son analizados por cromatografía y, tras integrar sus áreas (también se puede usar la altura de pico), se establece una recta de calibrado en la que se representan frente a la cantidad inyectada. Tras el tratamiento matemático acorde se obtiene una ecuación, que posteriormente se utilizará para calcular la concentración del analito en la muestra. Esta recta, proporciona también el valor del blanco de la metodología. Este valor debe tenerse en cuenta en muchos análisis, sin embargo, uno de los puntos positivos de la cromatografía es la capacidad de separar en el tiempo posibles impurezas que coeluyen con el analito de interés, de ahí que esto no sea un punto crítico en este tipo de análisis al no obtener generalmente señal analítica en el blanco.

Cuando el valor del blanco es cero y, la metodología usada sea lineal en el rango de concentraciones que se quiere medir, se puede llevar a cabo una cuantificación usando un único patrón externo, de forma que, obteniendo su área y dividiendo por la concentración, se puede calcular el factor de respuesta. Posteriormente, obteniendo el área del analito en la muestra se podrá inferir la concentración en ella. Para aplicar esta metodología es crucial que sea un método lineal de forma que el factor de respuesta será igual en cada punto de la recta de calibrado.

Este método se usa dada la simpleza tanto en la preparación de los patrones como posteriormente en la medida y procesado de los datos. Sin embargo, tiene ciertas desventajas que hacen necesario buscar alternativas. Por ejemplo, la presencia de efectos de matriz principalmente en muestras complejas.

*Método de las adiciones estándar*

El efecto de matriz se define como el efecto combinado de cualquier otro componente de la mezcla a excepción del analito, en la medida de la cantidad de este. Este efecto impide aplicar la cuantificación con patrón externo si no se usa la misma matriz en el análisis de este y del analito, ya que se obtendrían sensibilidades distintas y, por tanto, no habría una correlación directa entre señal y concentración.

En este modo de cuantificación, el patrón externo se adiciona directamente sobre la matriz donde se encuentra el analito a concentraciones crecientes y en pequeños volúmenes, de forma que se construye una recta de calibrado directamente sobre la muestra. Para conseguir el valor de la concentración se extrapola la curva de calibración a $y=0$. La evaluación de efectos de matriz se puede hacer fácilmente comparando las pendientes entre los calibrados externo y por adiciones estándar. Si ambas pendientes son estadísticamente iguales se concluirá que los efectos de matriz son nulos en la matriz para el analito analizado.

*Método del estándar interno*

Esta metodología también es útil para la corrección de efectos de matriz, pero, además, corrige posibles desviaciones aleatorias y sistemáticas de una metodología, siendo especialmente útil en el caso de la cromatografía de gases cuando existen derivas en la inyección, efectos de matriz o, derivas instrumentales.

Un estándar interno es un compuesto detectable por la metodología de análisis usada pero que no interfiere en la medida del analito, que se añade a un nivel de concentración conocida sobre la muestra y, sobre los patrones, bien sea en un análisis por calibrado externo o por adiciones estándar.

En este caso, la concentración de analito se calcula en función del patrón interno, de forma que, la evaluación de la señal de este y posterior incorporación en el proceso matemático, normaliza las posibles derivas comentadas antes, al sufrir las mismas el propio patrón. El proceso matemático con el que se aplica es usando como variables dependiente e independiente el ratio entre señales y concentraciones de analito y patrón interno.

En muchos casos, esta medida suele hacerse como se comentó, con un compuesto distinto al analito, sin embargo, esto puede llevar a nuevos errores ya que, al fin y al cabo, el analito no es exactamente el mismo que el patrón interno. Una de las ventajas que proporciona la espectrometría de masas es la capacidad de distinguir los isótopos de un mismo elemento, por lo que, la medida con patrón interno podría llevarse a cabo adicionando el mismo analito, pero con una composición isotópica diferente, de forma que su relación m/z cambie, y por tanto, se pueda diferenciar del analito de interés. Esto es lo que se conoce como dilución isotópica.

*Método de dilución isotópica*

La dilución isotópica consiste en la adición a la muestra de uno o varios[67] patrones internos de sustancias iguales al analito, conocidos como trazadores, pero con abundancias isotópicas modificadas, de forma que, mediante la

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[67] Suárez-Criado, L.; Queipo-Abad, S.; Rodríguez-Cea, A.; Rodríguez-González, P.; Alonso, J. I. G. "Comparison of GC-ICP-MS, GC-EI-MS and GC-EI-MS/MS for the determination of methylmercury, ethylmercury and inorganic mercury in biological samples by triple spike species-specific isotope dilution mass spectrometry". Journal of Analytical Atomic Spectrometry; **2022**; 37(7), 1462-1470.

espectrometría de masas se pueden diferenciar en función de su ratio m/z.[68] En la dilución isotópica se pueden diferenciar dos tipos, por un lado, la tradicional, en el que una o varias sustancias de concentración conocida se añaden cuantitativamente previo al análisis, a la disolución donde se encuentra el analito, o bien, la dilución isotópica en línea, en la que, en este caso, el patrón interno se añade en línea a lo largo de todo el análisis cromatográfico. Estos dos modos de medida implican multitud de diferentes ecuaciones que han sido desarrolladas a lo largo de los años, pero que quedan fuera del alcance de la presenta tesis doctoral.

Para llevar a cabo un análisis por dilución isotópica se requieren una serie de condiciones. Por un lado, una concentración bien establecida de trazador, requiriendo muchas veces una caracterización previa de este.[69] La adición del trazador debe ser en cantidades conocidas con la mayor exactitud posible y, la medida de la mezcla debe estar en condiciones de equilibrio, de forma que tanto trazador como analito estén correctamente homogeneizados.

En el caso de la dilución isotópica en línea, usada tanto para análisis total como de especiación, el flujo de la muestra se une en línea con otro flujo que contiene el trazador añadido de forma continua.[70] Posteriormente, la composición isotópica resultante se calculará en el espectrómetro de masas y, a través de una serie de operaciones matemáticas se obtendrá la concentración.

Otra clasificación que se suele hacer en dilución isotópica, tanto por la metodología analítica, como por el procesado matemático posterior es en

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[68] García Alonso, J. I..; Rodríguez-González, P.; González-Gago, A.; González-Antuña, A. "Determination of the uncertainties in the theoretical mass isotopomer distribution of molecules". Analytica chimica acta; **2010**; 664(1); 68-76.

[69] Cid-Barrio, L.; Calderón-Celis, F.; Costa-Fernández, J. M.; & Encinar, J. R. "Assessment of the potential and limitations of elemental mass spectrometry in life sciences for absolute quantification of biomolecules using generic standards". Analytical Chemistry; **2020**; 92(19); 13500-13508

[70] Heilmann, J.; Heumann, K. G. "Development of a Species-Unspecific Isotope Dilution GC– ICPMS method for possible routine quantification of sulfur species in petroleum products". *Analytical chemistry*; **2008**; *80*(6); 1952-1961.

función de la ionización, diferenciando entre dilución isotópica elemental, principalmente en espectrómetros de masas de ICP, o molecular. En el primer caso, el trazador usado será un isótopo enriquecido del analito, mientras que, en el segundo, se añadirá un compuesto marcado en alguno de los elementos que lo componen, normalmente con los isótopos $^{13}C$, $^{2}H$, $^{18}O$ o $^{34}S$. Las ecuaciones matemáticas par ambas metodologías son ligeramente diferentes, aunque de nuevo, no se entrará en detalle en este apartado.

Si bien la dilución isotópica es algo laboriosa de cara a pretratamiento de la muestra y tratamiento de los datos posterior, tiene grandes ventajas, como por ejemplo una gran exactitud y precisión o que sea un método absoluto directamente trazable al Sistema Internacional de Unidades[68] y, que por tanto no requiera de una calibración metodológica al medir ratios entre distintos isótopos. Esto no ocurre con otras metodologías requiriendo el uso de calibraciones como las explicadas anteriormente. Además, cualquier proceso, posterior al tratamiento de la muestra, que suponga una deriva en la señal, ya bien en la inyección o, en la medida de esta, será corregido.

### 4.2. Importancia de la cuantificación independiente de la especie.

Hasta el momento se han enumerado una serie de metodologías para cuantificación en las que en todas ellas se requiere el uso de patrones específicos del analito de interés, bien sea marcados isotópicamente o no. Este tipo de metodologías conlleva por tanto una desventaja inherente a su desarrollo, y es la necesidad de usar estos patrones específicos de cada analito.

Como ha quedado reflejado en apartados anteriores de la presente tesis doctoral y como quedará constatado a través de los distintos capítulos, la necesidad de usar patrones específicos supone un lastre en términos de aplicabilidad de las metodologías. Las muestras complejas, como los combustibles, contienen una cantidad de compuestos enorme, muchos de ellos todavía desconocidos y, el análisis de estos requiere metodologías cada vez más más sensibles con límites de detección más bajos. Las legislaciones, cada vez más estrictas, a su vez limitan las concentraciones de compuestos a niveles cada vez más bajos, lo que requiere a su vez una monitorización de cada vez más compuestos. Las cuantificaciones de estos, en el caso de cuantificaciones según métodos tradicionales, precisan, como se ha visto hasta ahora la utilización de patrones específicos que requieren una inversión económica para adquirirlos y también un procedimiento metodológico laborioso de cara a establecer calibraciones para cada uno de ellos.

Existen una alternativa a estas metodologías dependientes del uso de patrones específicos, y es el uso de patrones genéricos para llevar a cabo las cuantificaciones. Esto obviamente, requiere de una serie de condiciones que se evaluarán en los siguientes apartados.

### 4.2.1. Concepto de equimolaridad

La equimolaridad se define como la capacidad de un detector, o metodología de proporcionar una respuesta para distintos compuestos, proporcional a la cantidad, es decir al número de moles, de analito que se encuentra presente sin importar la estructura de este.[48] Así, durante todo el proceso analítico, toma de muestra, introducción de esta y el análisis mediante la metodología escogida, un método equimolar, permitirá procesar todos los analitos durante estos pasos sin obtener derivas debido a la estructura de estos. Este concepto, aunque se usa normalmente para técnicas cromatográfica y en concreto para la respuesta del detector, es aplicable a cualquier metodología analítica cuantitativa durante todas sus etapas. [71, 72, 69]

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[71] Rasanen, I.; Kyber, M.; Szilvay, I.; Rintatalo, J.; Ojanperä, I. ”Straightforward single-calibrant quantification of seized designer drugs by liquid chromatography–chemiluminescence nitrogen detection”. Forensic Science International; **2014**; 237; 119-125.

[72] Moreira-Álvarez, B.; Cid-Barrio, L.; Calderón-Celis, F.; Costa-Fernández, J. M.; Encinar, J. R. “Relative and Transport Efficiency-Independent Approach for the Determination of Nanoparticle Size Using Single-Particle ICP-MS”. Analytical Chemistry; **2023**; 95(27); 10430-10437.

En el caso de la cromatografía de gases, si se asume una transmisión cuantitativa de los analitos en el inyector y en la columna, se puede concluir que la parte, puramente cromatográfica, es equimolar. De esta forma en la mayoría de los casos, es el detector el que se evalúa de cara a obtener una respuesta equimolar. Teniendo en cuenta las propiedades de cada detector universal y selectivo, de los que se han explicado durante esta introducción, se pueden concluir ciertas tendencias en cuanto a la equimolaridad de cada uno. En el caso de los detectores universales, por ejemplo, la espectrometría de masas, tienen una respuesta dependiente de la especie debido al proceso de ionización y, requieren por tanto calibraciones específicas para cada compuesto. En otro detector universal como es el FID también se han observado derivas en la detección, requiriendo pequeñas modificaciones para obtener una respuesta equimolar.[38] El concepto de equimolaridad suele ir más asociado a los detectores selectivos ya que, por su propia condición, requieren de una respuesta independiente de la especie de cara a evaluar factores de respuesta en las especies. En muchos detectores se asocia una respuesta equimolar sin evaluar las condiciones, sin embargo, existen ciertas discrepancias al respecto en detectores como el AED, SCD o NCD,[30,64] pudiendo observarse desviaciones en el caso del AED debidas a efectos de matriz[20] y en el NCD variaciones dependiendo del grupo funcional en el que se encuentra el átomo de nitrógeno.

El único de los detectores dentro de la espectrometría de masas que se considera equimolar es el ICP-MS. Al igual que sucede en el FID, la capacidad de atomizar la molécula completamente permite que la ionización suceda sobre la misma especie en todos los compuestos.[73]

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[73] Freije-Carrelo, L.; García-Bellido, J.; Calderón-Celis, F.; Moldovan, M.; Encinar, J. R. "GC-ICP-MS/MS Instrumental Setup for Total and Speciation Sulfur Analysis in Gasolines using Generic Standards". Analytical chemistry; **2019**; 91(11); 7019-7024.

### 4.2.2. Cuantificación independiente de la especie en cromatografía de gases

La capacidad de una metodología de proporcionar una respuesta equimolar para diferentes compuestos abre la puerta a una de las propiedades largamente perseguidas en el desarrollo de detectores en cromatografía de gases, la cuantificación independiente de la especie, o calibración independiente de la especie.[20]

Un detector equimolar es capaz de proporcionar la misma respuesta para cualquier compuesto dependiendo solamente de su concentración. Esto permite cuantificar mediante la evaluación de un único y genérico, patrón, cualquier otro compuesto de esta que sea detectable por la metodología usada.[74] De esta forma, mediante la evaluación del factor de respuesta de este, se puede llevar a cabo la cuantificación del resto de compuestos de la mezcla de interés.

La cuantificación independiente de la especie permite simplificar el proceso analítico en varios puntos. Por un lado, reduce significativamente el coste debido a la multitud de patrones específicos que no sería necesario adquirir y, por otro lado, en el caso de usar la cuantificación con un patrón interno, se reduce también el procesado de la muestra y el tiempo del análisis, al solamente necesitar añadir un único *spike* del patrón a la muestra. Tras la evaluación del factor de respuesta de este, se cuantificará la muestra ahorrando laboriosos procesos de calibraciones analíticas. Además, la adición de un patrón interno, favorece la corrección de posibles efectos de matriz o derivas en la inyección.

En el grupo de investigación se han desarrollado diversas metodologías basadas en este principio, principalmente basadas en técnicas

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[74] Redeker, F. A.; Lesniewski, J. E.; Hahm, G.; McMahon, W. P.; Jorabchi, K. "High-Resolution Elemental Mass Spectrometry Using LC-ICP-Nanospray-Orbitrap for Simultaneous and Species-Independent Quantitation of Fluorinated and Chlorinated Compounds". Analytical Chemistry; **2022**; 94(34); 11865-11872.

cromatográficas asociadas a los detectores de ICP-MS/MS[69,75,76] y de GC-combustión-MS.[65,66] La cuantificación independiente de la especie, al igual que sucede con el término equimolaridad, aunque se asocia normalmente a la cromatografía de gases, es aplicable a cualquier metodología analítica. [38,69,77,78]

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[75] Calderón-Celis, F.; Sugiyama, N.; Yamanaka, M.; Sakai, T.; Diez-Fernández, S.; Calvete, J. J.; Encinar, J. R. “Enhanced universal quantification of biomolecules using element MS and generic standards: application to intact protein and phosphoprotein determination”. Analytical chemistry; **2018**; 91(1); 1105-1112.

[76] Freije-Carrelo, L.; Moldovan, M.; García Alonso, J. I.; Thanh VO, T. D.; Encinar, J. R. “Instrumental setup for simultaneous total and speciation analysis of volatile arsenic compounds in gas and liquefied gas simples”. Analytical chemistry,; **2017**; 89(11); 5719-5724.

[77] Vosse, C.; Thyssen, G. M.; Sperling, M.; Karst, U.; Hayen, H. “Complementary approach for analysis of phospholipids by liquid chromatography hyphenated to elemental and molecular mass spectrometry”. Analytical Science Advances; **2020**; 1(1); 46-55.

[78] Ma, Q.; Zhang, Q.; Li, X.; Gao, Y.; Wei, C.; Li, H.; Jiao, H. “The compound-independent calibration of five selenium species in rice using ion-pairing reversed phase chromatography coupled to inductively coupled plasma tandem mass spectrometry”. Journal of Chromatography A; **2022**; 1674; 463134.

# OBJETIVOS - OBJECTIVES

# OBJETIVOS

La idea de conseguir un detector universal, absoluto y con la capacidad de obtener una cuantificación que no requiera patrones externos y específicos ha sido largamente perseguida en el mundo de la cromatografía de gases. Si bien, el ICP-MS, aunque no se encuentra ampliamente establecido en análisis de rutina de cromatografía de gases, cumple en parte esta propuesta, hay un cierto número de elementos, por otro lado, muy importantes dada la naturaleza de los compuestos analizables por cromatografía de gases, que no son medibles por esta técnica. Elementos como el C y el H, forman parte de la estructura de la gran mayoría de los compuestos, mientras que otros como el N, el S o el O son de suma importancia de cara a caracterizar posibles impurezas y compuestos a concentraciones más bajas en diversas matrices. Por ello, la imposibilidad de tener una detección y cuantificación selectiva para estos elementos impide al ICP-MS establecerse como un detector con aplicación real en la cromatografía de gases.

Durante la introducción de la presente Tesis Doctoral, ha quedado patente la necesidad en el desarrollo de nuevas metodologías selectivas basadas en la cromatografía de gases en muestras complejas. Ante la creciente demanda de metodologías con sensibilidades cada vez mayores, que permitan caracterizar un mayor número de muestras y analitos, la presente Tesis Doctoral se plantea como una evaluación de nuevos detectores elementales en cromatografía de gases, haciendo uso de su capacidad selectiva y universal en cada uno de ellos, diferenciándose principalmente dos detectores, cuyas prestaciones se pretende evaluar en distintas muestras y analitos.

Este objetivo general se desarrolló en base a diferentes objetivos parciales que se detallan a continuación:

1. La evaluación de la técnica de GC-ICP-MS/MS para llevar a cabo un análisis en especiación y total de compuestos de cloro en muestras de

interés energético como son los aceites de pirólisis mediante el uso de cuantificación independiente de la especie.

2. El desarrollo de un nuevo detector universal y selectivo basado en la técnica de GC-combustión-MS, para el análisis simultáneo de compuestos que contienen C, H, N y S en su estructura.

3. La extensión del detector GC-combustión-MS como un detector selectivo de nitrógeno y su comparación con otro detector selectivo bien establecido para este compuesto como es el GC-NCD.

4. El último de los objetivos, basado también en la técnica de GC-combustión-MS, se orientó hacia la detección elemental selectiva de un elemento, hasta ahora, difícilmente caracterizable por GC, el oxígeno y la obtención de una respuesta independiente de la especie.

# OBJECTIVES

The idea of achieving a universal, absolute detector with the ability to obtain a quantification that does not require external and specific standards has long been pursued in the world of gas chromatography. Although ICP-MS, although not widely established in routine gas chromatographic analysis, partly fulfils this proposal, there are a number of elements, which are very important given the nature of the compounds analysable by gas chromatography, that are not measurable by this technique. Elements such as C and H form part of the structure of the vast majority of compounds, while others such as N, S or O are of great importance for characterising possible impurities and compounds at lower concentrations in various type of samples. Therefore, the impossibility of having a selective detection and quantification for these elements prevents ICP-MS from establishing itself as a detector with real application in gas chromatography.

During the introduction of this Doctoral Thesis, the need for the development of new selective methodologies based on gas chromatography in complex samples has become evident. In view of the growing demand for methodologies with increasingly higher sensitivities, which allow a greater number of samples and analytes to be characterised, the present Doctoral Thesis is proposed as an evaluation of new elemental detectors in gas chromatography, making use of their selective and universal capacity in each one of them, differentiating mainly between two detectors, whose performance is intended to be evaluated in different samples and analytes.

This general objective was developed on the basis of different partial objectives, which are detailed below:

1. The evaluation of the GC-ICP-MS/MS technique to carry out a speciation and total analysis of chlorine compounds in samples of energetic interest such as pyrolysis oils by means of the use of species-independent quantification.

2. The development of a new universal and selective detector based on GC-combustion-MS technique for the simultaneous analysis of compounds containing C, H, N and S in their structure.
3. The extension of the GC-combustion-MS detector as a selective detector for nitrogen and its comparison with another well-established selective detector for this compound such as GC-NCD.
4. The last of the objectives, also based on the GC-combustion-MS technique, was oriented towards the selective elemental detection of an element, until now, difficult to characterise by GC, oxygen, and obtaining a species-independent response.

# EXPERIMENTAL

# 1. Materiales, reactivos y muestras

## 1.1. Materiales de referencia

Durante el desarrollo de la presente tesis doctoral, con el objetivo de validar las distintas metodologías desarrolladas, se usaron diferentes materiales de referencia:

- *Organophophorous Pesticide Mix A,* (*Certified Reference Material; Part number 48391, Sigma Aldrich*). Este material consiste en una mezcla de ocho pesticidas organofosforados diluidos en una mezcla de Hexano/Acetona (9:1) a una concentración certificada de compuesto de aproximadamente 2000 µg/mL.
- *Nitrogen solution for High Boiling Solvents* (Certified Reference Material, Part Number D-4629-91-HB-CON, AccuStandards, Inc.). Este material de referencia está certificado para la concentración total de N en una dilución de Carbazol en una mezcla de Tolueno/Acetona (9:1) a una concentración de 998 µg/mL.
- B100 Biodiesel (*Soy-based)*, (*Standard Reference Material, Part number SRM2772, National Institute of Standards & Technology*). Consiste en un biodiesel 100% obtenido de soja.

## 1.2. Muestras reales

Durante la tesis doctoral, en gran parte realizada en colaboración con la empresa *TotalEnergies*, se han utilizado diferentes muestras reales, siendo la mayoría de ellas proporcionadas por la propia empresa. Así, en la siguiente lista se enumeran las muestras y, el origen de estas, que fueron usadas para el desarrollo de los distintos capítulos.

*Capítulo 1*

Durante el capítulo 1, con el objetivo de validar la metodología usada, se escogieron un tipo de muestras de creciente interés, muestras de aceite de pirólisis obtenido de materiales plásticos. Se usaron cuatro muestras distintas de origen desconocido proporcionadas por la empresa TotalEnergies

*Capítulo 2*

En el capítulo 2, se usaron muestras derivadas de petróleo, en este caso dos diéseles, con una concentración total de S y N conocida. Una tercera muestra, consistente en un coque de gasoil, fue fraccionada a través de un proceso de separación en fase sólida (SPE) en dos fracciones distintas, una alifática y otra aromática.

*Capítulo 3*

En el capítulo 3 se usó una muestra real de aceite de pirólisis obtenida a partir de biomasa además de un diésel. Ambas muestras fueron caracterizadas por NCD y calculado su contenido total de N.

*Capítulo 4*

En este capítulo se llevó a cabo la validación de la metodología mediante el análisis de una muestra real de diésel sin contenido de oxígeno. Esta muestra fue adicionada con varios compuestos de oxígeno y se llevó a cabo su cuantificación mediante la metodología propuesta.

# 2. Instrumentación

## 2.1. Cromatógrafos de gases

La cromatografía de gases es una de las técnicas más ampliamente usadas en el campo de la química analítica para analizar una gran cantidad de compuestos volátiles dado su poder de separación (resolución cromatográfica). La sencillez y robustez de la técnica, junto con el relativo bajo coste y, la versatilidad de sus métodos, han permitido una gran extensión de la técnica en diferentes campos, permitiendo el análisis tanto de moléculas "ligeras" con puntos de ebullición bajos, así como moléculas mucho más "pesadas" con altos puntos de ebullición.

La técnica, que permite separar un gran número de moléculas de una misma mezcla, consta, principalmente de tres partes principales, inyector, columna y detector. Haciendo uso de distintas configuraciones de cada una de las tres partes, podemos llevar a cabo separaciones muy complejas de mezclas muy dispares y obtener de esta forma información con respecto a la composición molecular de estas.

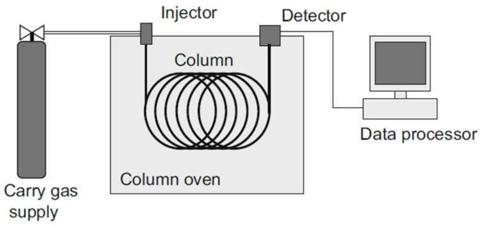

**Figura 10.** Esquema general de un cromatógrafo de gases.

El fundamento básico de la cromatografía de gases consiste en la separación de moléculas volátiles usando una fase móvil gaseosa, usualmente gases ligeros tales como $H_2$ o He, mediante su introducción en una columna, siendo las de tipo capilar las más usadas hoy en día.

La introducción de la muestra en la columna es la función básica del inyector. Este, ha de transmitir, de forma reproducible, el contenido de la alícuota inyectada en la columna cromatográfica. Este primer proceso de la separación cromatográfica es clave, ya que una inyección defectuosa limita el resto de procesos. Así, lo que se busca en una buena inyección, es introducir en el GC una representación fidedigna de la muestra original y, en caso de necesitar resultados cuantitativos es crítico que durante la inyección no se pierda ningún tipo de información relevante para el análisis.

En la presente tesis doctoral se usó un inyector de tipo Split/splitless. Este inyector, permite la introducción de la muestra en dos modos. Cuando inyectamos en modo *splitless*, usado normalmente en muestras con concentraciones bajas, se busca que todo el contenido inyectado sea introducido en la columna cromatográfica. En cambio, cuando se trabaja en modo *split* solo una parte de la muestra es introducida, y mediante la aplicación de un flujo de purga, se puede “dividir” el volumen inyectado en dos corrientes, permitiendo transmitir a la columna volúmenes de muestra mucho menores en caso de que la concentración sea elevada.

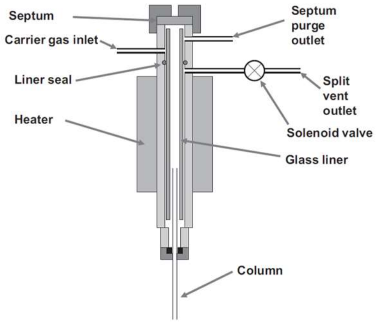

**Figura 11. Esquema general de un inyector tipo split/splitless.** [30]

Estas columnas, están recubiertas en su interior por diferentes fases estacionarias que interaccionan con los analitos de diferentes formas, favoreciendo una selectividad diferencial para cada analito. A su vez, la columna cromatográfica está situada en el interior de un horno cromatográfico que permite la aplicación de gradientes de temperaturas relativamente altas en la columna. Estas temperaturas, pueden aplicarse bien con un gradiente de temperatura que permite de nuevo una diferenciación en la separación de los analitos en función de su punto de ebullición, o en modo isocrático manteniendo una temperatura constante a lo largo de todo el análisis.

Durante la presente tesis doctoral, la cromatografía de gases ha sido el equipo común a todos los capítulos usándose tres equipos distintos:

- Cromatógrafo de gases 7890B de la compañía Agilent
- Cromatógrafo de gases 6890N de la compañía Agilent
- Cromatógrafo de gases Nexis™ GC-2030 de la compañía Shimadzu

Cada uno de ellos acoplado a un tipo de detector que se desarrollarán a continuación.

## 2.2. Espectrómetro de masas con plasma inductivamente acoplado como detector para cromatografía de gases

La espectrometría de masas elemental con fuente de plasma por acoplamiento inductivo, conocida como ICP-MS, se ha extendido en las últimas décadas como la técnica con mayor aplicación en numerosos campos, dada la alta sensibilidad, lo que se traduce en bajos límites de detección, así como la capacidad multielemental y su versatilidad en cuanto a análisis de muestras con matrices muy dispares.[79]

Este detector ha sido usado para el desarrollo experimental del primer capítulo de la presente Tesis Doctoral. En este caso, el modelo utilizado es el equipo de ICP-MS de triple cuadrupolo 8900 de la empresa Agilent. Sus características, así como el acoplamiento con el cromatógrafo de gases, se explicarán en profundidad en el primer capítulo de tesis doctoral.

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[79] Bolea-Fernandez, E.; Balcaen, L.; Resano, M.; & Vanhaecke, F. "Overcoming spectral overlap via inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS). A tutorial review". Journal of Analytical Atomic Spectrometry; **2017**; 32(9); 1660-1679.

En cuanto al cromatógrafo de gases utilizado se usó un modelo Agilent 7890B con modificaciones instrumentales llevadas a cabo en el grupo de investigación.[76] Este cromatógrafo, permite la entrada de muestras líquidas y gaseosas en línea, mediante la instalación de válvulas de inyección de 1 mL de capacidad. Esto permite el análisis no solo en especiación, a través de una columna cromatográfica estándar, sino del contenido total, mediante su introducción en una línea de transferencia que simplemente, traslada el contenido hasta la antorcha del ICP, sin llevar a cabo una separación cromatográfica. Esta metodología fue aplicada en el grupo de investigación para la determinación de compuestos de arsénico[76] y de azufre.[73]

**Figura 12. Cromatógrafo de gases Agilent 7890B acoplado al detector selectivo de ICP-MS/MS Agilent 8900.**

Además de estas modificaciones, el equipo se adaptó de cara a proporcionar una respuesta independiente de la especie. La interfase para acoplar un cromatógrafo de gases a un ICP-MS, consta además de una línea de transferencia calentada para evitar la pérdida de los compuestos que eluyen de la columna, de una línea extra a través de la cual se introduce un flujo de gas de dilución, en este caso argón. Dado que la diferencia en valor entre los flujos de columna y del plasma va desde los pocos mL/min para el primero, hasta los L/min en el segundo, se requiere de un aporte extra de gas que favorezca la introducción del primero en el segundo proporcionado por el flujo de dilución de Ar. Sin embargo, en el equipo comercial, esta línea auxiliar está introducida dentro del horno del GC, lo que hace que esté sometida a los cambios de temperatura de los gradientes. Se demostró experimentalmente, que esta fluctuación de temperatura afectaba directamente al flujo de He, proporcionado por el controlador de flujo másico al modificar la densidad de este. Esto se traduce en un efecto indeseado dado que compuestos que eluyen a temperaturas bajas tendrán un factor de respuesta diferente a los que eluyan a temperaturas más altas, lo que irremediablemente afecta al factor de respuesta y, por tanto, imposibilita llevar a cabo una cuantificación independiente de la especie. Para evitar este efecto, se llevó a cabo una modificación instrumental que consistió en la instalación de la interfase por donde circula el argón de dilución, en una plataforma calentada a temperatura constante, evitando así modificaciones de los flujos y obteniendo una señal independiente de la temperatura de elución.[73]

En función del tipo de análisis que se llevó a cabo, se instaló, la columna cromatográfica correspondiente, o la línea de transferencia usada, ambas, a un inyector de tipo split/splitless.

A lo largo del primer capítulo de la tesis doctoral se desarrollará más en profundidad las capacidades de los detectores de tipo cuadrupolo tándem. [80,81]

### 2.3. Cromatografía de gases – Combustión – Espectrometría de masas

El equipo de cromatografía de gases-combustión-espectrometría de masas fue la técnica fundamental usada en los capítulos 2, 3 y 4. Esta técnica, que como se comentó en la introducción ha sido desarrollada a lo largo de la última década en el grupo de investigación, y, durante la presente Tesis Doctoral se ha

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[80] Lum, T. S.; Leung, K. S. Y. "Strategies to overcome spectral interference in ICP-MS detection". Journal of Analytical Atomic Spectrometry; **2016**; 31(5); 1078-1088.

[81] Nelson, J.; Hopfer, H.;, Silva, F.; Wilbur, S.; Chen, J.;, Shiota Ozawa, K.; Wylie, P. L. "Evaluation of GC-ICP-MS/MS as a new strategy for specific heteroatom detection of phosphorus, sulfur, and chlorine determination in foods". Journal of agricultural and food chemistry; **2015**; 63(18); 4478-4483.

evolucionado y extendido todavía más. A continuación, se expondrán los dos prototipos utilizados.

### 2.3.1. Prototipo 1 (Agilent)

El primer prototipo usado y desarrollado en la presente tesis doctoral está basado en la modificación de un instrumento GC-MS comercial de la compañía Agilent (modelo GC 6890 acoplado a un detector MS modelo 5973Network).

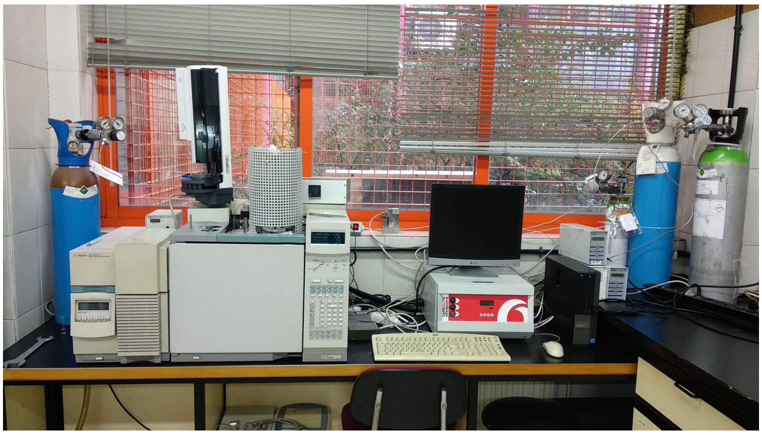

**Figura 13. Prototipo 1 del equipo de GC-combustión-MS usado durante los capítulos 2 y 3.**

Basado en la primera versión desarrollado por Sergio Cueto et al.[65], este prototipo se construyó mediante la instalación de una serie de modificaciones que se detallan a continuación:

a. Válvula de seis vías y dos posiciones de acero inoxidable

Mediante la instalación de una válvula manual de seis vías y dos posiciones (VICI Valco) el sistema permite el funcionamiento como un sistema GC-MS estándar, sin perder las propiedades cromatográficas adquiridas en la separación, o como un sistema GC-combustión-MS. La unión entre la válvula y el MS se llevó a cabó mediante un capilar inerte de 0.5 mm de diámetro externo y aproximadamente 20 cm de longitud. Tanto la columna cromatográfica como este capilar se unen a la válvula mediante unas conexiones tipo *column-to-valve*

IZERA 1.5M de VICI Valco. El resto de conexiones se llevaron a cabo con conexiones estándar metálicas de diámetro externo 1/16”.

b. Interfase de combustión

La modificación instrumental básica para transformar el equipo de GC-MS en un equipo de GC-combustión-MS consiste en la instalación de una interfase de combustión. Esta está formada por un horno tubular (Carbolite Hero MFT 12/25/250) en cuyo interior se aloja el corazón del sistema, un reactor (dimensiones, longitud 40 mm, diámetro externo 3 mm, diámetro interno 0.5 mm) en cuyo interior, a su vez, dos hilos de platino actúan como catalizadores de la reacción de combustión. Esta es una de las modificaciones llevadas a cabo con respecto al prototipo anterior desarrollado en el grupo, de forma que no se usaron hilos de cobre. Esto a su vez permitió una gran modificación que simplificó el sistema considerablemente, la introducción de un flujo constante de un gas de combustión. Las conexiones entre el reactor y el sistema cromatográfico se realizaron mediante conexiones reductoras de 1/8” a 1/16” en la parte superior del horno, con un tubo metálico de 1/16”, y de 1/8” a 1/32” en la inferior con un tubo metálico de 1/32”.

c. Sistema de introducción de flujo de gas de combustión.

En el prototipo anterior, la combustión de los compuestos tenía lugar previa oxidación del catalizador mediante un proceso que requería varias horas. En este prototipo, el catalizador de cobre se sustituyó por el de platino y, además, el aporte de oxígeno para llevar a cabo la combustión se realizó mediante un controlador de flujo másico (Bronkhorst) con el que se introdujo un flujo de $O_2$ diluido en He (0.3% v/v). Este oxígeno introducido en línea durante el análisis permite una combustión en línea de los compuestos que eluyen de la columna. La unión de ambos flujos, columna y combustión, se llevó a cabo mediante una conexión Tee (*Capillary flow intert Tee,* Agilent) localizada en el interior del horno.

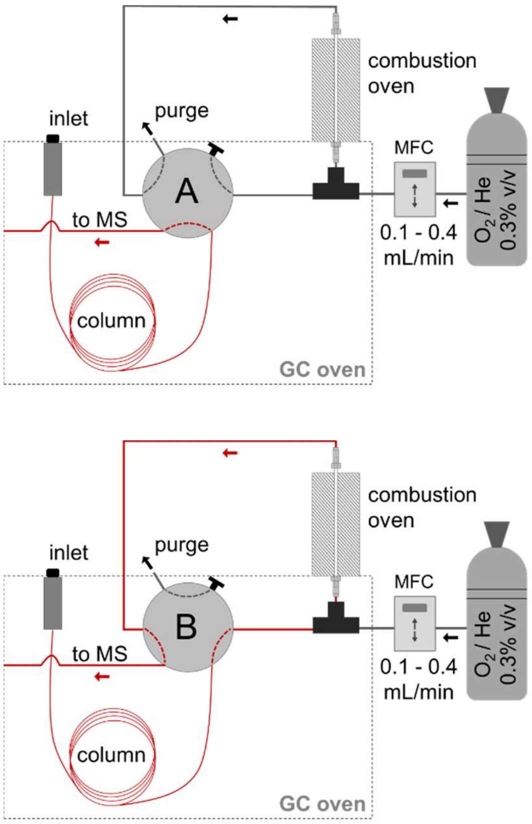

**Figura 14. Esquema del prototipo 1 basado en la modificación de un GC-MS comercial de Agilent. A) Válvula en posición GC-MS B) Válvula en posición GC-MS/MS**

### 2.3.2. Prototipo 2 (Shimadzu)

El proyecto de GC-combustión-MS avanzó sustancialmente durante los últimos tres años con la incorporación al proyecto de la compañía Shimadzu. En conjunto con esta empresa, se desarrolló un segundo prototipo cuyas prestaciones mejoran significativamente las del prototipo 1.

**Figura 15. Fotografía del prototipo 2 de GC-combustión-MS desarrollado junto a la empresa Shimadzu**

Por un lado, el hecho de basar las modificaciones en un cromatógrafo de gases actual, permitió conseguir mejores características en términos de sensibilidad que el anterior. Por otro lado, las modificaciones aplicadas en este instrumento permitieron conseguir un instrumento más cómodo para el usuario, haciendo más sencillo el reemplazo del reactor, además de aumentar la vida útil de este.

a. Válvula automática de seis vías

Una de las modificaciones más significativas en el prototipo 2 consistió en la instalación de una válvula de seis vías con las mismas características que la del anterior prototipo, pero, con un controlador que permite su manejo automático configurado a través del software del propio equipo. Esto permitió reducir considerablemente la interacción con el equipo por parte del usuario, o que también se tradujo en una vida más prolongada de la válvula.

b. Interfase de combustión

La interfase de combustión en este equipo se basó en un equipo comercial previamente diseñado por Shimadzu, para el detector selectivo de azufre, el Nexis SCD-2030. La principal diferencia con respecto al prototipo anterior es la orientación del horno, siendo en este caso horizontal. Otra modificación con respecto al prototipo anterior es la incorporación al sistema de un flujo extra de helio (*make-up*) como flujo protector alrededor del reactor de combustión. Este flujo es introducido en el sistema mediante un controlador extra instalado en el equipo. Se divide en dos flujos, uno introducido junto a la salida de la columna cromatográfica y, otro, introducido al final del reactor de forma que lo recorre purgando posibles volúmenes muertos para finalmente unirse al primer flujo. Con estas dos mejoras, el horno en posición horizontal y los flujos de gas auxiliares, se consiguió prolongar la vida útil de los reactores. Pese al mayor volumen de gas introducido en el MS, el sistema permite trabajar sin apenas perturbar el vacío del sistema.

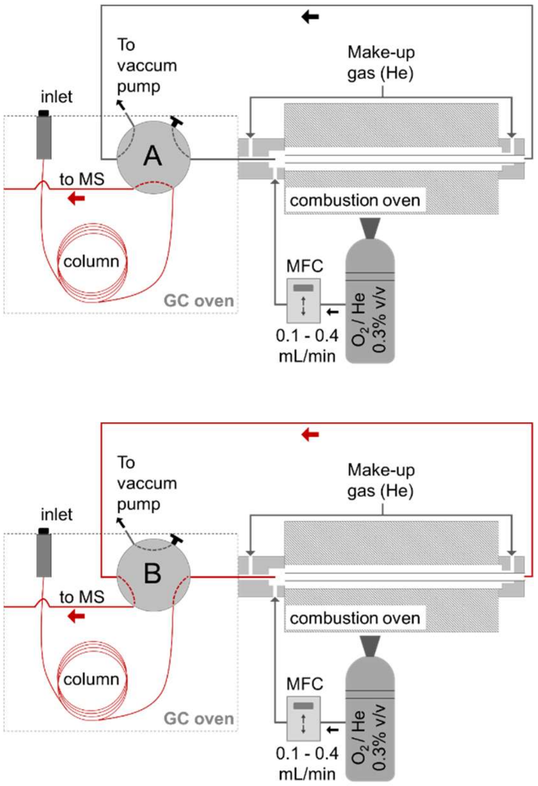

**Figura 16. Esquema del prototipo 2 (Shimadzu). A) Posición de GC-MS B) Posición de GC-combustión-MS**

a. Conexión a la bomba de vacío

En este sistema, la conexión que en el anterior prototipo estaba conectada a la purga, se conectó a la bomba de vacío, de forma que durante todo el análisis existe un vacío que permite reducir posibles fugas incluso cuando el sistema funciona en posición de GC-MS.

## 2.4. Cromatografía de gases bidimensional – espectrometría de masas

Durante la presente Tesis Doctoral se realizó una estancia breve en la Universidad de Messina, en el laboratorio del profesor Luigi Mondello, director de uno de los grupos de investigación con mayor experiencia en cromatografía multidimensional, tanto líquida como de gases.

El objetivo de la estancia, además de una introducción a la técnica de cara a una futura implementación en el prototipo de GC-combustión-MS, fue caracterizar mediante GC×GC las muestras analizadas durante los diferentes capítulos de la tesis doctoral con el objetivo de identificar los compuestos obtenidos selectivamente en cada una de las muestras y detectores. Para ello se llevó a cabo los análisis correspondientes mediante el uso de dos sistemas, un modulador de flujo y otro criogénico que se desarrollan brevemente a continuación.

### *2.4.1. Cromatografía de gases bidimensional con modulador de flujo*

Este modulador se basa en la instalación entre ambas dimensiones (columnas cromatográficas) de una válvula conectada a una plataforma con varios canales desarrollada por las empresas Chromaleont y Trajan. Estos están conectados de forma que el sistema opera en dos fases. En la posición de carga, posición A) de la Figura 14. Esquema del prototipo 1 basado en la modificación de un GC-MS comercial de Agilent. A) Válvula en posición GC-MS B) Válvula en posición GC-MS/MS los compuestos son enviados a un loop de acumulación (20 cm x 0.51 µm). Durante un tiempo establecido de acumulado, los compuestos se cargan en este loop y, cuando el sistema cambia a posición de inyectado, posición B) de la figura, el flujo cambia de sentido, de forma que los compuestos son enviados a la segunda dimensión. En esta segunda etapa de reinyección, el flujo total aumenta de forma considerable para que los compuestos eluyan rápidamente separándose con una separación prácticamente isoterma, de forma que se evita solapamientos entre los analitos que están siendo inyectados en la segunda columna y los analitos que eluyen al mismo tiempo de la primera. El tiempo de modulación total fue de 5 s, en los que se incluye 4.6 s de acumulación y 0.4 s de reinyección en la segunda dimensión.

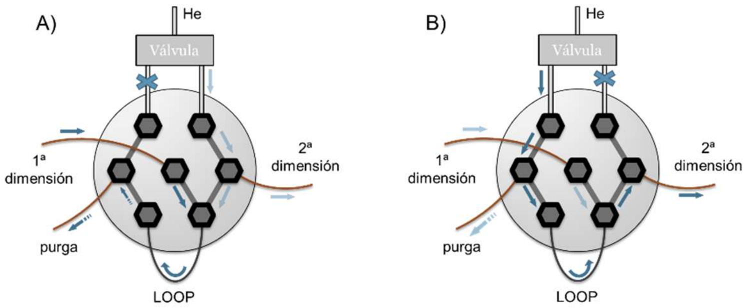

**Figura 17. Esquema de un sistema de GC×GC con un modulador de flujo**

Este sistema, tiene la ventaja de una gran versatilidad, al poder ser instalado en cualquier sistema GC-MS, y bajo coste, teniendo que realizar únicamente una serie de conexiones entre las columnas, además de la incorporación de la válvula.

En cuanto a la parte cromatográfica, en este sistema se utilizó un único horno cromatográfico (GC 2010, Shimadzu), situando ambas columnas en su interior sometidas al mismo gradiente, acoplado a un detector de MS (TQ-8040, Shimadzu)

### *2.4.2. Modulador criogénico.*

El segundo tipo de modulador usado se engloba en los moduladores basados en cambios de temperatura. En estos moduladores, la modulación se lleva a cabo mediante modificaciones en la temperatura de los analitos. En este caso, se usó un modulador tipo ZX2 de la empresa Zoex Corporation. Durante el paso de acumulación, un pulso de nitrógeno criogénico, a una temperatura aproximada de -80ºC, se aplica sobre un punto concreto de un loop reteniendo los analitos que llegan a este. Para el segundo paso de reinyección, se aplica durante un breve periodo de 0.3 s un flujo de nitrógeno caliente, que permite la liberación de los compuestos hacia la segunda dimensión. El loop está formado por un capilar estándar de 1.5 m y 0.18 mm de diámetro interno y consta de al menos dos zonas sometidas a los flujos de nitrógeno para asegurar que todos los analitos que se encuentran en el loop son modulados al menos una vez.

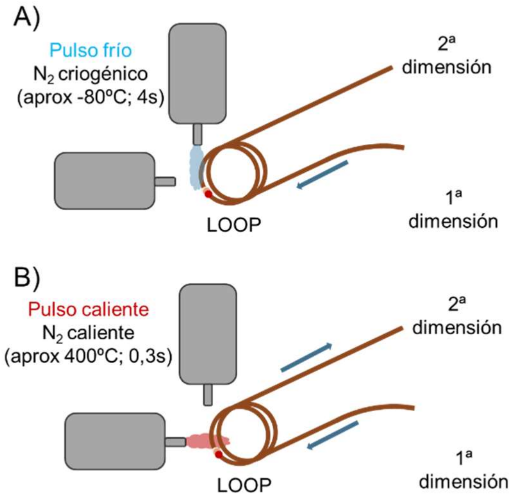

**Figura 18. Posiciones de un modulador de tipo criogénico. A) posición de acumulación con la aplicación de un pulso frío, B) posición de reinyección con la aplicación de un pulso caliente.**

Algunas de las ventajas de este modulador es el hecho de que el paso de acumulación, permite también una concentración de los analitos que son retenidos, mejorando la relación señal/ruido. Si bien, requiere de una operatividad más compleja que el modulador de flujo, al tener que instalar un sistema de nitrógeno líquido con un intercambiador de temperatura que implica mayor coste y espacio.

En este sistema, se utilizaron dos cromatógrafos de gases unidos en línea, uno para cada una de las dimensiones. Esta configuración permite la aplicación durante el cromatograma de *delays* en los gradientes de temperatura, pudiendo aplicar pequeñas diferencias en el tiempo de inicio de uno y otro horno, lo que permite mejoras en la separación cromatográfica en dos dimensiones.

Otra de las desventajas del modulador criogénico, es el hecho de que las temperaturas a las que se someten los analitos para llevar a cabo la modulación, al menos en este modelo, son fijas durante todo el análisis e impiden la aplicación a un rango muy extendido. Por lo general, se necesitan temperaturas 50ºC por debajo de los puntos de ebullición para retener el compuesto, y lo mismo, pero por encima para reinyectarlos, siendo, por un lado, difícil retener analitos muy volátiles y, por otro, difícil reinyectar compuestos con alto peso molecular y por tanto poco volátiles, teniendo que llegar a un compromiso de temperaturas cuando las mezclas a analizar son muy heterogéneas. En términos generales, un modulador criogénico permite una separación mejor en términos de resolución, pero en un rango más concreto, al contrario de lo que sucede con el modulador de flujo, dónde el rango de aplicación es completo, pero la separación es peor en términos cromatográficos.

Una ventaja respecto al modulador de flujo, es el hecho de que los analitos transcurren el mismo tiempo en la segunda dimensión independientemente del número de modulaciones que sufra el compuesto. En el caso del modulador de flujo, si la modulación no es simétrica, esto es que el pico a modular sea repartido en fracciones simétricas con respecto a su forma gaussiana, en cada periodo pueden entrar fracciones de tiempo diferentes, lo que hará que esta fracción de menor tiempo, recorra durante más tiempo la segunda dimensión, y eluya a un tiempo de retención diferente de la anterior fracción.

Otra diferencia importante entre ambos moduladores, es el control del flujo. Mientras que, en el modulador de flujo, cada dimensión requiere un cálculo especializado de la presión que debe utilizarse y depende de los diámetros de las columnas y el loop, en el modulador criogénico, se utiliza un único flujo para las dos dimensiones facilitando los cálculos.

# RESULTADOS/DISCUSIÓN

# CAPÍTULO UNO

## Avances en la cromatografía de gases acoplada a un detector de espectrometría de masas con plasma de acoplamiento inductivo como detector selectivo.

## Introducción

La cromatografía de gases ha sido ampliamente utilizada para análisis de especiación con diferentes detectores selectivos elementales, algunos de los cuales se han presentado en la Introducción de la presente Tesis doctoral. Algunos de estos son el detector de emisión atómica (AED), captura de electrones (ECD) o los detectores basados en la quimioluminiscencia de azufre (SCD) y nitrógeno (NCD). Sin embargo, la falta de sensibilidad y robustez asociada a estos detectores, sobre todo en muestras con matrices complejas, junto con los límites de concentración cada vez más bajos permitidos por la legislación, ha acrecentado el uso de la espectrometría de masas elemental una de las alternativas más usada de cara a una detección selectiva tras la separación cromatográfica.

La espectrometría de masas con plasma de acoplamiento inductivo, (ICP-MS) surge como una técnica con una alta sensibilidad para un gran número de elementos, robusta, con un amplio rango lineal y una alta selectividad hacia el elemento de interés. Esto, sumado al hecho de funcionar como un detector multielemental, capaz de dar información isotópica de los elementos, hace que los métodos basados en esta técnica se hayan desarrollado y extendido en el análisis de especiación desde los años 90 del pasado siglo hasta nuestros días, existiendo numerosos artículos, capítulos de libro y artículos de revisión que proponen multitud de análisis con una gran variedad de matrices y analitos.

El desarrollo inicial de estas técnicas estaba basado en el acoplamiento de un ICP-MS de cuadrupolo simple, lo que hasta cierto punto limitaba los resultados al estar presentes diferentes interferencias espectrales, limitando la aplicación de la técnica a compuestos con elementos con masas bajas como son los heteroátomos P, S, Si, Cl, Br, presentes en multitud de compuestos de interés. Estas interferencias, fueron acometidas en un primer lugar con el uso de equipos con celdas de colisión/reacción (*collision/reaction cell*, CRC) o equipos con resoluciones superiores como son los de doble enfoque.

La introducción en 2012 de los equipos de ICP-espectrometría de masas en tándem (ICP-MS/MS), con dos cuadrupolos consecutivos y una celda de colisión en el centro permitió una mejora en la sensibilidad de las técnicas, al aumentar considerablemente la relación señal-ruido para los elementos altamente interferidos. Si bien, la técnica de GC-ICP-MS ha sido ampliamente usada para compuestos organometálicos con elementos tales como Pb, Sn o Hg, su mayor dificultad es en la aplicación a elementos con energías de ionización más altas como As, Se, P, S, Si, Cl o Br.

En este contexto, en el grupo de investigación se han venido desarrollando diferentes metodologías basadas en el acoplamiento entre la cromatografía de gases y el ICP-MS/MS orientadas a la determinación de algunos de estos elementos en matrices complejas. En concreto, Freije-Carrelo et al. desarrollaron una metodología para la cuantificación de especies de As, en muestras de gaseosas y gaseosas licuadas. Para ello llevaron a cabo la modificación instrumental que permitió la introducción simultánea de muestras líquidas y gaseosas, permitiendo, mediante la instalación de dos líneas distintas el análisis en especiación de las muestras al mismo tiempo que se llevaba a cabo el análisis del contenido total. Partiendo de la base de esta metodología, también Freije-Carrelo et al. llevaron a cabo la optimización de una metodología orientada a un elemento cuyas características lo hacen si acaso más complejo para su determinación por ICP-MS, el S. Este elemento, además de estar presente en multitud de elementos del sistema, lo que repercute inexorablemente en un aumento de los fondos, se encuentra altamente interferido por iones poliatómicos de elementos muy abundantes en la atmósfera como el O y el N. Así, el uso de un ICP-MS/MS, redujo significativamente los requisitos para llevar a cabo el análisis de compuestos que contengan este elemento por ICP-MS, permitiendo su aplicación a muestras realmente complejas, como son los combustibles y concretamente la gasolina.

En la presente tesis doctoral, se ha llevado esta técnica a un elemento con una alta energía de ionización, el Cl, en muestras con una alta complejidad como son los aceites de pirólisis. Estas muestras, de creciente interés en el mundo actual, dada la alta generación de residuos que contienen plástico, son obtenidas a través de una revalorización de residuos plásticos mediante un procesado químico para obtener productos similares a los combustibles. La pirólisis es uno de estos procesos, en el que los plásticos son degradados a altas temperaturas bajo condiciones anaerobias, obteniendo posteriormente aceites con matrices muy complejas con un alto contenido de hidrocarburos, y cuya composición es muy variable en función tanto del origen de los residuos, como de las condiciones específicas del proceso de pirólisis.

Los aceites de pirólisis, requieren de un control de su composición, tanto de los compuestos mayoritarios como de elementos no deseados como N, S, O, Si o los halógenos, al igual que ocurre en otras muestras de tipo energético. En el caso del Cl, los plásticos se someten a procesos de pretratamiento de decloración con el objetivo de reducir el contenido de Cl, por lo que la presencia de compuestos que contengan este elemento es importante. Aunque existen diferentes metodologías que permiten su detección, estas están principalmente orientadas al contenido total de Cl, como son la cromatografía iónica de combustión, la fluorescencia de rayos X, teniendo límites de detección por encima de las partes por billón en el mejor de los casos. En cuanto a análisis de especiación se refiere, el uso del AED y la MS de cuadrupolo simple, como detector selectivo para evaluar el contenido de compuestos de cloro durante la pirólisis de plásticos con contenido en policloruro de vinilo (PVC).

En este capítulo, se evalúa y propone el ICP-MS/MS como detector selectivo para la determinación de compuestos de Cl en muestras de aceite de pirólisis, haciendo uso de la capacidad de discriminación de interferencias del sistema tándem MS y mediante la incorporación de un gas de reacción en la celda, en concreto el $H_2$, para reaccionar con el $^{35}Cl^+$ y formar el correspondiente ion poliatómico $^{35}Cl^1H_2^+$, libre de interferencias. Además, se propone el uso de la cuantificación independiente de la especie para llevar a cabo la cuantificación, dada la capacidad de equimolaridad del sistema, llevando a cabo tanto el análisis de especiación, como de contenido total para distintas muestras de aceite de pirólisis.

Como se ha comentado durante la introducción, una de las limitaciones de los detectores selectivos y en concreto del ICP-MS, es la falta de capacidad para elucidar la estructura de los compuestos que son capaces de detectar. Además, la complejidad de las matrices, así como la baja concentración de los analitos, dificultan la identificación mediante GC-MS tradicional. En este capítulo, en el marco de una de las estancias predoctorales realizadas durante la Tesis Doctoral, se llevó a cabo la caracterización de las muestras analizadas por GC-ICP-MS/MS, mediante GC × GC-MS, con el objetivo de correlacionar los perfiles selectivos obtenidos mediante la primera con la identificación obtenida en la segunda.

Artículo científico I.

**Título***: Recent advances in GC-ICP-MS: Focus on the current and future impact of MS/MS technology*

**Autores**: Javier García-Bellido, Laura Freije-Carrelo, Mariella Moldovan, Jorge Ruiz Encinar

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# Recent advances in GC-ICP-MS: Focus on the current and future impact of MS/MS technology

Javier García-Bellido, Laura Freije-Carrelo, Mariella Moldovan**, Jorge Ruiz Encinar*

*Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain*

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Compound independent calibration

A B S T R A C T

Because of its intrinsic characteristics, ICP-MS is widely considered to be a powerful element-selective detector in gas chromatography analysis for diverse applications. In particular, GC-ICP-MS has proven very useful for classical organometallics analysis (i.e. Sn, Pb, Hg and As) in environmental-, biological- and industrial-related samples. However, it suffered so far, from severe spectral interferences on certain key elements (i.e. P, S, Si and Cl) that limited significantly its potential application fields. In this review, we would like to introduce the potential and improvements in GC-ICP-MS speciation analysis as a result of the arrival of MS/MS configuration which has been established as the best instrumental choice for the extremely sensitive and robustness analysis of such highly-interfered elements present in numerous potential targets (i.e. pesticides, siloxanes, petroleum- and biogas-related products) in complex sample matrices. Recent advances (2010–2020) and expected new exciting applications now affordable as a consequence of the use of GC-ICP-MS/MS are summarized and discussed.

## 1. Introduction

Gas chromatography (GC) is widely being used for speciation purposes in combination with different element specific detectors such as atomic emission (AED), electron capture (ECD) or chemiluminescence based sulfur (SCD) and nitrogen (NCD) detectors. However, their lack of sensitivity and robustness when considering analysis of complex samples along with the increasingly low levels of organometallic compounds currently demanded by Environmental Protection Agencies and Legislation Bodies, has turned elemental mass spectrometry into the best alternative for element-selective detection after GC separation [1]. In fact, ICP-MS (inductively coupled plasma mass spectrometry) provides high sensitivity for most elements, robustness, wide linear dynamic range, high specific response together with multi-elemental analysis capabilities and isotopic information. In this context, GC-ICP-MS based methods have been widespread used in speciation analysis since the 90's as demonstrated by the extensive existing bibliography and the number of book chapters [2,3] and reviews [4–8] published in this regard.

GC-Quadrupole (Q) ICP-MS instruments were more often used in initial studies. However, the formation of polyatomic spectral interferences within the plasma hampered the applicability of quadrupole instruments to potential target elements such as P, S, Si and Cl that are present in a vast number of compounds of interest. ICP-MS instruments equipped with a collision/reaction cell (CRC) system, as well as double focusing (DF) ICP-MS, offered significant control of polyatomic spectral interferences for such elements but, still did not provide the low detection limits (DL) and robustness required for their analysis in many real samples.

However, the introduction in 2012 of ICP-MS/MS (ICP-tandem mass spectrometer) instruments opened new ways to overcome the previously cited limitations. With two quadrupoles in tandem and a collision-reaction cell in between, ICP-MS/MS instrument provides better sensitivity due to the huge increase in the signal to noise ratio observed in the detection of several highly interfered elements, especially S and P among others [9].

Several articles have been already published showing exciting applications of GC-ICP-MS/MS, and still many others are expected in the coming years due to its great analytical capabilities. This review aims to provide a flavour of the potential benefits that the use of ICP-MS/MS instruments, instead of traditional ICP-MS, can bring in speciation analysis using GC. For that purpose, a brief overview and update of the GC-ICP-MS approaches applied to the determination of traditional speciation elements (Pb, Sn, Hg) as

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* Corresponding author.
** Corresponding author.
*E-mail addresses:* moldovanmariella@uniovi.es (M. Moldovan), ruizjorge@uniovi.es (J.R. Encinar).

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well as more interfered elements (As, Se, P, S, Si, Cl and Br) is given first in the second section. The analytical performance of ICP-MS/MS instruments is presented in the third section, meanwhile the future benefits of GC-ICP-MS/MS for the emerging ICP detectable elements (P, S, Si, Br and Cl) to be analyzed are presented within the fourth section. Finally, the future impact of ICP-MS/MS technology is discussed in the last section.

## 2. GC-ICP-MS applications in 2010–2020 using Q, CRC and DF technologies

Meanwhile the coupling of high performance liquid chromatography (HPLC) with ICP-MS is an easy task by means of a direct connection between the end of the column and the nebulizer, the GC-ICP-MS coupling becomes more complex and critical. Since analytes elute from the GC column in gaseous form, it is necessary to avoid their condensation along the way between the exit of the GC column and the entrance to the ICP plasma. As a result, the GC-ICP-MS interface is usually a heated inert transfer line aiming, not only at transporting quantitatively the analytes unaltered, but also at maintaining the excellent GC efficiency (i.e. peak sharpness and resolution).

Several approaches were made during the 90's to undertake these demands and consequently different types of interfaces were designed. The publication of García Alonso and Ruiz Encinar [2] covered the evolution of GC-ICP-MS interfaces until year 2001, which was then updated by Wuilloud et al. [5] in 2004. At that time, such new speciation technique was already established and its application fields well defined. As a consequence, instrumental companies developed their own GC-ICP-MS interfaces during the 00's. Basically, such GC-ICP-MS interfaces can be grouped under two design categories: (i) transfer lines; and (ii) mixed interfaces. In the transfer line approach, the effluent of the column is directly inserted through the central channel of the torch into the plasma, after removing the ICP-MS spray chamber. The main advantage of such design is that plasma energy is not wasted in the volatilisation of the solvent, being the energetic efficiency of the plasma higher than that compared with liquid-phase analysis. Non- [10], partially- [11], and fully-heated [12] transfer lines were developed and used. Nowadays, most of the commercial designs are based on entirely heated transfer lines to avoid condensation of high boiling point compounds. In this regard, first commercial interface was released by Agilent in 2002 and updated in 2010. In a similar way, Perkin Elmer also settled a heated transfer line compatible with its GC and ICP-MS instruments. On the contrary, in GC-ICP-MS mixed interfaces the spray chamber equipped with a concentric nebulizer remains placed for obtaining an aerosol that, before the plasma, is mixed with the GC effluent by means of a T-connection. Based on this design, in 2005 Thermo developed a commercial interface able to work under both wet and dry plasma conditions [13]. In summary, several instrumental developments have been made since the groundbreaking paper by Van Loon et al. [14] in 1986 until the current commercial interfaces. In fact, GC-ICP-MS coupling is well-established nowadays for both research and routine analysis.

One of the analytical characteristics which makes the ICP-MS unique in comparison with other element selective detectors is the possibility of direct measurement of isotope ratios. Such exceptional feature opened the door to carry out traceability and provenance studies through the measurement of precise isotope ratios. Additionally, highly accurate and precise quantifications could be then performed by means of isotope dilution analysis, mostly species-specific applications [15].

Initially, the application of GC-ICP-MS to elemental speciation was evaluated for a large number of elements. This list included: As, Bi, Br, Cd, Cl, Cu, Fe, Ge, Hg, I, In, Mn, Mo, Ni, P, Pb, S, Sb, Se, Si, Sn, Te, V, W and Zn as reviewed by Wuilloud et al. [5] However, the number of elements with clear interest for GC-ICP-MS analysis is much shorter nowadays probably due to a lack of real samples applications and stable and reliable standards to carry out the corresponding quantifications.

In the next sub-sections we will focus on the last advances of GC-ICP-MS analysis using Q- and DF-based instruments of the most relevant elements. The selected elements are divided in three groups: (i) Pb, Sn and Hg, named as traditional elements; (ii) As and Se; and, (iii) P, S, Si, Cl and Br, named as non-traditional elements.

### *2.1. Lead, tin and mercury*

Lead speciation analysis is significant as a result of the past use of alkyllead compounds as antiknocking agents in gasolines, what contributed to the spread of organolead compounds in the environment and their accumulation in different biological systems in the 80's and 90's. Tetramethyllead ($Me_4Pb$), trimethylethyllead ($Me_3EtPb$), dimethyldiethyllead ($Me_2Et_2Pb$) and methyltriethyllead ($MeEt_3Pb$) could be mentioned among the environmental emitted lead species. Nowadays, industrial emissions and runoff waters are the main lead sources and, for instance, triethyllead ($Et_3Pb$) and diethyllead ($Et_2Pb$) are the most common organolead compounds present in water samples. Organotin compounds (OTCs), and tributyltin (TBT) in particular, were commonly present in antifouling paints, pesticides, wood preservatives until their regulation at the end of the 90's. Unfortunately, monobutyltin (MBT) and dibutyltin (DBT) are still present in many industrial uses such as polymer additives and flooring among many others. In general, OTCs are classified by the type (i.e.: methyl-, butyl-, phenyl- and octyl-) and the number (i.e.: tri-, di- and mono-) of organic functional groups bond to the tin atom. Tri-substituted OTCs present higher toxicity than di- and mono-ones. Regarding mercury emissions, different natural processes such as volcanic eruptions and anthropogenic activities like fossil fuel combustion release mercury, mainly $Hg^0$ and $Hg^{2+}$, to the atmosphere. Further methylation in the aquatic environment originates the organomercury species, being methylmercury (MeHg) the most toxic one. At present, mercury compounds are present in a large variety of biological and environmental samples.

During the past three decades, a considerable amount of literature on the use of GC-ICP-MS for the speciation analysis of Pb, Sn and Hg organometallic compounds have been reported in many sample types, for example ocean and river waters, sediments and sludges. As a result of their high mass/charge ratio, their corresponding most abundant isotopes ($^{208}Pb$, $^{120}Sn$ and $^{202}Hg$) are not usually affected by polyatomic or isobaric spectral interferences. For this reason, the determination of their species was, and is still, performed mainly by single quadrupole ICP-MS instruments without the need of more complex instrumentation.

Regarding sample preparation, Pb, Sn and Hg compounds have to be extracted from the sample matrix and derivatized to form thermally stable volatile species. In this regard, exciting strategies that make use of isotopically enriched species for species-specific isotope dilution analysis (IDA) quantification were elaborated to assess extraction and derivatization procedures, as they were clearly identified as the "*Achilles heel*" of speciation methods [4]. Interestingly, the synthesis and use of multiple organometallic species labelled with different enriched isotopes is extremely useful to evaluate and correct for degradation and interconversion processes during chemical analysis, especially for Sn [16] and Hg [17] compounds.

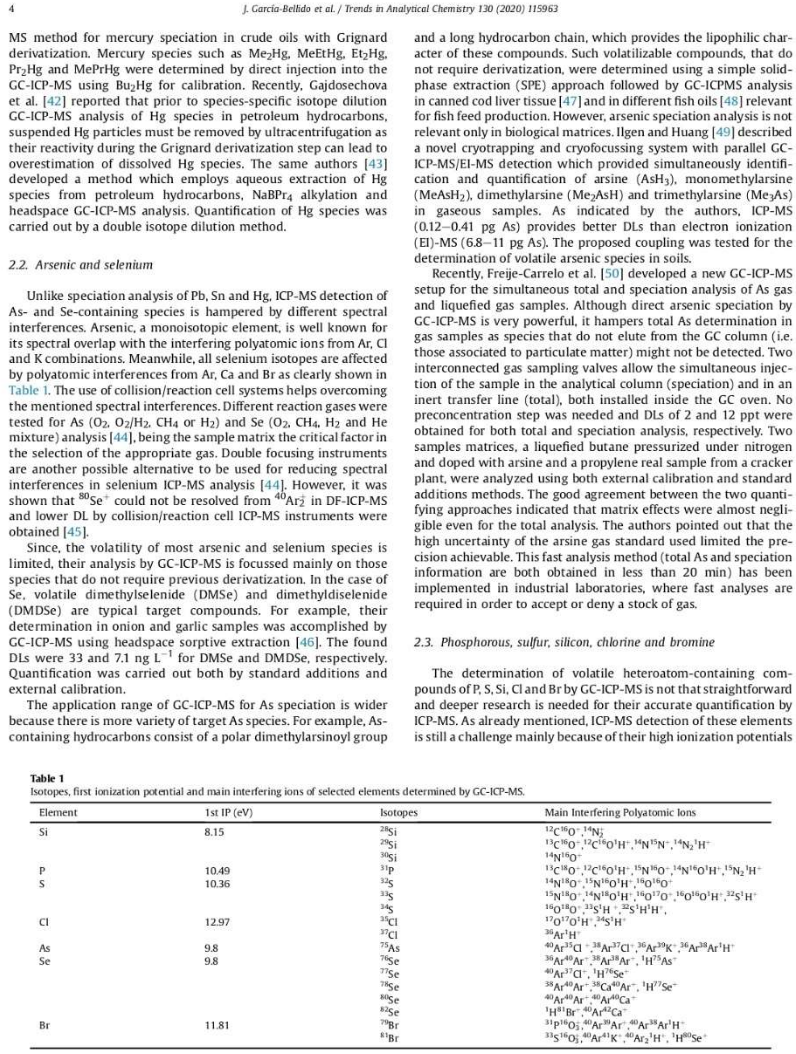
The isotopic information achieved by GC-ICP-MS also allows the accurate and precise measurement of isotope ratios for provenance studies. In this sense, Ruiz Encinar et al. [18] reported that the

measurement of lead isotope ratios by quadrupole ICP-MS in individual organolead species present in collected airborne particles and urban dust, could be used to track the organolead emission sources. Afterwards, the coupling of GC to multicollector (MC) ICP-MS instruments opened the door to the measurement of more precise and accurate isotope ratios. In this way, Krupp et al. [19] illustrated the use of wet plasma conditions, where thallium solution was introduced for mass bias correction, for the determination of lead isotope ratios on short transient signal obtained by GC-MC-ICP-MS. Later, the GC-MC-ICP-MS method described by Sanabria-Ortega et al. [20] combined both sample preparation and ICP-MS operation operated under wet plasma conditions for mass bias correction by means of a thallium standard solution. This optimized method was applied for the Pb isotopic characterization of complex geochemical matrices such as black shale, asphaltene, crude oil and kerogen, as a tool for precise dating of crude oil studies. GC-MC-ICP-MS measurement of Hg isotope ratios allows, in a similar way, studying the biogeochemical cycle of mercury. In this regard, the method developed by Masbou et al. [21] offered isotopic data on inorganic Hg (iHg) and MeHg in different biological samples which could be useful for understanding mercury dynamics. Interestingly, Bouchet et al. [22] described the use of compound-specific isotope analysis of Hg species using GC-MC-ICP-MS at ultratrace levels. On-line preconcentration and subsequent transfer to the column were achieved by a programmed temperature vaporization (PTV) injector fitted with a packed liner. The further analysis of several reference materials demonstrated the applicability to environmental samples with species concentrations in the low ng g$^{-1}$ content.

Most of the efforts during the 2010–2020 decade were indeed focused on the development of sample preparation methods to improve methodological detection limits for the determination of Pb, Sn and Hg compounds in diverse real-life samples. In this context, and as a result of the implementation of the European Water Framework Directive (WFD) 2000/60/EC, together with the related directives 2008/105/EC and 2013/39/EU, the water maximum limits for Sn and Hg organometallic species were established at low ng L$^{-1}$ levels, and at μg L$^{-1}$ for Pb organometallic compounds, forcing the required detection limits for their analysis to be lowered. Consequently, analytical methods were reported stressing the necessity of a reliable preconcentration step for the quantification of organometallic species in water at such low content level. As an example, Alasonati et al. [23] proposed solid phase extraction (SPE) followed by GC-ICP-MS for the quantification of TBT (tributyltin) in mineral water in order to meet the stringent 0.2 ng L$^{-1}$ required in the WFD. Species-specific isotope dilution was carried out and the methodological DL achieved for TBT fell down to the pico-trace levels. Later, the same research team [24] described the TBT quantification at sub ng L$^{-1}$ level in whole water bodies, this is, non-filtered water samples with humic substances and suspended particulate matter. In parallel to single-element water speciation analysis, Hu et al. [25] described the ultra-trace determination of tin (TBT, DBT and MBT) and mercury species (MeHg and inorganic mercury, iHg) in waters, including seawater, by GC-ICP-MS. The required preconcentration step of the analytes was performed by dynamic headspace in-tube extraction and quantification by species-specific isotope dilution, except for MBT whose quantitation was carried out by standard addition calibration, with $^{117}$Sn-enriched DBT serving as the internal standard species. The proposed method accomplished detection limits below 1.1 pg g$^{-1}$, as Sn or Hg, for all studied species. Similarly, Terán-Baamonde et al. [26] proposed a very sensitive method for the simultaneous determination of Pb, Sn and Hg organometallic compounds in natural waters by an online automated preconcentration method using large volume injections through a PTV injector fitted with a sorbent packed liner coupled to GC-ICP-MS. The authors reported detection limits at pg L$^{-1}$ level.

The concern about the environmental and health effects of Sn and Hg organometallic compounds has also draw attention to their monitoring in more complex real-life matrices. For instance, landfill leachates are an important sink of OTCs as a result of their presence in different plastic and manufactured household waste products. Vahčič et al. [27] described an analytical procedure for the simultaneous routine determination of up to ten OTCs in landfill leachates by GC-ICP-MS in conjunction with standard addition calibration. Lately, the same research group presented a method which made use of isotopically enriched tin tracers to study of the degradation and biomethylation processes of OTCs in landfill leachates [28]. Interestingly, the same isotope dilution methodologies together with sensitive GC-ICP-MS measurements were used to produce and characterize a sewage sludge quality control material (QCM) with well-established reference values and uncertainties for DBT and TBT, ready for use in day-to-day quality control purposes [29]. The monitoring of organotin compounds in food and beverages samples continues being relevant as well, in order to estimate the dietary exposure to OTCs. In this sense, recently, Chung et al. [30] described a novel method for the simultaneous determination of ten organotin compounds, including butyltins, octyltins, phenyltins, and tricyclohexyltin, in fishes, crustaceans, and mollusks using GC-ICP-MS and external calibration in the range 0.5–50 μg Sn L$^{-1}$. Additionally, Vacchina et al. [31] developed and validated a GC-ICP-MS method with internal correction for monitoring the presence of methyl-, butyl-, phenyl- and octyltin in red wine. The method was applied to the analysis of more than 100 worldwide samples, and the results indicated that monobutyl-, dibutyl- and monooctyltin were the main species.

Over the last decade, the presence of Hg species has been studied in a wide range of real-life samples. For instance, great efforts have been made for the accurate and fast quantification of Hg compounds in biological samples such as whole blood [32–36], urine [32] and hair [32,37]. Urine samples usually indicate exposure to inorganic mercury, meanwhile blood and hair are the suitable samples for evaluating the exposure to the highly toxic organomercury compounds. As already pointed out for organotin compounds, the monitoring of food contamination is compulsory in order to limit the dietary intake of toxic organometallics. In this sense, fish and seafood are the main source of MeHg human uptake. Clemens et al. [38] determined MeHg and total mercury in seafood by species-specific isotope dilution analysis and GC-ICP-MS. The selected method offered DLs of 1.2 μg Hg kg$^{-1}$ for MeHg and 1.4 μg Hg kg$^{-1}$ for total mercury. Analysis of more than 60 seafood samples indicated low MeHg content in the range 1.9–588 μg kg$^{-1}$. Then, Esteban et al. [39] applied double spike isotope dilution analysis with GC-ICP-MS for studying species interconversions due to sample preparation. Soft ultrasound assisted extraction procedure was selected. The obtained results indicated negligible species transformations in the analysis of a lyophilized certified reference material, while a small demethylation factor was observed in the case of real samples. In a recent paper, Renedo et al. [40] described a method for the determination of mercury compounds in seabirds feathers, samples that contains mainly methylmercury and represent the main route for mercury elimination. Spike addition before the microwave nitric acid extraction and GC-ICP-MS analyses using species-specific isotope dilution were carried for the determination of MeHg and iHg content. In petroleum industry, mercury species can affect the behaviour of refining catalysts and can have hazardous effects to the workers. As a result, technologies for the elimination of mercury from crude oil and related products are continuously being improved. Pontes et al. [41] proposed a GC-ICP-



MS method for mercury speciation in crude oils with Grignard derivatization. Mercury species such as $Me_2Hg$, MeEtHg, $Et_2Hg$, $Pr_2Hg$ and MePrHg were determined by direct injection into the GC-ICP-MS using $Bu_2Hg$ for calibration. Recently, Gajdosechova et al. [42] reported that prior to species-specific isotope dilution GC-ICP-MS analysis of Hg species in petroleum hydrocarbons, suspended Hg particles must be removed by ultracentrifugation as their reactivity during the Grignard derivatization step can lead to overestimation of dissolved Hg species. The same authors [43] developed a method which employs aqueous extraction of Hg species from petroleum hydrocarbons, $NaBPr_4$ alkylation and headspace GC-ICP-MS analysis. Quantification of Hg species was carried out by a double isotope dilution method.

### 2.2. Arsenic and selenium

Unlike speciation analysis of Pb, Sn and Hg, ICP-MS detection of As- and Se-containing species is hampered by different spectral interferences. Arsenic, a monoisotopic element, is well known for its spectral overlap with the interfering polyatomic ions from Ar, Cl and K combinations. Meanwhile, all selenium isotopes are affected by polyatomic interferences from Ar, Ca and Br as clearly shown in Table 1. The use of collision/reaction cell systems helps overcoming the mentioned spectral interferences. Different reaction gases were tested for As ($O_2$, $O_2/H_2$, $CH_4$ or $H_2$) and Se ($O_2$, $CH_4$, $H_2$ and He mixture) analysis [44], being the sample matrix the critical factor in the selection of the appropriate gas. Double focusing instruments are another possible alternative to be used for reducing spectral interferences in selenium ICP-MS analysis [44]. However, it was shown that $^{80}Se^+$ could not be resolved from $^{40}Ar_2^+$ in DF-ICP-MS and lower DL by collision/reaction cell ICP-MS instruments were obtained [45].

Since, the volatility of most arsenic and selenium species is limited, their analysis by GC-ICP-MS is focussed mainly on those species that do not require previous derivatization. In the case of Se, volatile dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) are typical target compounds. For example, their determination in onion and garlic samples was accomplished by GC-ICP-MS using headspace sorptive extraction [46]. The found DLs were 33 and 7.1 ng $L^{-1}$ for DMSe and DMDSe, respectively. Quantification was carried out both by standard additions and external calibration.

The application range of GC-ICP-MS for As speciation is wider because there is more variety of target As species. For example, As-containing hydrocarbons consist of a polar dimethylarsinoyl group and a long hydrocarbon chain, which provides the lipophilic character of these compounds. Such volatilizable compounds, that do not require derivatization, were determined using a simple solid-phase extraction (SPE) approach followed by GC-ICPMS analysis in canned cod liver tissue [47] and in different fish oils [48] relevant for fish feed production. However, arsenic speciation analysis is not relevant only in biological matrices. Ilgen and Huang [49] described a novel cryotrapping and cryofocussing system with parallel GC-ICP-MS/EI-MS detection which provided simultaneously identification and quantification of arsine ($AsH_3$), monomethylarsine ($MeAsH_2$), dimethylarsine ($Me_2AsH$) and trimethylarsine ($Me_3As$) in gaseous samples. As indicated by the authors, ICP-MS (0.12–0.41 pg As) provides better DLs than electron ionization (EI)-MS (6.8–11 pg As). The proposed coupling was tested for the determination of volatile arsenic species in soils.

Recently, Freije-Carrelo et al. [50] developed a new GC-ICP-MS setup for the simultaneous total and speciation analysis of As gas and liquefied gas samples. Although direct arsenic speciation by GC-ICP-MS is very powerful, it hampers total As determination in gas samples as species that do not elute from the GC column (i.e. those associated to particulate matter) might not be detected. Two interconnected gas sampling valves allow the simultaneous injection of the sample in the analytical column (speciation) and in an inert transfer line (total), both installed inside the GC oven. No preconcentration step was needed and DLs of 2 and 12 ppt were obtained for both total and speciation analysis, respectively. Two samples matrices, a liquefied butane pressurized under nitrogen and doped with arsine and a propylene real sample from a cracker plant, were analyzed using both external calibration and standard additions methods. The good agreement between the two quantifying approaches indicated that matrix effects were almost negligible even for the total analysis. The authors pointed out that the high uncertainty of the arsine gas standard used limited the precision achievable. This fast analysis method (total As and speciation information are both obtained in less than 20 min) has been implemented in industrial laboratories, where fast analyses are required in order to accept or deny a stock of gas.

### 2.3. Phosphorous, sulfur, silicon, chlorine and bromine

The determination of volatile heteroatom-containing compounds of P, S, Si, Cl and Br by GC-ICP-MS is not that straightforward and deeper research is needed for their accurate quantification by ICP-MS. As already mentioned, ICP-MS detection of these elements is still a challenge mainly because of their high ionization potentials

**Table 1**
Isotopes, first ionization potential and main interfering ions of selected elements determined by GC-ICP-MS.

| Element | 1st IP (eV) | Isotopes | Main Interfering Polyatomic Ions |
|---|---|---|---|
| Si | 8.15 | $^{28}Si$ | $^{12}C^{16}O^+, ^{14}N_2^+$ |
| | | $^{29}Si$ | $^{13}C^{16}O^+, ^{12}C^{16}O^1H^+, ^{14}N^{15}N^+, ^{14}N_2{}^1H^+$ |
| | | $^{30}Si$ | $^{14}N^{16}O^+$ |
| P | 10.49 | $^{31}P$ | $^{13}C^{18}O^+, ^{12}C^{16}O^1H^+, ^{15}N^{16}O^+, ^{14}N^{16}O^1H^+, ^{15}N_2{}^1H^+$ |
| S | 10.36 | $^{32}S$ | $^{14}N^{18}O^+, ^{15}N^{16}O^1H^+, ^{16}O^{16}O^+$ |
| | | $^{33}S$ | $^{15}N^{18}O^+, ^{14}N^{18}O^1H^+, ^{16}O^{17}O^+, ^{16}O^{16}O^1H^+, ^{32}S^1H^+$ |
| | | $^{34}S$ | $^{16}O^{18}O^+, ^{33}S^1H^+, ^{32}S^1H^1H^+,$ |
| Cl | 12.97 | $^{35}Cl$ | $^{17}O^{17}O^1H^+, ^{34}S^1H^+$ |
| | | $^{37}Cl$ | $^{36}Ar^1H^+$ |
| As | 9.8 | $^{75}As$ | $^{40}Ar^{35}Cl^+, ^{38}Ar^{37}Cl^+, ^{36}Ar^{39}K^+, ^{36}Ar^{38}Ar^1H^+$ |
| Se | 9.8 | $^{76}Se$ | $^{36}Ar^{40}Ar^+, ^{38}Ar^{38}Ar^+, ^1H^{75}As^+$ |
| | | $^{77}Se$ | $^{40}Ar^{37}Cl^+, ^1H^{76}Se^+$ |
| | | $^{78}Se$ | $^{38}Ar^{40}Ar^+, ^{38}Ca^{40}Ar^+, ^1H^{77}Se^+$ |
| | | $^{80}Se$ | $^{40}Ar^{40}Ar^+, ^{40}Ar^{40}Ca^+$ |
| | | $^{82}Se$ | $^1H^{81}Br^+, ^{40}Ar^{42}Ca^+$ |
| Br | 11.81 | $^{79}Br$ | $^{31}P^{16}O_3^+, ^{40}Ar^{39}Ar^+, ^{40}Ar^{38}Ar^1H^+$ |
| | | $^{81}Br$ | $^{33}S^{16}O_3^+, ^{40}Ar^{41}K^+, ^{40}Ar_2{}^1H^+, ^1H^{80}Se^+$ |

and the existence of severe polyatomic interferences at the corresponding m/z of interest (Table 1).

The first attempts for the speciation analysis of these elements were performed using high resolution mass spectrometry. For instance, the determination of volatile sulfur compounds in human breath [51] and the determination of total homocysteine (after derivatization) in human serum [52] were achieved by double focusing sector-field instruments. The development of collision and/or reaction cell (CRC) technology enabled reducing polyatomic spectral interferences of the aforementioned elements, as well. Polyatomic ions have larger collision cross-sections than analyte ions so they collide more frequently with the non-reactive gas (i.e. He) leading to a greater loss of their kinetic energy. As a result, such lower energy ions can be discriminated with the adequate potential barrier at the cell exit so they are not able to pass to the mass analyzer, turning into interference attenuation. For example, the use of He in the cell has been applied to the simultaneous quantification of P, Br, Cl, I and S in pesticides, both by external calibration and compound independent quantification [53]. On the contrary, when the cell is filled in with a reactive gas (i.e. $H_2$, $NH_3$ or $O_2$) the cell gas reacts either with the analyte ions providing a new m/z to be monitored (mass shift mode) or with the interfering ions shifting them to a different m/z (on mass mode). Silicon speciation, with significant importance for oil and gas industry, could be performed in this regard by GC-ICP-MS using $H_2$ as reaction gas as it was able to attenuate the interferences $^{14}N^{14}N^+$ and $^{12}C^{16}O^+$ on the $^{28}Si$ isotope [54]. However, poor detection limits were obtained. Additionally, the observed GC-ICP-MS non-universal response for the different studied silicon compounds forced the requirement to calculate response coefficients for each silicon compound.

On the other hand, the addition of $N_2$ as optional gas in the central argon plasma channel was found to enhance phosphorus ionization improving its detection limits. The determination of 10 organophosphates, used as flame retardants and plasticizers, in sediment samples was described García-López et al. [55] The introduction of a 0.5 mL $min^{-1}$ $N_2$ flow provided low background signal and adequate response for organophosphates what brought about that the use of collision cell with He was not required anymore.

In the case of S speciation, its most abundant isotope ($^{32}S^+$) is severely interfered by the polyatomic ion $^{16}O_2^+$. The presence of oxygen traces within the argon gas plasma causes an unspecific background signal at m/z 32. For instance, the use of a gas purification system for oxygen removal from the argon gas in combination with cold plasma ICP-MS conditions made possible the determination of S-containing compounds, without the need for CRC or SF instruments, in petroleum products by GC-ICP-MS using on-line [56] or species-specific [57] isotope dilution approaches. As well, particular attention must be also paid to the possible presence of traces of S-containing compounds in the argon gas plasma and/or different GC-ICP-MS components such as, for example, o-rings.

Polybrominated diphenyl ethers (PBDEs) have been extensively used as flame retardants in a wide range of consumer goods. The use, recycling, landfill disposal or incineration of such materials release Br-containing compounds into the environment. Bergant et al. [58] described a reliable and sensitive GC-ICP-MS (quadrupole instrument) method for the determination of six PBDE congeners in human serum applying standard addition for their quantification. Alternatively, PBDEs quantification methodologies based on the species-specific isotope dilution are available as well since $^{81}Br$ isotopically enriched analogues are commercially available. For instance, PBDEs were determined by species-specific isotope dilution GC-ICP-MS in different sample matrices, such as sewage sludge [59], fish [60], or water [61–63]. In order to improve the detection limits, González-Gago et al. [64] found that the addition of an additional flow of 40 mL $min^{-1}$ of He to the plasma gas, along with species-specific isotope dilution analysis, facilitate the determination of 6 priority PBDEs included into the WFD at sub ng $L^{-1}$ levels.

Finally, chlorine in polychlorinated biphenyls (PCBs) compounds have been measured by GC-ICP-MS employing He as collision gas in order to attenuate spectral interferences on Cl isotopes. However, poor sensitivity mainly due to its high ionization potential was still obtained when real samples were analyzed, hampering the reliable quantification [65].

At the same time, the precise isotope ratio information provided by the MC-ICP-MS instruments has expanded the GC-ICP-MS application range for Br, Cl and S. In this way, stable bromine isotopic composition provides the baseline for the discrimination between the anthropogenic and naturally produced brominated organic compounds (BOCs), as indicated by Sylva et al. [66] The GC-MC-ICP-MS approach, operated in low resolution mode ($m/\Delta m = 400$), allowed the measurement of stable bromine ratios on individual BOCs at a precision of 0.30‰ when 0.3 nmol of Br is injected. Compound-specific bromine isotope compositions were reported in further studies [67,68] In this regard, chlorine isotope analysis is beneficial in environmental and forensic fields in order to investigate the fate of these substances in the environment. However, the achievement of such isotopic analysis is hampered by the Cl high first ionization potential (13.01 eV) and the $^{1}H^{36}Ar^+$ polyatomic ion produced in the Ar plasma acting as significant mass-to-charge (m/z) interference for $^{37}Cl^+$. Van Acker et al. [69] indicated that running the MC-ICPMS at a high mass resolution ($m/\Delta m = 10000$) facilitates the removal of ArH interference on Cl. Later, Horst et al. [70] studied the feasibility of working under dry plasma conditions, without any nebulization, and low resolution mode ($m/\Delta m = 400$) for minimizing the hydrogen-based interferences. Krupp et al. [71] reported, for the first time, precise isotope ratio values at the ng-level for the gaseous sulfur compound $SF_6$ by GC-MC-ICP-MS working at high resolution, allowing the study of sulfur isotope fractionation in nature. Some years later, the publication of Amrani et al. [72] described the use of dry plasma, this is, without aqueous vapour added to the gas streams, for minimizing the interference of $O_2^+$ on all monitored sulfur masses using medium resolution ($m/\Delta m = 5000$) MC-ICP-MS detection. The optimized method facilitated the isotopic characterization of individual organosulfur compounds in complex samples, such as low sulfur crude oil. The work by Said-Ahmad et al. [73] showed the possibility of compound-specific sulfur isotope analysis of trace species in gaseous samples (i.e. 10% v/v $H_2S$) produced by pyrolysis of sulfur-rich kerogen. A modified vapour GC with passivated lines equipped with a valve to transfer the gas to a thermal conductivity detector (TCD) was used, enabling a precise $H_2S$ peak cut, with no effect on the other target sulfur peaks.

In summary, the use of GC-ICP-MS in combination with DF (medium, pseudo-high and high resolution) and CRC technologies represents an important advance in the determination of difficult elements (P, S, Si, Cl and Br) forbidden by quadrupole ICP-MS. However, further improvements are still pending for the final sensitive determination of species containing these elements present at ultratrace levels in complex samples.

## 3. ICP-MS/MS technology

The introduction of tandem ICP-MS (ICP-MS/MS) instruments in 2012 turned out to be a revolution in the analysis of highly-interfered elements [74]. ICP-MS/MS instrument includes an additional quadrupole mass analyzer (Q1) before the collision/reaction cell. When a particular m/z is fixed in Q1, only that m/z is allowed to enter into the CRC, what results in a complete control over the processes occurring in the cell comparing to single quadrupole ICP-

MS and thus a more efficient interference correction even for the analysis of real samples with complex matrices [9,44]. Another main difference between CRC operated with MS/MS with regards to single MS technologies is that in the first case, the two Q provide two specificity levels that reduce drastically the chemical noise levels, leading to much better DLs. One important aspect of this instrument is the multiple operation modes available, thereby providing flexibility. For specific applications, ICP-MS/MS can be used in a similar way than quadrupole ICP-MS, this is, by using the first quadrupole Q1 as ion guide. However, the key factor of this instrument is the possibility of using both quadrupoles as mass filters, providing therefore a double mass selection.

When the mass-shift mode is selected, Q1 filters only the looked m/z ratio that will further enter into the CRC filled with a reaction gas [75]. Under optimum conditions analyte ions will react efficiently with gas molecules while the interference ions will not react at all. The new m/z formed after the reaction in the CRC is then fixed in Q2. This allows the analyte ion to be converted into a reaction product ion with a different m/z ratio than the original interference.

When the on-mass mode is selected, the same m/z is fixed on both quadrupoles and spectral interferences are removed by making them react or collide efficiently with the reaction gas. As a result, interfering polyatomic species either will be converted into new species with m/z ratio different than the one of the analyte, or they will undergo a decreasing in their kinetic energy that enabled their discrimination before the second quadrupole that is sat to the same m/z as Q1.

The choice between one or another mode depends on the enthalpy of reaction that indicates the reaction efficiency and whether the reaction of the cell gas with the analyte (mass-shift) or the interferent (on-mass) will proceed forward.

Fig. 1 shows a scheme of the main operation modes in ICP-MS/MS, including on-mass and mass-shift with $H_2$ and $O_2$ as reaction gases. Elements typically measured with each mode are also indicated. Since its introduction, publications are mostly focused either on the direct sample introduction with conventional nebulization or on the coupling with liquid chromatography. However despite its great potential, only few analytical works are so far available based on GC-ICP-MS/MS. Therefore, it is expected an increasing number of new GC-ICP-MS/MS applications in the near future.

## 4. GC-ICP-MS/MS applications

As highlighted in previous sections, the development of accurate, sensitive and fast GC-ICP-MS analytical methods for low levels determination of P, S, Si, Cl and Br compounds in a large variety of matrices is important in several real life applications. In this sense, the first important step in order to ensure the reliability of the analytical results and to provide the required DLs is to control the spectral interferences described above (see Table 1). In this regard, MS/MS instruments provide a more flexible controlled and efficient removal of the spectral interferences during the analysis of those difficult elements in real samples.

### *4.1. GC-ICP-MS/MS approaches*

Different cell gases are available in ICP-MS/MS applications. Under standard configuration, He, $H_2$, $NH_3$/He or $O_2$ could be selected and introduced in the collision/reaction cell. Even so, the use of $N_2O$ and $CH_3F$ has also been evaluated in recent publications [75]. The main ICP-MS/MS operation modes with their corresponding gases of the recently published GC speciation applications are listed in Table 2.

Oxygen is the most commonly used ICP-MS/MS reaction gas. One of the oxygen atoms is transferred from the $O_2$ molecule by means of reaction with the analyte ion ($M^+$) forming an oxide ion ($MO^+$). Therefore, the analyte ion $M^+$ is mass shifted by 16 amu to a new m/z different from the one of the original interference. Such O-atom transfer mechanism proceeds when the enthalpy of the gas phase reaction is negative ($\Delta H_r < 0$). The reaction of P with oxygen is favoured due to the negative enthalpy of the reaction (−3.17 eV) and the polyatomic ion $^{31}P^{16}O^+$ is efficiently formed. Thus, fixing Q1 at m/z 31 and Q2 at m/z 47 (mass-shift mode) is the main chosen ICP-MS/MS mechanism for phosphorus determination. A similar reaction is produced for sulfur analysis, where the formation of the polyatomic ion $^{32}S^{16}O^+$ also has a negative reaction enthalpy (−0.34 eV). As a result, Q1 is fixed at m/z 32 and Q2 at m/z 48. Therefore, these reactions separate the target analytes from their interferences as a result of their favoured reaction with oxygen compared to the interfered elements (see Fig. 1). Additionally, the two selectivity levels provided by the two used mass analyzers (Q1 and Q2) leads to a significant decrease in the noise and in turn in the DLs.

Nelson et al. [76] described the GC-ICP-MS/MS simultaneous determination of P- and S-containing pesticides in several vegetable based food matrices (baby food purees, fresh vegetables and loose tea) allowing the interference-free measurement of S and P as their respective oxides. The obtained results pointed to the fact that the proposed methodology is particularly sensitive for P giving DLs in the low ppt level that are at least one order of magnitude lower than those obtained previously using other ICP-MS instruments. Unfortunately, due to the system contamination from $H_2S$/Ar gas used for tuning the instrument, the attained DLs for S were similar to those previously reported [56].

Recently, a new method for both total and speciation sulfur quantification in petroleum derivates has been described by Freije-Carrelo et al. [77] The modification of a commercial GC-ICP-MS/MS interface solved the matrix effects originated because of the high-carbon-containing sample matrix and allowed compound-independent quantification for both total and individual sulfur species in gasolines. Within this instrumental design, shown in Fig. 2, the GC inlet can be connected either to a transfer line for total analysis or to a column for speciation analysis. As a result, one single instrumental setup carries out both types of sulfur quantifications. Working under mass-shift configuration, sulfur was monitored as $^{32}S^{16}O^+$ and, the use of GC-ICP-MS/MS provided an interference- and matrix-free sulfur measurement. Such operation mode made possible the use of a higher RF power (1550 W), in contrast to previous GC-ICP-MS applications for S analysis [56,57] where it was mandatory to operate under cold plasma conditions (700 W). Notably, it allowed as well the continuous on-line addition of an optional flow ($Ar{:}O_2$) to help burning the petroleum matrix reducing matrix effects significantly. Carbon-matrix effects correction in the plasma was evaluated by injecting dimethyl sulfide (DMS) gas standard through the transfer line meanwhile increasing flows (from 2 to 5 mL/min) of a $CO_2$/Ar mixture (10:90) were added online through a T connected to the dilution/option port. Both S and C (as $^{29}CO^+$) signals were monitored. The obtained results pointed out that within the tested range (107–268 μg/min C) the occurrence of carbon-matrix effects was negligible since both the RSD of the S areas for individual triplicates at each carbon content and the RSD for all injections were around 1%. The GC-ICP-MS/MS instrumental setup provided detection limits in the very low ppb level, which was more than one order of magnitude lower than those previous published for other selective S detectors [1,76]. The proposed method was successfully applied to total and speciation sulfur analysis of a commercial gasoline sample and validated with a certified reference material (ERM-EF213) gasoline.

The alternative of making use of $H_2$ as cell gas could be advantageous for the analysis of Cl and Si compounds (see Fig. 1). Along

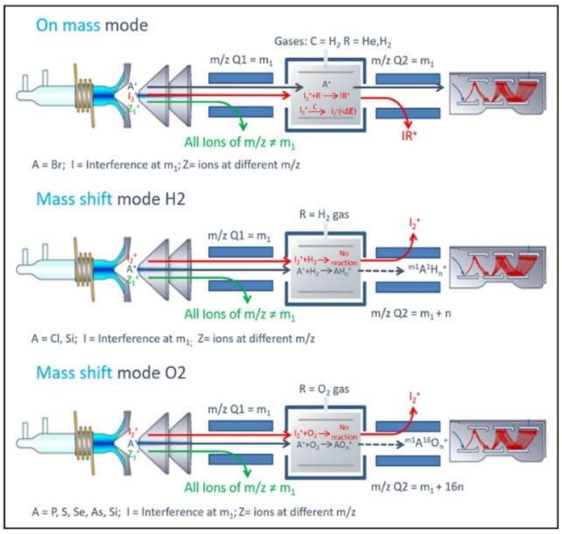


**Fig. 1.** Schematics of the main measurement modes in ICP-MS/MS.

**Table 2**
Compilation of GC-ICP-MS/MS works published in the literature indicating the selected cell gas, acquired ion and ICP-MS/MS operation mode for each application.

| Element | Cell Gas | Acquired Ion (operation mode) | Ref. |
|---|---|---|---|
| P | $O_2$ | $^{31}P^{16}O^+$ (mass-shift) | [76,83] |
| S | $O_2$ | $^{32}S^{16}O^+$ (mass-shift) | [76,77,83] |
| Cl | $H_2$ | $^{35}Cl^1H_2^+$ (mass-shift) | [65,76] |
| Si | $H_2$ | $^{28}Si^+$ (on-mass) | [79,83] |
| Br | He | $^{79}Br^+$,$^{81}Br^+$ (on-mass) | [81], |
|  | $N_2O$ | $^{79}Br^{16}O^+$,$^{81}Br^{16}O^+$ (mass-shift) | [82] |
| As | $O_2$ | $^{75}As^{16}O^+$ (mass-shift) | [83] |
| Ge | $O_2$ | $^{74}Ge^{16}O^+$ (mass-shift) | [83] |

these lines, the abovementioned study of Nelson el al [76]. described the use of $H_2$ as reaction gas to form $^{35}ClH_2^+$ ion (mass-shift mode) to monitor the transition from m/z 35 in Q1 to 37 in Q2 for Cl-containing pesticides determination. The obtained Cl DLs were in the low ppb, better than with single quadrupole ICP-MS with He as collision gas [78]. In this context, Somoano-Blanco et al. [65] compared the performance of GC-NCI-MS, GC-EI-MS, GC-EI-MS/MS, GC-ICP-MS and GC-ICP-MS/MS for the quantification of polychlorinated biphenyls. Interestingly, chlorine quantification by GC-ICP-MS was hampered by the low sensitivity that the method exhibited when He was used as collision gas. However, GC-ICP-MS/MS offered accurate results. Both Cl isotopes were measured in mass-shift mode with $H_2$ as reaction gas ($^{35}Cl \rightarrow {}^{35}Cl^1H_2$ and $^{37}Cl \rightarrow {}^{37}Cl^1H_2$). Nevertheless, the poor signal to noise ratio for the $^{35}Cl \rightarrow {}^{35}Cl^1H_2$ transition forced carrying out the quantification only by using the transition $^{37}Cl \rightarrow {}^{37}Cl^1H_2$.

In addition to mass-shift mode, $H_2$ could also be used as cell gas in on-mass mode, as recently described by Rademacher et al. [79] for Si compounds analysis. The quantification of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) in bream fish samples from the German Environmental Specimen Bank was carried out by GC-ICP-MS/MS. The use of $H_2$ allows the elimination of $^{14}N_2^+$ and $^{12}C^{16}O^+$ interferences on the monitoring $^{28}Si^+$ isotope since Si does not react with $H_2$ molecules while $^{14}N_2^+$ and $^{12}C^{16}O^+$ species are transformed into the corresponding $^{14}N_2H^+$ and $^{12}C^{16}OH^+$ species.

Given the fact that the resolution of quadrupole ICP-MS instruments is limited (1 amu), a possible interference on the monitored mass could be caused by the tailing of adjacent masses with a difference of ±1 amu to that of interest. In the case of Br, this is an important source of error, since both Br isotopes are at 1 amu from one of the major polyatomic ions formed by Ar, $^{40}Ar_2^+$, whose peak tails can contribute to the signal of Br isotopes ($^{79}Br$ and $^{81}Br$). Notably, the MS/MS operation of two mass analyzers (Q1 and Q2) yields extremely high abundance sensitivity ($10^{-10}$), several orders of magnitudes lower than in single Q ($2 \times 10^{-5}$) and even in sector field instruments operated in medium resolution ($3 \times 10^{-7}$) [80], that turned out to be critical for removing such tailing interference. In that way, Zhang et al. [81] optimized the on-mass mode employing He as cell gas for the compound-independent calibration (CIC) determination of PBDEs by GC-ICP-MS/MS. Different

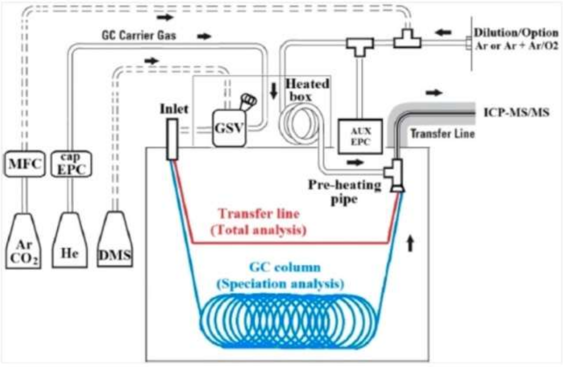


**Fig. 2.** Scheme of the GC-ICP-MS/MS setup for total analysis (transfer line, red line) or speciation analysis (GC column, blue line). Dimethyl sulfide gas standard (DMS) and Ar/$CO_2$ (dotted lines) were only used for the assessment of species-independent responses and matrix effects. [*Reproduced by permission of the American Chemical Society.*].

instrumental parameters were considered in order to evaluate Br independent elemental response. Later, the same research group, evaluated the quantification of PBDEs by GC-ICP-MS/MS using isotope dilution [82]. The use of three reaction gases, $O_2$, $H_2$ and $N_2O$, were tested for Br monitoring. Among them, $N_2O$ offered the highest sensitivity and argon plasma spectral interference could be efficiently corrected by monitoring the $BrO^+$ product (mass-shift) with a different mass/charge ratio from the $HAr_2^+$ interference. This free-interference method was then applied by the authors to the analysis of six PBDEs congeners in fish samples.

Geiger et al. [83] reported the comparison of two ICP-MS Agilent instruments, single Q and MS/MS, for the determination of six low molecular weight gases volatile compounds $AsH_3$, $PH_3$, $H_2S$, COS (carbonyl sulphide), $GeH_4$ and $SiH_4$. These compounds are considered as contaminants in high purity gases employed in petrochemical and semiconductor industries. In all cases the ICP-MS/MS was operated in mass-shift mode. After optimization, $O_2$ was finally selected as reaction gas for the measurement of As, P, S and Ge species, while $H_2$ was used for Si determination.

As can be understood from the aforementioned publications, it is evident that GC-ICP-MS/MS capabilities can be exploited for expanding considerably the application field of GC speciation analysis both in terms of target species and real-life applications.

### *4.2. Analytical characteristics*

The main problems related to the analysis of environmental-, biological- and industrial-related samples are: (i) the large number of potential species to be monitored and quantified; (ii) the low concentration level at which they could be present, most of the cases, in complex sample matrices; and, (iii) the lack of individual stable and commercial analytical standards for all of them. In fact, the GC-ICP-MS/MS applications developed so far have mostly pursued to meet the stringent sensitivity requirements established by legislation bodies whilst at the same time provide compound independent calibration (CIC) that facilitate quantification strategies.

**Detection Limits**. The improved detection limits provided by the GC-ICP-MS/MS coupling is an important advantage over other ICP-MS configurations, as clearly shown in Table 3. In general, significant lower detection limits are reported by using ICP-MS/MS instruments. A clear example is, for example, the P detection limit in the range 0.4–35.9 ng $L^{-1}$ provided by GC-ICP-MS/MS for P-containing pesticides [76] compared with those 1–2 order of magnitude higher (130–300 ng $L^{-1}$) obtained for the determination of P-containing compounds in petroleum products [84] (200–300 ng $L^{-1}$) or organophosophorus flame retardants and plasticizers [55] (130–300 ng $L^{-1}$) using collision cell GC-ICP-MS. Similar observations could be highlighted for the determination of Br-containing compounds. Again, DLs achieved by GC-ICP-MS/MS were 1–2 order of magnitude lower for the determination of PBDEs in fish tissue [81] (0.03–0.56 ng $L^{-1}$) compared to the ones reported in the analysis of PBDEs by GC-ICP-MS in human serum [58] (0.6–3.0 ng $L^{-1}$). However, such DLs evident improvement was not observed for the analysis of Cl-containing compounds. For instance, the reported GC-ICP-MS/MS DLs for the analysis of Cl-pesticides in food (150–510 ng $L^{-1}$) were only 2–3 times lower than those obtained for the determination of trihalomethanes by GC-ICP-MS in water [78] (350 ng $L^{-1}$).

Geiger et al. [83] reported GC-ICP-MS/MS detection limits as low as ~ 2 ppt, ~10 ppt, ~10 ppt, ~10 ppt, ~4 ppt and ~200 ppt for $AsH_3$, $PH_3$, $H_2S$, COS, $GeH_4$ and $SiH_4$, respectively. Interestingly, DLs obtained using the same GC setup but quadrupole ICP-MS for the same analytes were ~6 ppt, ~77 ppt, ~22 ppt, ~21 ppt, ~1 ppt and ~1180 ppt, respectively. Notably, the accurate quantification at ultratrace level of such volatile compounds is crucial for avoiding adverse effects on the manufacturing process in petrochemical and semiconductor industries.

Special attention must be given to instrumental aspects that could diminish the great potential sensitivity of GC-ICP-MS/MS. For instance, as indicated by Nelson el al., [76], in the analysis of S-containing compounds it its crucial to correctly choose the right introduction place of the tuning standard into the GC-ICP-MS/MS system. The introduction of a mixture of 100 ppm $H_2S$

**Table 3**
Detection limits comparison between GC-ICP-MS and GC-ICP-MS/MS in selected publications.

| Element | DL (ng $L^{-1}$) | Mass Analyzer | Analyte//Sample | Ref. |
|---|---|---|---|---|
| P | 200–300 | ICP-MS – collision cell (He) | P-compounds//petroleum – mid oil distillate | [84] |
| | 130–300 | ICP-MS – collision cell (He) | P-compounds//flame retardants and plasticizers | [55] |
| | 0.4–35.9 | ICP-MS/MS – mass-shift ($^{31}P^+ \rightarrow ^{31}P^{16}O^+$) | P-pesticides//vegetable based foods | [76] |
| S | 8000–33000 | SF-ICP-MS – resolution 3000 | volatile S compounds//human breath | [51] |
| | 3000–11000 | ICP-MS – collision cell (He) | S-compounds//petroleum – mid oil distillate | [84] |
| | 70–9500 | ICP-MS/MS – mass-shift ($^{32}S^+ \rightarrow ^{32}S^{16}O^+$) | S-pesticides//vegetable based foods | [76] |
| | 380–420 | ICP-MS/MS – mass-shift ($^{32}S^+ \rightarrow ^{32}S^{16}O^+$) | sulfur compounds//petroleum - gasoline | [77] |
| Cl | 1000–3000 | ICP-MS – collision cell (He) | Cl-compounds//petroleum – mid oil distillate | [84] |
| | 350 | ICP-MS – collision cell (He) | trihalomethanes//drinking water | [78] |
| | 150–510 | ICP-MS/MS – mass-shift ($^{35}Cl^+ \rightarrow ^{35}Cl^1H_2^+$) | Cl-pesticides//vegetable based foods | [76] |
| Si | 24–136 | ICP-MS – reaction cell ($H_2$) | siloxanes//petroleum – light products | [54] |
| | 3.9–30.9 | ICP-MS/MS – on-mass ($^{28}Si^+ \rightarrow ^{28}Si^+$) | cyclotetrasiloxane//bream fish | [79] |
| Br | 3–9 | ICP-MS | PBDEs//natural water | [60] |
| | 1.6–3.9 | ICP-MS | PBDEs//human serum | [58] |
| | 0.03–0.56 | ICP-MS/MS – on-mass ($^{79,81}Br^+ \rightarrow ^{79,81}Br^+$) | PBDEs//SRM- 1947 (fish tissue) | [81] |
| | 0.03–0.09 | ICP-MS/MS – mass-shift ($^{79,81}Br^+ \rightarrow ^{79,81}Br^{16}O^+$) | PBDEs//fish tissue | [82] |

in Ar gas through the gas chromatograph EPC modules and carrier gas lines resulted in the contamination of the flow system. Authors indicted that the $H_2S$ previously adsorbed during the tuning process was continuously released during the entire duration of the experiments. The authors indicated that, one solution might be to introduce the $H_2S/Ar$ mixture as close as possible to the ICP torch. Another example is the possible continuous release of Si from different GC and/or ICP parts. Chainet et al. [54] indicated that silicon release from the ICP torch and the injector, made of quartz, increases the background noise limiting considerably the detection limits achievable for silicon compounds. However, when the possible silicon contamination from the GC parts (silicone-based injection port, septa or chromatographic column) is minimized by replacement by silicon-free materials, lower DL for Si determination could be achieved. In this way, DLs of 3.1–16.8 ng $g^{-1}$, 2.9–12.3 ng $g^{-1}$, and 3.9–30.9 ng $g^{-1}$ for D4, D5 and D6, respectively enabled the determination of these three siloxanes at trace level in fish samples from the German Environmental Specimen Bank collected between years 1995 and 2017 [79].

Chlorine could be measured according to the $Cl^+ + H_2 \rightarrow ClH_2^+$ reaction, however it is important to highlight that good ICP-MS/MS resolution is required for avoiding the overlap of the $^{35}Cl^1H_2^+$ with $^{37}Cl^+$ ions. However, the high signal to noise ratio results in poor precision results in comparison, for instance, with GC-EI-MS/MS as reported by Somoano-Blanco et al. [65].

**Compound-Independent Calibration**. Under certain conditions a complete compound-independent ionization can be obtained when ICP is used as ion source and, as a result, instrumental sensitivity is made independent from the chemical structure of the analyzed compounds. This allows compound-independent calibration for quantifying organic compounds with various chemical structures present in the sample using a single generic compound. Compound-independent calibration is an attractive alternative since eliminates the need for specific and often expensive, not stable or not commercially available standards, which are still rare for many applications. This is particularly interesting nowadays that the analyte list is continuously growing. Finally, it speeds up analyses as well by reducing standards preparation and analysis time.

It is clear that the more efficient the interference removal, the better the CIC attainable. This is particularly true for the analysis of real samples where the coelution with matrix components can affect the interference rate (especially polyatomics) disturbing the element response factor and so, worsening the CIC capabilities. In fact, the better interference correction provided by the MS/MS configuration opens the door to operate the plasma in more aggressive conditions that compensate better matrix effects. For example, Freije-Carrelo et al. [77] resorted to hot plasma (1550W) and the addition of a continuous flow of oxygen to the ICP plasma to deal better with the high matrix during the CIC analysis of S-containing compounds in petroleum derivates. The optimization of the GC conditions, the assessment of the quantitative transmission within the injector and column for all S-containing compounds, and the evaluation of the lack of carbon matrix effects in sulfur detection provided the adequate conditions for CIC quantification. In this sense, the authors observed that sulfur response could be directly influenced by the temperature change along the GC gradient when the Ar carrier is previously heated by means of a pre-heating pipe located inside the GC oven. In order to evaluate such influence, it was shown that moving the pipe outside the oven (see Fig. 2) and placing it in a gas-sampling valve (GSV) box, the temperature of the Ar carrier can be kept heated at constant 200°C temperature. Such instrumental approach provided sulfur stable areas (RSD = 3%) along the GC gradient, and therefore S response, with temperature. In contrast, when the pre-heating pipe was located inside the oven, sulfur areas increased significantly (RSD = 55%). It is therefore critical to carefully optimize the instrumental conditions to achieve CIC quantification. In this way, dibuthylsulfide was selected as sulfur generic standard for the quantification of ethyl phenyl sulphide, benzothiophene, methylbenzothiophenes and dibenzothiophene. As already indicated in a previous section, the analysis of the certified reference material (ERM-EF213) gasoline with the proposed method reported results in good agreement with the certified values.

Compound-independent calibration of PBDEs using GC-ICP-MS/MS has also been investigated [81]. About 11 PBDEs were selected and the effects of injection conditions, carrier gas, flow rate and matrix effects on the Br response were evaluated. Under optimized chromatographic conditions Br response in the tri-to hepta-brominated PBDEs was found to be independent of the structure of the analyte, and the reported detection limits, ranging from 0.03 to 0.56 ng $g^{-1}$, indicates the advantages of the CIC method for the measurement of PBDE.

In spite of its great quantitative potential, we should be aware that in order to carry out CIC other requirements besides species-independent ionization in the ICP plasma must be fulfilled. In this sense, quantitative transmission of the target compounds and the generic standards must be assure along the GC process and, therefore, adsorptions and degradations (especially in the injector) should be avoided or al least kept under control [76,77].

**Table 4**
Detection limits comparison, in selected publications, when element selective detectors were employed after gas chromatography separation.

| Element | DL (ng $L^{-1}$) | Element Selective Detector | Ref. |
|---|---|---|---|
| P | 280 | GC-FPD | [87] |
| S | 100000–220000 | GC-SCD | [88] |
| | 4280 | GC-FPD | [87] |
| | 1500 | GC-AED | [89] |
| | 1000 | GC-combustion-MS | [90] |
| Cl | 9500 | GC-AED | [89] |
| | 20000–4700000 | GC-ECD | [91] |
| Br | 11000 | GC-AED | [89] |
| | 6–73 | GC-ECD | [92] |

### *4.3. Comparison with other techniques*

In order to describe better the potential of ICP-MS/MS as detector in GC we should compare it not only with traditional ICP-MS instruments (already done in previous sections) but also with other established GC detectors.

GC-MS with electron ionization has become one of the most widely used detectors in research and routine laboratories all over the world. Its peculiarity lies in the fact that it can provide universal detection and structural identification as well as species-specific detection when operated in scan and SIM modes, respectively. However, such selective quantitative mode is limited to screen for specific families (i.e. element-containing compounds) in complex samples because of important isobaric overlaps and matrix effects. The specificity level provided by GC-EI-MS/MS instruments overcomes such limitations leading to much lower DL and reliable quantifications in real samples. However, GC-ICP-MS/MS is an interesting alternative for selective and sensitive quantifications of such element-containing compounds in complex samples because it offers the possibility for CIC as described in the previous section. As an example, in the specific case of organophosphorus pesticides, instrumental detection limits attainable using GC-ICP-MS/MS [76] can be up to one order of magnitude lower than those obtained using GC-EI-MS/MS [85,86], depending on the specific compound. Moreover, GC-ICP-MS/MS does not require retention time-based acquisition methods (SIM windows) or the selection of the adequate parent ion fragment transitions when developing MRM methods.

Element selective detectors such as AED, ECD, NPD, FPD and SCD offer sensitive detection of heteroatom-containing compounds. In this sense, a brief comparison of DLs for the determination of P- [87], S- [87–90], Cl- [89,91] and Br-containing [89,92] compounds using different element selective detectors after GC separation is given in Table 4. As it can be observed, although such reported DLs are all at the low trace level, they are at least one order of magnitude higher than those attainable using GC-ICP-MS/MS (Table 3). However, such spectroscopic-based detectors suffer from significant quenching effects when analysing complex unresolved samples [1]. Moreover, such matrix effects limit the application of CIC in real samples can be made if specific standards are available.

Recently, Freije-Carrelo et al. [90] introduced a single detection approach that enabled sensitive and compound-independent quantification of N-, S– C- and H-containing compounds in complex samples, while maintaining the structural elucidate power of mass spectrometry. It consists of the online conversion in a combustion furnace (ca. 1000°C) of all the eluting compounds from the GC into the same volatile species (NO, $SO_x$, $CO_2$ and $H_2O$) before their entrance to the EI-MS detector. Unfortunately, such innovative approach only enables detection of the specific elements mentioned above. Moreover, although DLs are in the very low ppb range they cannot compete with those of GC-ICP-MS/MS.

## 5. Outlook

As clearly demonstrated in this review, the introduction of ICP-MS/MS has meant a great improvement in the performance of ICP-

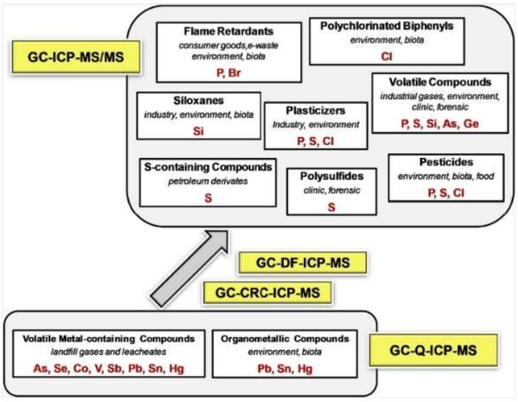


**Fig. 3.** Traditional and new application fields with the corresponding target analytes of GC-ICP-MS and GC-ICP-MS/MS speciation analysis.

MS instruments as specific detectors in GC speciation analysis. The possibility to efficiently overcome spectral interferences for hampered elements such as P, S, Si, Cl and Br is a clear advantage, and open the door to the analysis a wide range of industrial-, environmental- and biological-related analytes.

Fig. 3 clearly summarizes the idea conveyed throughout this article. A clear trend could be established from traditional GC-ICP-MS (Q, CRC, DF) to GC-ICP-MS/MS instruments when the determination of specific analytes in different sample matrices is compared. Despite the advances in instrumental techniques, the GC-ICP-MS scope seems to be restricted. Single quadrupole ICP-MS instruments are robust and reliable enough for the determination of traditional organometallic compounds and volatile metal-containing compounds in several environmental-, biota- and food-related samples. However, nowadays there is a growing trend towards quantifying species containing elements badly detectable so far by traditional ICP-MS instruments (i.e. P, S, Si, Cl, Br) as a response to the increasing concern about their presence, already at ultratrace levels, in a considerable amount of real-life samples. Currently, special attention should be paid to speciation studies, for instance, in petroleum derivates, industrial products, consumer goods, environment, biota or food samples in order to assess more reliably their presence, transformation and effect in nature. The GC-ICP-MS/MS advent is crucial for this analytical challenge.

Some GC-ICP-MS/MS based methods have been already reported obtaining significantly lower detection limits and better tolerance to matrix effects compared to using GC-ICP-MS facilitating the development of quantitative values without the need for specific standards, which are scarce and unstable in most cases. These results push forward to a deeper optimization of the GC-ICP-MS/MS approach, enabling analysis of more complex matrices and elements not so well studied until now because of the limitations commented in this review. In fact, ICP-MS/MS technology as a GC detector is still clearly in its early stages but although the number of papers based on GC-ICP-MS/MS is still scarce, they have already shown its enormous potential. The potential of ICP-MS/MS as a GC detector should lead the way to future exciting applications with stringent analytical requirements in many kinds of samples (see Fig. 3).

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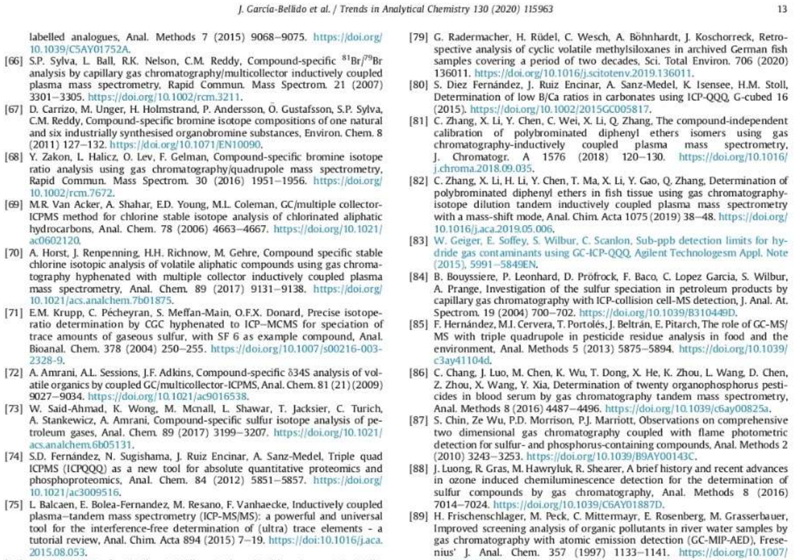

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Artículo científico II.

**Título**: *Assessing GC-ICP-MS/MS determination of chlorine-containing compounds in plastic-based pyrolysis oil samples*

**Autores**: Javier García-Bellido, Montserrat Redondo-Velasco, Mariosimone Zoccali, Luigi Mondello, Pierre Giusti, Mariella Moldovan and Jorge Ruiz Encinar

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# Assessing GC-ICP-MS/MS determination of chlorine-containing compounds in plastic-based pyrolysis oil samples

**Javier García-Bellido,[a] Montserrat Redondo-Velasco,[a] Mariosimone Zoccali,[b] Luigi Mondello,[c,d] Pierre Giusti,[e] Mariella Moldovan,[a*] and Jorge Ruiz Encinar[a]**

[a] Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain

[b] Department of Mathematical and Computer Science, Physical Sciences and Earth Sciences, University of Messina, Messina, Italy

[c] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[d] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[e] TotalEnergies, TotalEnergies Research& Technology Gonfreville, 76700 Harfleur, France

* Mariella Moldovan: moldovanmariella@uniovi.es

## ABSTRACT

Pyrolysis technology processes are a promising alternative for converting plastic waste into oil, which can later be processed to obtain chemicals or fuel-like products. The presence of additives, organic waste or metals introduce unwanted heteroatoms, such as Si, P, S, Cl and Br, in the obtained plastic-based pyrolysis oil. Such impurities, even at ultra-trace levels, can negatively affect the thermal processing, as well as degrading the quality of the pyrolysis oils and derivatives produced, which would also affect their subsequent industrial refinement. Thus, it is critical to determine both total concentration and speciation analysis of the individual species of these elements, but without specific standards as most of them are not available.

A GC-ICP-MS/MS based method for the analysis of chlorine in real pyrolysis plastics oil samples is presented. The speciation and total quantification analysis of real samples was accomplished using generic Cl-containing standards after development and optimization of a compound-independent quantification methodology. Quantitative speciation analysis was carried out using a regular chromatographic column and an internal standard spiked in the samples. In the case of total chloride content, the analysis was performed after changing the column by an inert transfer line and resorting to external calibration with a chlorine-containing generic standard. Sensitivity and detection limit were also evaluated with the introduction of different gases (Ar:$N_2$, Ar:He, Ar:Ne and Ar:$CO_2$) to the plasma. Detection limit as low as 1 ng/g was obtained under optimal conditions. For validation of both total and speciation methodologies a certified reference material was used. Several real pyrolysis oils obtained from diverse plastic sources were analyzed with the methodology proposed.

**Keywords**

Plastic pyrolysis oil; Chlorine, GC-ICP-MS/MS, Flow Injection Analysis, Speciation Analysis.

## 1. INTRODUCTION

Nowadays, there is an increasing interest in the development of new technological solutions for plastic waste management, in order to reduce environmental pollution and to meet circular economy strategies. Plastic waste is generally classified as: (i) post-industrial, clean and with a well-defined composition; and, (ii) post-consumer, dirty and usually contaminated with glass, metals, food residues, labels, adhesives, etc.[1] Low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP) and polystyrene (PS), and a smaller fraction of polyethylene terephthalate (PET), polyvinyl chloride (PVC) or poly(methyl methacrylate) (PMMA) are reported as the most common constituents of post-consumer plastic waste.

Chemical recycling converts plastic waste into valuable feedstocks to obtain new chemicals or fuel-like products.[2] The most frequently applied chemical recycling process is pyrolysis where high molecular polymer particles are degraded under anaerobic conditions at relatively high temperature. The primary product of the pyrolysis is plastic pyrolysis oil, which is a very complex hydrocarbon matrix whose composition depends on the type of feedstock material used and pyrolysis parameters, such as temperature, type of reactor, residence time and type of catalyst.[3] The further use of plastic pyrolysis oil will depend on the presence of contaminants arising from the presence of unwanted elements (metals, N, S, O, Si, and halogens) in the plastic waste.

Chlorine is an undesired component in the plastic pyrolysis oil.[4] Cl-containing compounds can poison the catalysts both during the pyrolysis and in further application of the obtained plastic pyrolysis oil, and cause significant corrosion during storage, transportation and/or combustion.[5] A maximum limit of 10 ppm of chlorine in plastic pyrolysis oil intended for petrochemical feedstock has been established[6], although recently an even lower value of 3 ppm in industrial steam cracker feedstocks has been reported.[7] Although prior pyrolysis, plastic waste undergoes dechlorination pre-treatment steps, chlorine concentrations within the pyrolysis oils can be still relevant. Therefore, attention must be given to the presence of chorine, and Cl-compounds, in plastic pyrolysis oil before further applications.

Various methods have been reported for the determination of chlorine content in plastic pyrolysis oil: chlorine trace analyzer (TCL-100),[8] total titration according to ASTM 2361m method,[9] acid-base titration according to ASTM D4208-8 3 method,[10] EPA 5050 method[11,12] combustion ion chromatography[13] and fluorescent x-ray (XRF).[5] However, these methods determine total chlorine concentration rather than identifying individual Cl-containing compounds. Next to the mentioned lack of specific chlorine species information, the limit of detection (LOD) is relatively high. Kusenberg et al.[7] indicated a literature EDXRF chlorine LOD in the low ppm level, whereas the combustion ion chromatography, the most widely applied chlorine analysis technique has a LOD of 0.02 µg/mL, this is, in the low ppb level.[14]

GC-AED[15] and GC-MS[16] have been used to evaluate Cl-compounds formation during of the pyrolysis of PVC-containing mixed plastics. In both studies, authors were able to identify few chlorinated hydrocarbons indicating that their distribution strongly depends on the plastic feedstock. Although this approach is interesting, there is still a lack of knowledge regarding the composition and concentration of Cl-compounds in plastic pyrolysis oil.

The determination of chlorine by ICP-MS is challenging because of its high first ionization potential of 12.967 eV and the presence of relevant spectral interferences, such as for instance $^{18}O^{16}O^{1}H$, $^{17}O^{18}O$, $^{17}O^{17}O^{1}H$ or $^{34}S^{1}H$, on $^{35}Cl$ isotope, the most abundant chlorine isotope.[17] To overcome this limitation, the use of ICP-MS/MS technology is an effective alternative. Such system is working as a double filter by setting in each of the two quadrupoles different m/z. The introduction of a collision/reaction cell (CRC) between both quadrupoles allows interference removal.[18] For chlorine ICP-MS/MS detection, mass shift mode using $H_2$ as CRC reaction gas has been revealed to be the best approach.[19,20] Setting Q1 at m/z 35, only the analyte ion and the interfering ions with the same m/z enter into the CRC. The introduction of $H_2$ converts the $^{35}Cl^+$ analyte ion into the interference free $^{35}Cl^1H_2^+$ ion that will be pass to the detector as Q2 is set at m/z 37.[21]

Complex sample matrices, with high carbon content, can significantly hamper chlorine determination by ICP-MS/MS. Recently, direct total analysis of Cl in petroleum crude samples was investigated[20] and, good agreement was observed when compared to previously instrumental neutron activation analysis.

While the combination of GC with ICP-MS/MS improves selectivity and detection limit, chlorine speciation analysis in plastic pyrolysis oils is hindered by the variety of occurring Cl-compounds. Compound-independent calibration enables the application of a generic standard to quantify compounds, and thus can reduce costs and time-consuming procedures.

The main objective of this study is to discuss the use of GC-ICP-MS/MS as single instrumental setup for total and speciation analysis of chlorine in plastic-based pyrolysis oil using a generic chlorine standard for quantification purposes. The proposed methodology was successfully applied to the analysis of real plastic pyrolysis oil samples. GC×GC-MS analysis helped on Cl-compounds identification.

## 2. MATERIAL AND METHODS

### 2.1. Materials

1,2-dichloroethane (99%), 5-chloro-2-pentanone (99.1%), 1-chloroheptane (99.9%), benzylchloride (99.7%), 3-chlorophenol (99.2%), dichlorvos (99%), 2,3-dichlorobiphenyl (99%), methyl (2,4,5-trichlorophenoxy) acetate (98.7%), dichlofenthion (99%), fenchlorphos (99%), chlorpyrifos (99%), carbophenothion (99%) were purchased from Sigma Aldrich.. The pesticide certified reference material (Organophosphorous Pesticides Mix A) was purchased from Sigma Aldrich. The gas mixtures $CO_2$/Ar (10:90), $N_2$/Ar (10:90), He/Ar (10:90) and Ne/Ar (10:90) were supplied by Air Liquide.

### 2.2. Instrumentation

A gas chromatograph (7890B, Agilent Technologies) coupled to an ICP-MS/MS instrument (8900, Agilent Technologies) through the commercial heated GC-ICP-MS interface was used (Agilent Technologies). As illustrated in Figure S-1 (Supplementary Information), total and speciation chlorine analysis can be performed on a single setup. Speciation analysis was carried out using an RXi-1ms column (30 m x 0.32 mm i.d. x 1 µm; Restek) meanwhile a transfer line (deactivated fused silica; 5 m x 0.32 mm i.d.; Agilent Technologies) was installed for total chlorine analysis. A split/splitless standard injector was employed, and samples were injected manually. A mixture of Ar:$O_2$ (80:20; v:v) was used as option gas. For sensitivity optimization, different mixtures of gases were introduced online with dilution gas with a tee connection and a mass flow controller PR4000B (MKs Instruments). Table S-1, from Supplementary Information, summarizes the GC-ICP-MS/MS instrumental parameters.

The identification of the Cl-compounds was performed using a 2D-GC system consisting of two gas chromatographs, Shimadzu GC-2010, connected on line and a QP2020 single quadrupole mass spectrometry as detector. The gradient used was 35ºC and a ramp of 5ºC/min up to 380 ºC (for both chromatographic ovens). A split/splitless injector, held at a temperature of 280ºC, was equipped into the first GC system, and a split of 1:200 was used injecting 1 μL of sample. The capillary column used were SLB-5MS (30 m × 0.25 mm ID × 0.25 μm) and SLB-35 (1m × 0.1 mm ID × 0.1 μm) for $^1$D and $^2$D, respectively. The modulation was performed in an uncoated capillary column loop (1.5 m × 0.18 mm ID). Constant linear velocities of about 20.4, 46.6 and 235.1 cm/min were used during the analysis, being the flow of approx. 1.4 mL/min using He as carrier gas. A unit ZX2 (Zoex Corporation) was used as a modulation system by using a liquid nitrogen free chiller. This modulator consists of a vertical cold jet, operating continuously during the analysis while every modulation period, 4.0 s, a hot pulse of (280 ºC) was applied during 0.3 s to release the compounds into the $^2$D.

### 2.3. Plastic Pyrolysis Oil Samples

Plastic pyrolysis oil samples were provided by TOTAL TRTF (Total Research and Technology, Feluy, Belgium). Total chlorine concentration, determined by combustion ion chromatography, was provided as reference value.

Samples PO-1 and PO-2 were obtained from a mixture of plastic material (99 wt %) together with metals and fibrous (1 wt %) subjected to a pyrolysis process at 410 ºC under an inert atmosphere without any additive additions. The plastic material consisted of PE (84 wt %) and PP (11 wt %) and in less quantity of PS (1 wt %), PVC (1 wt %), and PET (2 wt %).[22] Due to their solid appearance, samples PO-1 and PO-2 were first heated up to 60 ºC, and then diluted in cyclohexane.

Then, in order to separate the insoluble fraction, samples were centrifuged at 300 G during 10 minutes at room temperature, and the liquid phase was conveniently diluted for analysis.

Samples PO-3 and PO-4 were directly diluted in hexane. Figure S-2 shows the color and appearance of the analyzed samples.

## 3. RESULTS AND DISCUSSION

### 3.1. Assessment of Chlorine Species Independent Response

The increasing concern on the fate of chlorine during the pyrolysis of plastic waste results in a need for reliable quantification analytical procedures. For a complex sample matrix, such as the plastic-based pyrolysis oil, the main drawback is the variable number of unknown Cl-compounds present in a wide range of concentrations. Under these conditions, classic quantification approaches result complicated because of the multiple variety of target analytes and the difficulty in assuring individual standards available for each of them. As a result, compound-independent calibration would be the most appropriate solution allowing the use of a generic Cl-containing standard to quantify other Cl-compounds present in the sample, when the selective detector provides an identical response regardless of its chemical structure.

The independent response of the used GC-ICP-MS/MS setup was evaluated by the analysis of a mixture of twelve different chlorine-compounds covering different relevant chlorine substance groups. Each Cl-compound had a concentration of approx. 15 µg Cl /g and the mixture was injected into the GC with a split 1/10. The complete chromatographic separation of the Cl-containing compounds is shown in Figure 1.

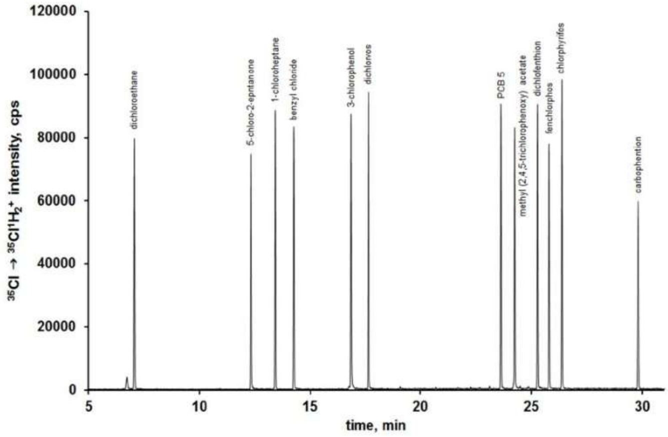

**Figure 1**. GC-ICP-MS/MS chromatogram of the twelve Cl-containing compounds mixture.

The equimolarity of the system was evaluated by computing all Cl-compounds response factor (m/z 37 area vs concentration of Cl) and by calculating the relative standard deviation of the values. Dichlorvos was used as internal standard for the quantification of the remaining Cl-compounds. As summarized in Table S-2, the calculated response factors (area Cl / concentration of Cl) obtained for five replicates is around 6%, and recoveries were in the range of 89-110 % for all Cl-compounds indicating that the proposed instrumental setup is offering good equimolarity, and proving that a single generic chlorine standard can be used for compound independent quantification purposes.

The limit of detection (LOD) of the proposed GC-ICP-MS/MS method, based on a signal-to-noise ratio (S/N) of 3, was calculated to be about 1 ng Cl /g for all studied chlorine compounds. To the best of our knowledge, this is the lowest LOD reported in the literature for chlorine selective detection, much lower than the ones reported for the Cl determination by ICP-MS/MS in crude oils, 12 ng Cl /g,[20] or complex hydrocarbon matrices, 30 ng Cl /g.[23]

### 3.2. Assessment of Matrix Effect on Chlorine Detection

In the literature, different approaches have been reported to enhance the sensitivity of high energy ionization elements. Plasma properties, such as temperature or electron density, can be modified when a gas different to argon is added to the central channel or auxiliary gas to the plasma. For GC-ICP-MS analysis, Vonderheide et. al claims that the addition of 60 mL/min of $N_2$ can improve more than one order of magnitude the ionization of P,[24] meanwhile Pröfock et al. outlines that the addition of 20 mL/min of He increases the ionization of elements such P, S, Cl, Br and I.[25]

In an attempt to enhance chlorine ICP-MS/MS detection, four different gases ($N_2$, He, Ne and $CO_2$) were introduced continuously through a T connection (see Figure S-1) with the dilution gas. All gases were introduced into the setup as 10:90 (v/v) in Ar mixtures, and in order to maintain the additional flow added at the end of the column (0.4 mL/min) the dilution gas was decreased according the increase of auxiliary gas. The evaluated flow ranged between 0 and 150 mL/min. A mixture of six Cl-compounds (1,2-dichloroethane, 5-chloro-2-pentanone, 1-chloroheptane, benzylchloride, 3-chlorophenol and dichlorvos) at a concentration of 300 µg Cl /g each was injected through the chromatographic column. Benzylchloride was used as quantification internal standard of the other, so that the calculated recoveries could be compared in order to evaluate chlorine specific detection. As shown in Figure S-3, only a slightly increase was observed when adding $N_2$ to the plasma, although this is not a statistical difference given the standard deviation (1SD). Thus, the presence of chlorine-free high-carbon content compounds should present no problem in the determination of the chlorine target analytes, and the introduction of additional gases for Cl ionization purpose is not required. This is in agreement with the GC-ICP-MS/MS working conditions for the measurement of sulfur-containing compounds in petroleum derivates.[26]

### 3.3. Validation of the Methodology

Due to the lack of a certified reference material (CRM) for chlorine in plastic pyrolysis oil, the proposed methodology was validated by the analysis of the Organophosphorous Pesticide Mix A material, a mixture of eight pesticides in hexane:acetone (9:1). Within the mixture, the presence of four pesticides (dichlorvos, fenchlorphos, chlorpyrifos and tokuthion) that contain chlorine in their molecular structure, allows validation of the proposed methodology for both total and speciation chlorines analysis. Certified individual compound concentration within the mixture listed in the certificate of analysis was about 2000 µg/mL, and chlorine concentration was calculated considering the number of chlorine atoms in the molecule structure.

For speciation chlorine GC-ICP-MS/MS analysis, using the GC column, 5-chloro-2-pentanone was added to the pesticides mix as internal chlorine standard prior dilution. The obtained chromatogram is shown in Figure S-4, and the quantitative results for the present Cl-containing pesticides are summarized in Table 1. Total chlorine content, determined by GC-ICP-MS/MS speciation analysis, was calculated as sum of areas of all individual chlorine peaks.

In total chlorine GC-ICP-MS/MS analysis, using a 5m x 0.32 mm i.d. deactivated fused silica transfer line, all Cl-compounds eluted in a single peak without chromatographic separation. Quantification was carried out by building an external calibration curve using flow injections analysis (FIA) of increasing 5-chloro-2-pentanone concentrations as generic chlorine standard. A 1:10 split was selected in order to avoid excessive carbon loading. Figure S-5a displays the FIAgram for total chlorine analysis by external calibration. This method shows a clear linear response over a concentration range up to 100 µg Cl/g, this is more than three orders of magnitude, as displayed in Figure S-5b. An absolute detection limit of 20 ng Cl/g was achieved for the analysis of the total chlorine content. The obtained quantitative results are presented in Table 1.

**Table 1.** Organophosphorous Pesticide Mix A certified concentrations. Certified Cl concentration was obtained from the compound concentration and the number of Cl atoms within the molecule. Found values for GC-ICP-MS/MS total and speciation analysis are also shown. All concentrations are expressed in µg/mL. Standard deviation was expanded at 95% of confidence level

| | **Certified compound concentration** | **Certified Cl concentration** | **Found Cl concentration** |
|---|---|---|---|
| Dichlorvos | 2004 ± 64 | 643 ± 21 | 675 ± 34 |
| Mocap | 2001 ± 27 | --- | --- |
| Disulfoton | 2000 ± 30 | --- | --- |
| Methyl Parathion | 2000 ± 34 | --- | --- |
| Fenchlorphos | 2000 ± 79 | 662 ± 26 | 709 ± 63 |
| Clorphyrifos | 2000 ± 24 | 607 ± 7.3 | 595 ± 41 |
| Tokuthion | 1999 ± 35 | 411 ± 7.2 | 401 ± 47 |
| Guthion | 2000 ± 66 | --- | --- |
| Total Cl certified concentration | --- | 2322 ± 35 | --- |
| Total Cl found concentration<br>GC-ICP-MS/MS<br>Speciation (sum of peaks) | --- | --- | 2381 ± 94 |
| Total Cl found concentration<br>GC-ICP-MS/MS<br>Total (FIA) | --- | --- | 2260 ± 48 |

The correlation between certified and found chlorine concentrations offers powerful evidence for reliable quantification using a generic chlorine standard of Cl-compounds by the proposed GC-ICP-MS/MS setup.

### 3.4. Total Chlorine Analysis in Plastic Pyrolysis Oil by GC-ICP-MS/MS

Four plastic pyrolysis oils were examined for their total chlorine and Cl-containing compounds content. Total chlorine analysis was carried out by introducing the sample into the GC-ICP-

MS/MS setup through the transfer line directly connected to the GC inlet. A Cl-containing compound was used as generic standard, and flow injections (FIA) of increasing concentration of the generic chlorine standard allowed building up the corresponding external calibration curve.

Figure 2 displays a FIAgram-based external calibration along with the signal of one of the analyzed plastic pyrolysis oil samples. The external calibration curve, constructed within the concentration range 0.2-10 µg/g chlorine using 5-chloro-2-pentanone chlorine as generic standard, presented a linear behavior ($R^2 = 0.9995$). For the pyrolysis oil sample, all chlorine compounds elute in a single peak since there is no chromatographic separation within the transfer line. Table 2 presents the obtained total chlorine concentration of the studied plastic pyrolysis oil samples compared with the reference concentrations achieved by combustion ion chromatography. As it could be observed, the found recoveries, from 82.0 to 115.1 %, show good agreement with the reference chlorine quantification method, which validates externally our proposed method.

It is important to highlight the significant reduction of the required analysis time, about 1 minute for the proposed GC-ICP-MS/MS method, compared to the more than 15 minutes needed when combustion ion chromatography is used to determine the chlorine content.[27] Such analytical feature has the advantage that the proposed GC-ICP-MS/MS can be easily used for the fast determination of the total chlorine concentration in a sample of interest.

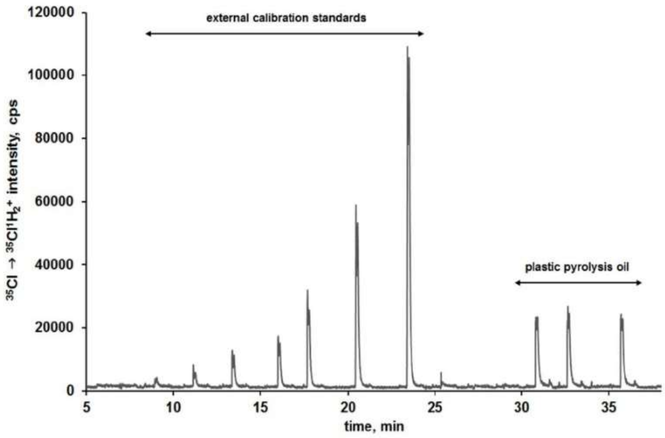

**Figure 2**. FIAgram of the total chlorine analysis of sample PO-3 plastic pyrolysis oil by GC-ICP-MS/MS. The external standard calibration curve was prepared from a concentration of 0.2 to 9.5 µg/g of 5-chloro-2-pentanone as chlorine generic standard.

**Table 2**. Quantitative results for total chlorine content in real plastic-based pyrolysis oil samples. Concentration is expressed as ppm (µg Cl / g plastic-based pyrolysis oil). Speciation analysis result is obtained as sum of all the compounds. Uncertainty was expanded to 95% confidence level (n=3).

| | Combustion Ion Chromatography Reference Value | GC-ICP-MS/MS Total Analysis | GC-ICP-MS/MS Speciation Analysis |
|---|---|---|---|
| Sample PO-1 | 262 ± 26 | 246 ± 13 | 238 ± 23 |
| Sample PO-2 | 233 ± 23 | 191 ± 12 | 182 ± 28 |
| Sample PO-3 | 560 ± 56 | 550 ± 49 | 522 ± 21 |
| Sample PO-4 | 497 ± 50 | 572 ± 27 | 452 ± 39 |

### 3.5. Speciation Chlorine Analysis in Plastic Pyrolysis Oil by GC-ICP-MS/MS

Although for dechlorination purposes the total chlorine plastic pyrolysis oil concentration is a key parameter, detailed information on the present chlorine species could be crucial for the enhancement of the dechlorination yield. Therefore, speciation chlorine analysis contributes to increasing that knowledge.

Samples PO-1 and PO-2 were diluted 1:10 in cyclohexane and spiked with 5-chloro-2-pentanone as internal standard; meanwhile, fenchlorphos was choose as internal standard for samples PO-3 and PO-4 which were diluted 1:20 in hexane. Both internal standards were chosen so, that they did not co-elute with any of the chlorine compounds present in the samples.

Speciation chlorine analysis was carried out by replacing the transfer line with the GC column. The added internal standard to each plastic pyrolysis oil sample, was used to compute the Cl response factor employed for quantification of the Cl species present. Figure 3 shows two of the obtained chromatograms for plastic pyrolysis oils.

Total chlorine contents determined by speciation analysis, calculated using the area sum of all the chlorine peaks obtained in the chromatogram, are given in Table 2. These results were found to be statistically indistinguishable from those determined in the direct total flow-injection analyses, for both the four analyzed samples validating internally our results, and displaying once more the negligible influence of the matrix in the direct analysis of total Cl contents. Detailed information about the found individual concentration of each detected Cl-compound peak is given in Tables S-4 to S-7.

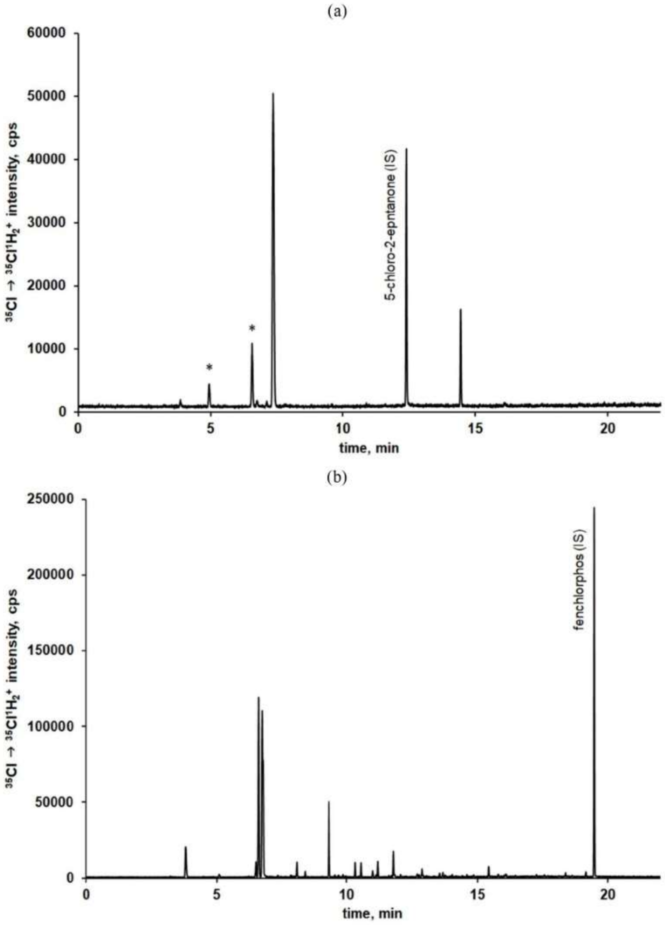

**Figure 3.** GC-ICP-MS/MS chromatogram of plastic pyrolysis oils: (a) sample PO-1 using 5-chloro-2-pentanone as internal standard; and, (b) sample PO-3 using fenchlorphos as internal standard. Peaks marked with (*) indicate solvent contamination.

The different observed pyrolysis oil Cl-compounds chromatographic profiles, both from the point of view of the number of compounds and their concentration, can be supported by the fact that both plastic pyrolysis oils have a different feedstock source and pyrolysis oil production. In the literature, it has been already reported that chlorine-compounds are mainly generated during the pyrolysis process depending on the initial amount of formed HCl and/or $Cl_2$ from the original plastic waste material.[28]

### 3.6. GC × GC-MS Analysis

In order to provide a better understanding of the samples analysed, an identification of compounds detected by GC-ICP-MS/MS would be interesting. Of course, the destructive technique GC-ICP-MS/MS cannot provide any molecular information, and only the retention time can be used as an identification parameter. However, given the unknown composition of the samples, the option of comparing the retention time with pure standards would be highly expensive and troublesome. As an alternative, the analysis by GC-MS was proposed. Nevertheless, the complexity of the samples, with highly concentrated hydrocarbons and non Cl-containing compounds co-eluting with Cl-containing compounds, avoid a reliable identification. Also, the concentration below the limit of detection of GC-MS could be an explanation.[29] Therefore, a more comprehensive technique was necessary for the identification, and bidimensional GC × GC-MS was chosen for that purpose. Using a cryogenic modulator with two parallels GC, the identification of some major compounds could be achieved. With this system, compounds are modulated in a loop by retaining them with a cold flow of cryogenic nitrogen, during the modulation time (4s), and then released into the $^{2}D$ with a hot pulse at 280 ºC (300 ms).[30,31] Match quality of the identification over 85% was considered valid when was compared with NIST20 library.

In the case of samples PO-3 and PO-4 two compounds were identified, 2-chloroethanol acetate at 9.3 min and benzyl chloride at 11.6 min (retention times are referred to GC-ICP-MS/MS profile). Identification of both compounds was confirmed by comparing retention time when injecting pure standard in GC-ICP-MS/MS. Samples PO-1 and PO-2 were also analysed by GC × GC-MS and one compound could be identified, 1-chloro-2,3-dihydro-1H-indene at 14.5 min with a 93% of coincidence. In this case, correlation between GC × GC-MS and GC-ICP-MS/MS profile was assumed by injecting the Internal Standard used to quantify those sample (5-chloro-2-pentanone) and comparing order of elution in both approaches. Since only one peak after the IS was observed we can assume that is the identified compound. Figure 4 illustrates the obtained results for both samples PO-1 and PO-3.

# Assessing GC-ICP-MS/MS determination of chlorine-containing compounds in plastic-based pyrolysis oil samples

**Javier García-Bellido,[a] Montserrat Redondo-Velasco,[a] Mariosimone Zoccali,[b] Luigi Mondello,[c,d] Pierre Giusti,[e] Mariella Moldovan,[a*] and Jorge Ruiz Encinar[a]**

[a] Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain

[b] Department of Mathematical and Computer Science, Physical Sciences and Earth Sciences, University of Messina, Messina, Italy

[c] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[d] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[e] TotalEnergies, TotalEnergies Research& Technology Gonfreville, 76700 Harfleur, France

* Mariella Moldovan moldovanmariella@uniovi.es

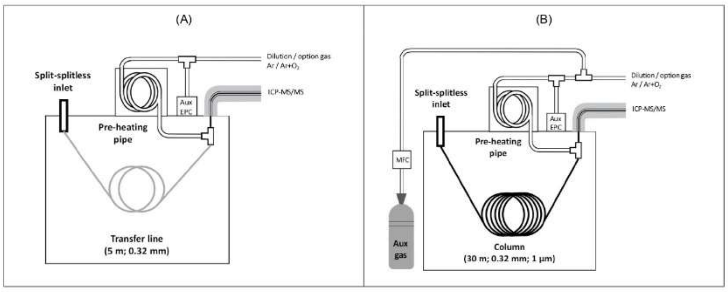

**Figure S-1.** Scheme of the GC-ICP-MS/MS setup for total analysis (A) and speciation analysis (B). Auxiliary gases were introduced in a tee connection with dilution/option gas.

**Table S-1.** GC and ICP-MS/MS instrumental conditions for both speciation and total chlorine analysis.

| **ICP-MS/MS parameters** | |
|---|---|
| RF power | 1550 W |
| Sample Depth | 8 mm |
| Option Gas (Ar:$O_2$) | 8 % |
| Dilution Gas | 0.4 mL/min |
| Cell flow ($H_2$) | 2.5 mL |
| Cones type | Pt |
| ICP torch | GC-ICP-MS Interface Agilent Torch |
| Monitored m/z | $^{35}Cl \rightarrow ^{35}Cl^{1}H_2$ |
| **GC parameters** | |
| Inlet temperature | 250 ºC |
| Interface temperature | 250 ºC |
| **Total Analysis** | |
| Transfer line flow | 1 mL/min |
| Oven temperature | 250 ºC |
| Injection mode | pulsed splitless |
| Pressure pulse | 10 psi |
| Injection volume | 0.6 µL |
| **Speciation Analysis** | |
| Column | Rxi-1MS (30 m; 0.32 mm; 1 µm) |
| Column Flow | 1.5 mL/min |
| Oven Gradient | Standards: 40 ºC (3 min), 20 ºC/min to 280 ºC<br>Samples: 35 ºC (3 min), 10 ºC/min to 280 ºC |

sample PO-1 and PO-2

sample PO-3

sample PO-4

**Figure S-2.** Analyzed plastic-based pyrolysis oils.

**Table S-2.** Found response factors and recoveries of Cl-containing compounds standards mixture obtained by GC-ICP-MS/MS. Dichlorvos was used as generic internal standard. The uncertainties indicate standard deviations for n = 5 injections.

| | Response Factor | Recovery, % |
|---|---|---|
| 1,2-dichloroethane | 590 | 95 |
| 5-chloro-2-pentanone | 551 | 89 |
| 1-chloroheptane | 620 | 100 |
| benzylchloride | 611 | 99 |
| 3-clorophenol | 657 | 106 |
| dichlorvos **(IS)** | 620 | 100 |
| 2,3-dichlorobiphenyl | 687 | 111 |
| methyl (2,4,5-trichlorophenoxy) acetate | 666 | 107 |
| dichlofenthion | 640 | 103 |
| fenchlorphos | 661 | 107 |
| chlorpyrifos | 611 | 99 |
| carbophenothion | 575 | 93 |
| **average ± standard dev.** | 624 ± 40 | 100.8 ± 6.4 |
| **RSD** | 6.4 | 6.4 |

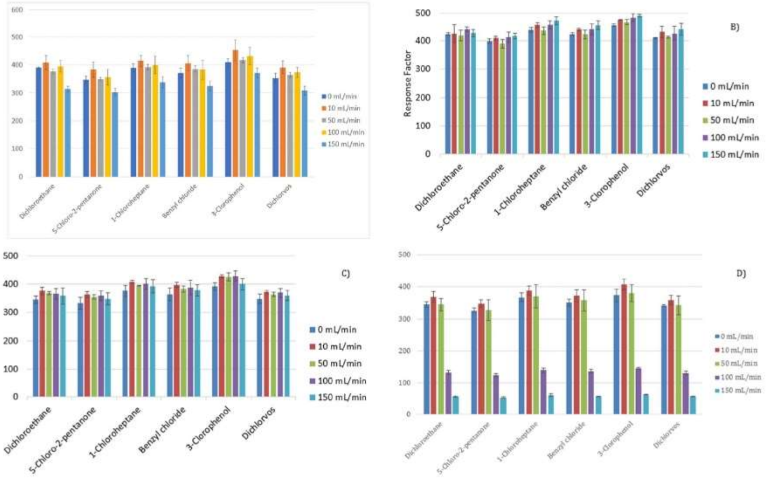

**Figure S-3.** Response factors of a mixture of six Cl-compounds evaluated with the additions of different auxiliary gases: (A) $N_2$, (B) He, (C) Ne, and (D) $CO_2$.

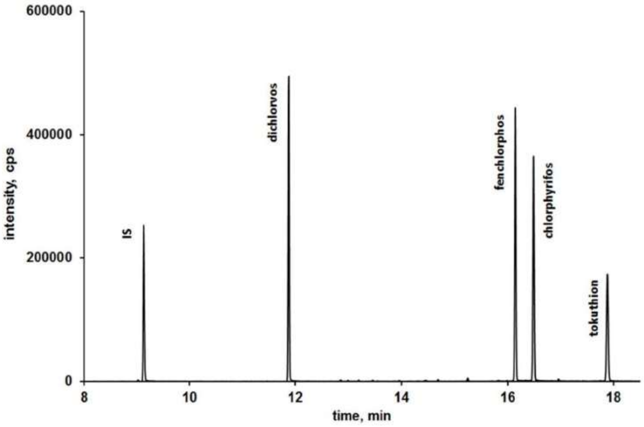

**Figure S-4.** GC-ICP-MS/MS speciation chlorine analysis of the CRM.

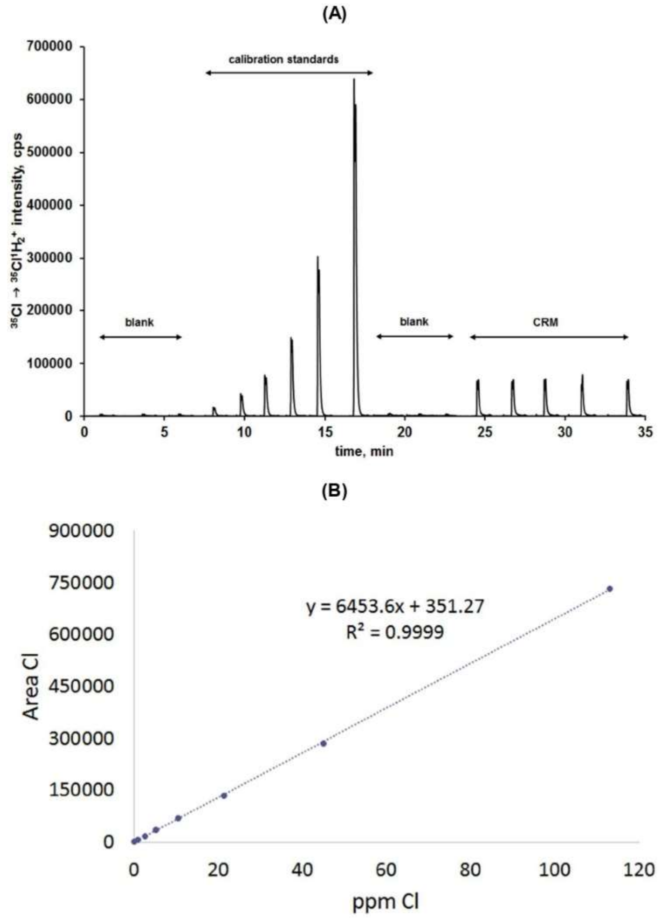

**Figure S-5. (a)** GC-ICP-MS/MS total chlorine analysis of the CRM. (b) …

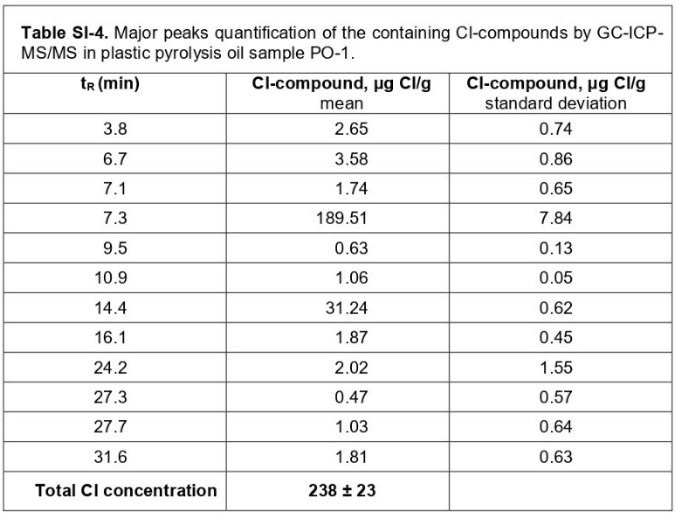

**Table SI-4.** Major peaks quantification of the containing Cl-compounds by GC-ICP-MS/MS in plastic pyrolysis oil sample PO-1.

| **$t_R$ (min)** | **Cl-compound, µg Cl/g** mean | **Cl-compound, µg Cl/g** standard deviation |
|---|---|---|
| 3.8 | 2.65 | 0.74 |
| 6.7 | 3.58 | 0.86 |
| 7.1 | 1.74 | 0.65 |
| 7.3 | 189.51 | 7.84 |
| 9.5 | 0.63 | 0.13 |
| 10.9 | 1.06 | 0.05 |
| 14.4 | 31.24 | 0.62 |
| 16.1 | 1.87 | 0.45 |
| 24.2 | 2.02 | 1.55 |
| 27.3 | 0.47 | 0.57 |
| 27.7 | 1.03 | 0.64 |
| 31.6 | 1.81 | 0.63 |
| **Total Cl concentration** | **238 ± 23** | |

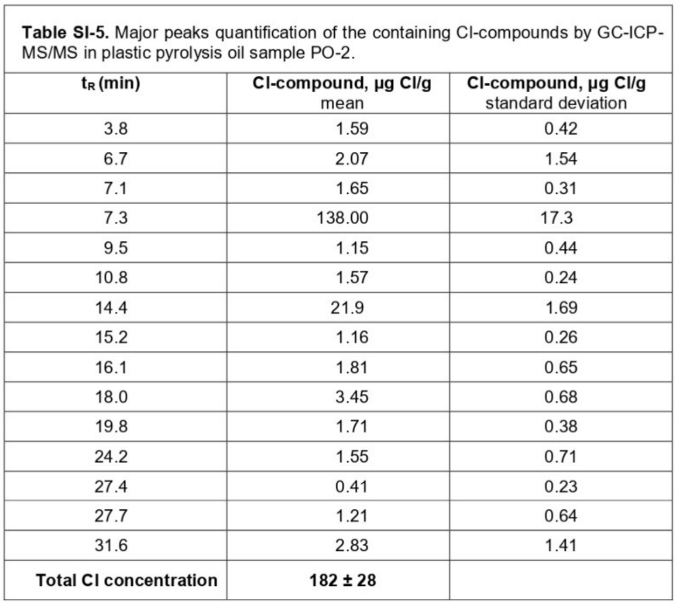

**Table SI-5.** Major peaks quantification of the containing Cl-compounds by GC-ICP-MS/MS in plastic pyrolysis oil sample PO-2.

| $t_R$ (min) | Cl-compound, µg Cl/g mean | Cl-compound, µg Cl/g standard deviation |
|---|---|---|
| 3.8 | 1.59 | 0.42 |
| 6.7 | 2.07 | 1.54 |
| 7.1 | 1.65 | 0.31 |
| 7.3 | 138.00 | 17.3 |
| 9.5 | 1.15 | 0.44 |
| 10.8 | 1.57 | 0.24 |
| 14.4 | 21.9 | 1.69 |
| 15.2 | 1.16 | 0.26 |
| 16.1 | 1.81 | 0.65 |
| 18.0 | 3.45 | 0.68 |
| 19.8 | 1.71 | 0.38 |
| 24.2 | 1.55 | 0.71 |
| 27.4 | 0.41 | 0.23 |
| 27.7 | 1.21 | 0.64 |
| 31.6 | 2.83 | 1.41 |
| **Total Cl concentration** | **182 ± 28** | |

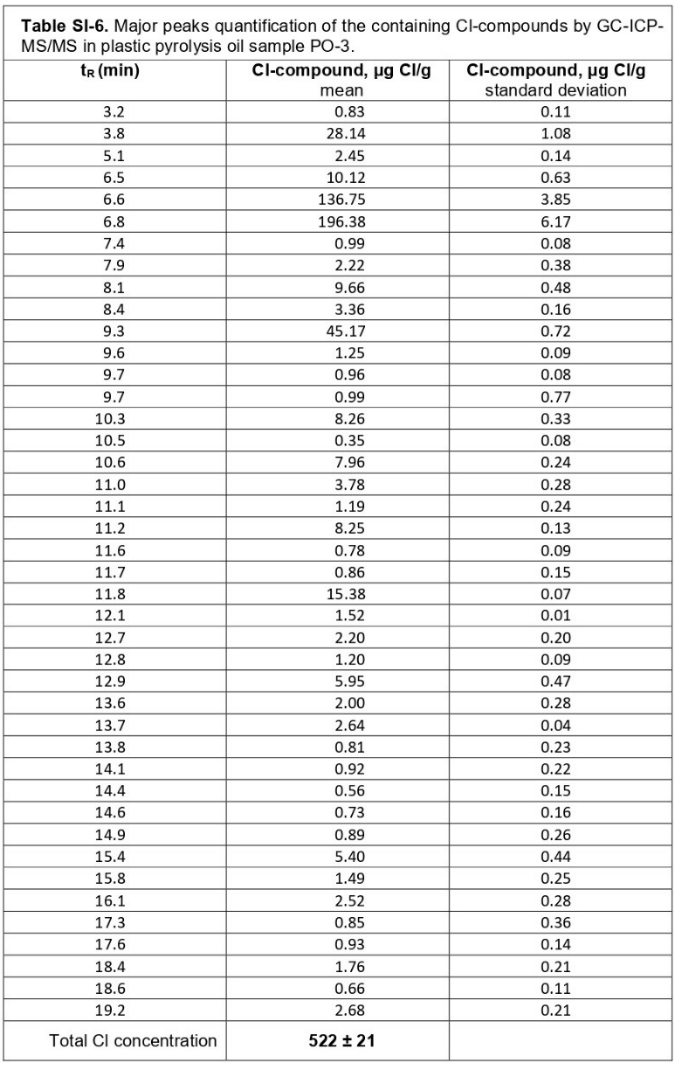

**Table SI-6.** Major peaks quantification of the containing Cl-compounds by GC-ICP-MS/MS in plastic pyrolysis oil sample PO-3.

| $t_R$ (min) | Cl-compound, µg Cl/g mean | Cl-compound, µg Cl/g standard deviation |
|---|---|---|
| 3.2 | 0.83 | 0.11 |
| 3.8 | 28.14 | 1.08 |
| 5.1 | 2.45 | 0.14 |
| 6.5 | 10.12 | 0.63 |
| 6.6 | 136.75 | 3.85 |
| 6.8 | 196.38 | 6.17 |
| 7.4 | 0.99 | 0.08 |
| 7.9 | 2.22 | 0.38 |
| 8.1 | 9.66 | 0.48 |
| 8.4 | 3.36 | 0.16 |
| 9.3 | 45.17 | 0.72 |
| 9.6 | 1.25 | 0.09 |
| 9.7 | 0.96 | 0.08 |
| 9.7 | 0.99 | 0.77 |
| 10.3 | 8.26 | 0.33 |
| 10.5 | 0.35 | 0.08 |
| 10.6 | 7.96 | 0.24 |
| 11.0 | 3.78 | 0.28 |
| 11.1 | 1.19 | 0.24 |
| 11.2 | 8.25 | 0.13 |
| 11.6 | 0.78 | 0.09 |
| 11.7 | 0.86 | 0.15 |
| 11.8 | 15.38 | 0.07 |
| 12.1 | 1.52 | 0.01 |
| 12.7 | 2.20 | 0.20 |
| 12.8 | 1.20 | 0.09 |
| 12.9 | 5.95 | 0.47 |
| 13.6 | 2.00 | 0.28 |
| 13.7 | 2.64 | 0.04 |
| 13.8 | 0.81 | 0.23 |
| 14.1 | 0.92 | 0.22 |
| 14.4 | 0.56 | 0.15 |
| 14.6 | 0.73 | 0.16 |
| 14.9 | 0.89 | 0.26 |
| 15.4 | 5.40 | 0.44 |
| 15.8 | 1.49 | 0.25 |
| 16.1 | 2.52 | 0.28 |
| 17.3 | 0.85 | 0.36 |
| 17.6 | 0.93 | 0.14 |
| 18.4 | 1.76 | 0.21 |
| 18.6 | 0.66 | 0.11 |
| 19.2 | 2.68 | 0.21 |
| Total Cl concentration | **522 ± 21** | |

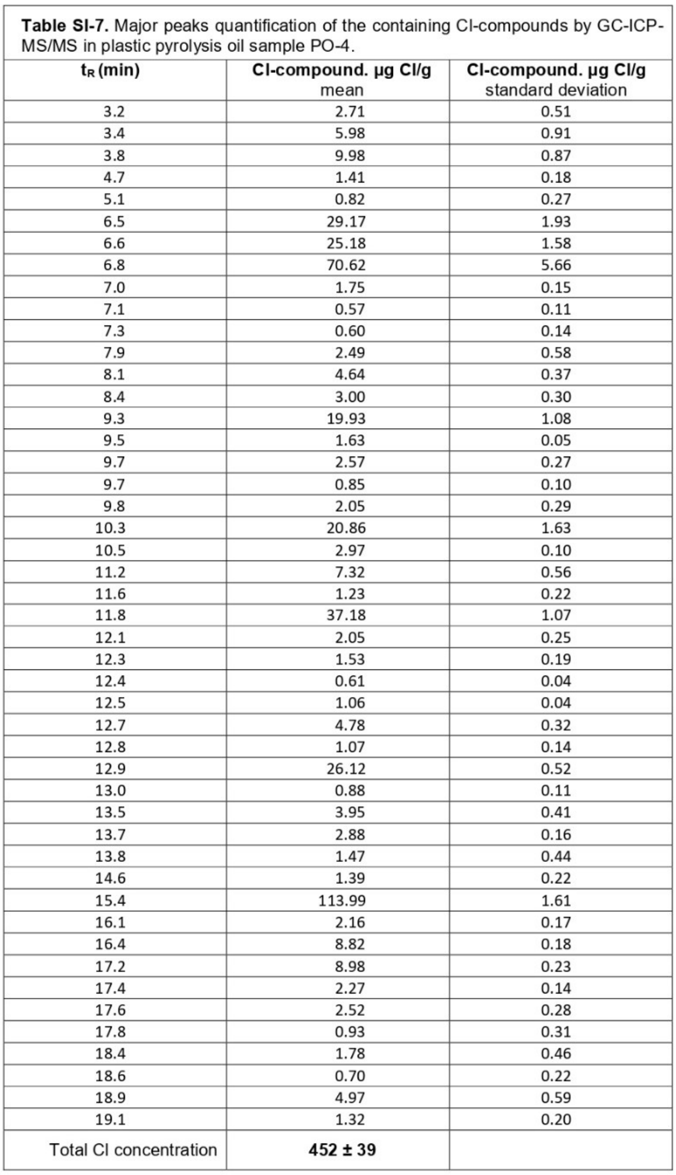

**Table SI-7.** Major peaks quantification of the containing Cl-compounds by GC-ICP-MS/MS in plastic pyrolysis oil sample PO-4.

| **$t_R$ (min)** | **Cl-compound. µg Cl/g** mean | **Cl-compound. µg Cl/g** standard deviation |
|---|---|---|
| 3.2 | 2.71 | 0.51 |
| 3.4 | 5.98 | 0.91 |
| 3.8 | 9.98 | 0.87 |
| 4.7 | 1.41 | 0.18 |
| 5.1 | 0.82 | 0.27 |
| 6.5 | 29.17 | 1.93 |
| 6.6 | 25.18 | 1.58 |
| 6.8 | 70.62 | 5.66 |
| 7.0 | 1.75 | 0.15 |
| 7.1 | 0.57 | 0.11 |
| 7.3 | 0.60 | 0.14 |
| 7.9 | 2.49 | 0.58 |
| 8.1 | 4.64 | 0.37 |
| 8.4 | 3.00 | 0.30 |
| 9.3 | 19.93 | 1.08 |
| 9.5 | 1.63 | 0.05 |
| 9.7 | 2.57 | 0.27 |
| 9.7 | 0.85 | 0.10 |
| 9.8 | 2.05 | 0.29 |
| 10.3 | 20.86 | 1.63 |
| 10.5 | 2.97 | 0.10 |
| 11.2 | 7.32 | 0.56 |
| 11.6 | 1.23 | 0.22 |
| 11.8 | 37.18 | 1.07 |
| 12.1 | 2.05 | 0.25 |
| 12.3 | 1.53 | 0.19 |
| 12.4 | 0.61 | 0.04 |
| 12.5 | 1.06 | 0.04 |
| 12.7 | 4.78 | 0.32 |
| 12.8 | 1.07 | 0.14 |
| 12.9 | 26.12 | 0.52 |
| 13.0 | 0.88 | 0.11 |
| 13.5 | 3.95 | 0.41 |
| 13.7 | 2.88 | 0.16 |
| 13.8 | 1.47 | 0.44 |
| 14.6 | 1.39 | 0.22 |
| 15.4 | 113.99 | 1.61 |
| 16.1 | 2.16 | 0.17 |
| 16.4 | 8.82 | 0.18 |
| 17.2 | 8.98 | 0.23 |
| 17.4 | 2.27 | 0.14 |
| 17.6 | 2.52 | 0.28 |
| 17.8 | 0.93 | 0.31 |
| 18.4 | 1.78 | 0.46 |
| 18.6 | 0.70 | 0.22 |
| 18.9 | 4.97 | 0.59 |
| 19.1 | 1.32 | 0.20 |
| Total Cl concentration | **452 ± 39** | |

# CAPÍTULO DOS

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## Desarrollo y aplicación de un nuevo detector de Cromatografía de gases basado en la Combustión – Espectrometría de Masas para la detección universal y selectiva de heteroátomos

## Introducción y contexto

La detección universal de compuestos en cromatografía de gases, como se ha discutido en la introducción de la Tesis Doctoral, es a menudo realizada mediante el detector FID. Sin embargo, dada su respuesta no completamente independiente de la especie, siendo además necesario el uso de correcciones matemáticas en función de los grupos funcionales presentes, se hace patente la necesidad de usar patrones específicos para llevar a cabo la cuantificación. La MS se propone también como alternativa como método de detección universal ampliamente usado en la GC, con la característica, además, de poder proporcionar información estructural de los compuestos. También, usando el modo SIM, se puede llegar a una detección selectiva, aunque limitada, para el cribado de familias concretas de compuestos, como pueden ser aquellos que contienen S y/o N en su estructura, debido a posibles interferencias isobáricas. Esto, unido a la alta dependencia en la ionización en función de la estructura de la especie, suponen unas limitaciones importantes para la GC-MS.

Durante los últimos años, en el grupo de investigación se ha venido desarrollando una metodología basada en la modificación de un GC-MS convencional para la cuantificación universal de compuestos orgánicos al mismo tiempo que se proporciona información cualitativa. Sergio Cueto et al. consiguieron mediante la instalación de un horno de combustión entre la columna cromatográfica y el MS, la conversión cuantitativa de todos los compuestos a $CO_2$, manteniendo un equilibrio isotópico con el trazador enriquecido añadido en continuo ($^{13}CO_2$), de forma que, en este caso, la ionización es independiente del compuesto, al transformarse todos al mismo compuesto tras la combustión. La aplicabilidad de esta metodología se limita a obtener información cuantitativa y cualitativa de compuestos con C en su estructura, siendo un gran paso en comparación con los detectores universales descritos previamente. Sin embargo, existen como se ha comentado anteriormente una serie de elementos cuya determinación a niveles de traza tiene una importancia relevante en ciertos campos de la química analítica.

Compuestos que contienen S o N en su estructura son difícilmente detectados por detectores universales, ya que requieren de una extracción selectiva de los abundantes hidrocarburos de la matriz, siendo necesario técnicas basadas en la cromatografía de gases multidimensional o el uso de espectrometría de masas de alta resolución. Es aquí donde entran en juego los detectores selectivos definidos durante la introducción de la Tesis Doctoral. Detectores como los de quimioluminiscencia (SCD y NCD) han sido ampliamente utilizados, sin embargo, carecen de información estructural de los compuestos, además de que la equimolaridad en muchos casos está bajo discusión. Otro elemento de interés en la GC, es el H, siendo únicamente detectable por el AED. Este detector multielemental que permite también una respuesta selectiva para S y N, además de una respuesta universal para C, tiene una selectividad menor comparada con los anteriores para estos elementos y, además, la respuesta equimolar está ampliamente discutida. Esto, sumado a los efectos de *quenching* por la matriz hacen que este detector tenga una aplicabilidad limitada. Otro detector a evaluar, sería el GC-ICP-MS/MS, utilizado durante el primer capítulo de la Tesis Doctoral. Este detector, que ha sido usado incluso dentro del grupo de investigación para la detección selectiva de compuestos de S en muestras de gasolina, se encuentra muy limitado, dadas sus características internas al trabajar a presión atmosférica, para detectar elementos presentes en la atmósfera, como el C, H, N u O.

Continuando la línea de investigación abierta en el grupo de investigación por Sergio Cueto y Jorge Ruiz en el año 2009, en este capítulo se pretende evaluar el prototipo, ya no solo como detector universal, si no como detector multielemental selectivo para H, N y S, cubriendo así un rango amplio de elementos presentes en compuestos analizables por GC. Para ello, al igual que se forma $CO_2$ tras la combustión de los compuestos orgánicos, es de esperar que se formen otras especies volátiles conteniendo el resto de elementos de estos. Estas especies, bajo una cantidad de oxígeno suficiente, serán los óxidos correspondientes de cada uno ($NO_x$, $SO_x$ y $H_2O$). Así, se evaluó, mediante el análisis de una serie de patrones la generación de estos a distintas condiciones de temperatura y cantidad de oxígeno del sistema. La aplicación de la metodología se realizó sobre una serie de muestras diésel, analizadas previamente por los detectores de SCD y NCD, de cara a confirmar los resultados obtenidos. También se plantean posibles aplicaciones futuras que permitiría la detección selectiva de hidrógeno, como es el uso del detector para el cálculo del grado de insaturación de muestras reales.

Una de las limitaciones de la GC-MS es llevar a cabo la identificación de especies a bajas concentraciones cuando coeluyen con compuestos altamente concentrados, como ocurre en muestras de tipo combustibles. Como se explicó durante la introducción general de la Tesis Doctoral, es necesario llevar a cabo procesos de separación de la matriz, bien ya sean previos al análisis o, haciendo uso de una separación cromatográfica de esta mediante la GCxGC. En este contexto, dada la baja concentración a la que se encuentran los analitos de interés en las muestras analizadas, se recurrió a la técnica de la cromatografía de gases bidimensional para llevar a cabo la identificación de las muestras. Esto pone en relevancia también el detector desarrollado y la alta sensibilidad que posee de cara a detectar compuestos en muy baja concentración.

Artículo científico III.

**Título**: *Quantitative multiplexed elemental (C, H, N, and S) detection in complex mixtures using gas chromatography*

**Autores**: Laura Freije-Carrelo, Javier García-Bellido, Laura Alonso Sobrado, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti and Jorge Ruiz Encinar

**Referencia**: *Chemical Communications, **2020**, 56, 2905*

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# Quantitative multiplexed elemental (C, H, N and S) detection in complex mixtures using gas chromatography†

Laura Freije-Carrelo,[a] Javier Garcia-Bellido,[a] Laura Alonso Sobrado,[a] Mariella Moldovan,[a] Brice Bouyssiere,[b] Pierre Giusti[c] and Jorge Ruiz Encinar*[a]

**We present a novel and single detection approach that enables sensitive, accurate and compound-independent quantification of N, S and H in the individual compounds present in complex samples. Integration of the whole chromatographic profile gives the total content of the elements. Simultaneous universal detection is also achieved using the C profile.**

Universal detection of volatile organic compounds in GC is often performed using a Flame Ionization Detector (FID).[1] However, its carbon response is not fully compound-independent; so, calibration relies on the use of specific standards for each analyte or on the use of correction factors based on the different functional groups present.[2] Besides, it cannot provide any structural information. Another widely used universal GC detection method is Mass Spectrometry (MS). It can provide either universal detection and structural identification (scan mode) or compound-specific detection (SIM mode). However, such a selective SIM mode is highly limited to screen for specific families (*i.e.*, S and N compounds) in complex samples because of important isobaric overlaps. Additionally, ionization is highly compound-dependent, so specific standards for each analyte are again needed.[3] A new detection GC system able to provide both qualitative information and generic universal quantification of organic compounds was presented in 2009.[4,5] A combustion interface was installed in a quadrupole GC-MS instrument for the conversion of all the compounds eluting from the column into $CO_2$ providing isotopic equilibration between such compounds and the isotopically enriched tracer ($^{13}CO_2$) continuously added. In this way, ionization became compound-independent. Despite their usefulness, universal detectors are still limited in the quantitative analysis of complex samples as they require complete chromatographic isolation of the targeted analytes. Furthermore, information on specific families of compounds cannot be provided. Given the relevance of N- and S-containing compounds in the petrochemical, environmental and pharmaceutical industries, selective detectors such as Sulfur Chemiluminescence Detectors (SCDs) and Nitrogen Chemiluminescence Detectors (NCDs) have been developed.[6] Remarkably, no adequate H selective detectors exist. Atomic Emission Detectors (AEDs) can provide both universal detection for organic compounds when monitoring C as well as S, N and H selective detection through their corresponding emission lines. However, the selectivity of AEDs is lower compared to SCDs and NCDs[1] and their equimolar response is under discussion.[7] Finally, similar to most spectroscopic detectors, AEDs suffers from significant quenching effects when analysing complex unresolved samples.[1]

Interestingly, the same way as the combustion of an organic compound at the exit of a GC column produces $CO_2$ molecules detectable by MSD, other volatile species such as $H_2O$, $SO_x$ and $NO_x$ (if S and N are present) should be produced and potentially detected as well. Such an innovative combustion-MS setup could turn out to be the first GC detector able to provide simultaneous universal (C) and selective (S, N, H) compound-independent quantification of organic compounds. A schematic of the process and reactions occurring during combustion is shown in Fig. S1 (ESI†). Even more, structural elucidation capabilities of conventional MS can be maintained by the simple introduction of a six-way valve. A scheme of the instrumental setup is shown in Fig. S2 (ESI†). The combustion interface consisted of a ceramic tube (400 mm length, 3 mm width and 0.5 mm internal diameter) in which 2 platinum wires were introduced. Combustion was carried out online at temperatures ranging from 850 to 1150 °C while adding a flow of $O_2$ diluted in He (0.3% v/v) provided by a mass flow controller (MFC) and mixed online with an eluting flow from the column using a capillary flow "T". The six-way valve installed inside the GC oven allows operation either in a conventional GC-MSD or

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[a] *Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain. E-mail: ruizjorge@uniovi.es; Tel: +34 985103069*

[b] *Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Materiaux, UMR5254, Hélioparc, 64053 Pau, France*

[c] *TOTAL Raffinage Chimie, TRTG, BP 27, 76700, Harfleur, France*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9cc09842a

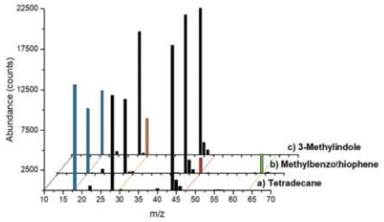

Fig. 1 Mass spectra (blank subtracted) obtained using GC-combustion-MS for (a) tetradecane, (b) methylbenzothiophene and (c) 3-methylindole. Intensity for *m*/*z* 44 has been divided by 6 for clarity.

GC-combustion-MS mode (see Fig. S2, ESI†). First, we studied the different volatile species which originated after combustion (925 °C and 0.4 mL min$^{-1}$ $O_2$/He) of the eluting species depending on their elemental composition. A mixture of tetradecane (exclusively containing C and H), methylbenzothiophene and 3-methylindole (S- and N-containing compounds, respectively) was analysed by GC-combustion-MS while scanning at 18–300 *m*/*z*. No trace was observed at the corresponding specific fragments (NIST library) of each compound proving that the combustion is complete.

In contrast, signals originating from the volatile species could be detected as shown by different MS spectra obtained for each compound (Fig. 1). The most intense signal detected corresponded to $^{12}C^{16}O_2$ at *m*/*z* 44. The trace signal observed at *m*/*z* 45 corresponds mostly to $^{13}C^{16}O_2$ (contribution of $^{17}O$ can be neglected). In fact, the average 45/44 ratio obtained for the 3 compounds (0.012 ± 0.001) matched pretty well with the theoretical $^{13}C/^{12}C$ isotope ratio. A signal at *m*/*z* 28 was also generally observed. As these spectra were background subtracted, it could not be assigned to the contribution of $N_2$ background. It was detected in every spectrum, so it is not specific for the N-containing compounds either. It is likely due to the in-source fragmentation of $^{12}C^{16}O_2$ to $^{12}C^{16}O$ as borne out by the fact that the 44/28 ratio is the same for the three species (0.10 ± 0.02) and similar to that observed in $CO_2$ NIST spectra (0.098). The last common signal observed was at *m*/*z* 18 and corresponded to the $H_2O$ molecules produced after combustion due to the presence of H atoms in every molecule. Interestingly, both the N- and S-containing compounds provided element specific MS signals. Nitrogen present led to the formation of NO detected at *m*/*z* 30 (Fig. 1c). $NO_2$ formation was not significant. In fact, a small trace at *m*/*z* 46 was detected for every compound likely corresponding to the isotopologue of the $CO_2$ molecule. As shown in Fig. 1b, S was selectively detected as SO (*m*/*z* 48) and $SO_2$ (*m*/*z* 64). Libraries predict that $SO_2$ fragments into SO to give a SO/$SO_2$ ratio of 0.5. In our case, the ratio observed was slightly higher (0.7), which seems to indicate that both SO and $SO_2$ are produced after combustion. However, S detection as an $SO_2$ signal is favoured in terms of sensitivity. Fig. S3 (ESI†) shows the extracted ion chromatograms for the 3 compounds at *m*/*z* 44, 18, 30 and 64, which proves that each and every element (C, H, N and S, respectively) can be selectively detected. Interestingly, although C and H detections are still element selective, both atoms are present in the majority of compounds, so their detection can be considered universal. N and S are in contrast present in much fewer compounds typically at low level concentrations (*i.e.* in petroleum derivatives).

The next experiments were designed to find the optimum operational conditions under which the instrument could provide an equimolar response for the four elements, that is, independent of the chemical structure of the compounds. In this context, once quantitative conversion of every organic compound into $CO_2$, $H_2O$, NO and $SO_x$ is ensured, a compound independent response will be achieved. We selected EI-MSD because it is operated under vacuum thereby guaranteeing low backgrounds for the final simple volatile species to be detected. Importantly, it can also provide structural information of the target compounds when the combustion furnace is bypassed (Fig. S2, ESI†). Complete conversion into $CO_2$ of the carbon present in simple organic compounds (alkanes, aromatics) at combustion temperatures above 850 °C has been demonstrated already.[4,8] Therefore, we selected an alkane (tetradecane) to compute the carbon response factor (peak area at *m*/*z* 44 divided by C concentration). It was spiked as an Internal Standard (IS) to model mixtures containing different types of compounds where the S and N are present in very different chemical forms (Fig. S4, ESI†). Direct comparison of different C response factors obtained for each compound and the IS provided the individual recoveries. Four different temperatures (850, 925, 1000 and 1150 °C) with three different online post-column flows of $O_2$/He (0.1, 0.2 and 0.4 mL min$^{-1}$) were tested. As can be seen in Fig. S5 and S6 (ESI†), excellent results were obtained at any T and at 0.2–0.4 mL min$^{-1}$ $O_2$/He flows. In fact, control of the flow at a low level of 0.1 mL min$^{-1}$ was very difficult. Average quantitative recoveries were obtained for 8 N-(97%) and 5 S-(98%) containing compounds under such conditions. The only compound with a recovery below 90% was dibenzothiophene (89 ± 1%). However, it should be noted here that the recovery computed with reference to the IS is not only affected by the combustion process itself but by different chromatographic transmissions (injector and column) as well. In fact, C recovery for dibenzothiophene using a low film thickness column (BD-EN14103, 30 m × 0.32 mm × 0.25 μm instead of Rxi-1 ms, 30 m × 0.32 mm × 1 μm) was quantitative (102 ± 3%). In the case of N-compounds, quinoleine and carbazole (91 ± 1 and 90 ± 1%, respectively) showed lower but still adequate recoveries, which could not be improved using different columns. It seems clear that the presence of those heteroatoms does not cause any significant detrimental effect on the formation of $CO_2$ during combustion. The detection limit obtained was 20 ng C g$^{-1}$, which is barely distinguishable from the one obtained with online C isotope dilution and those obtained using FID or MSD in scan mode.[4] The linear range covers more than 2 orders of magnitude (0.05–12 μg C g$^{-1}$, Fig. S7, ESI†).

Likewise, we could use the H response factor (peak area at *m*/*z* 18 divided by H concentration) computed for the IS tetradecane to calculate the H recoveries for the S- and N-compounds under the operational conditions tested. The complete sets of results are given in Fig. S8 and S9 (ESI†). Again, quantitative recoveries were obtained at all T tested and 0.2–0.4 mL $min^{-1}$ $O_2$/He except for dibenzothiophene (87 ± 3%). However, such a recovery became quantitative (99 ± 4%) once again when the BD-EN14103 column was used. The DL obtained was 8 ng H $g^{-1}$ and the linear range obtained covers 2 orders of magnitude (0.020 to 3 μg H $g^{-1}$, Fig. S10, ESI†). Notably, this is the first time that the same detector can measure H and C simultaneously in every isolated chromatographic peak with a similar sensitivity. Therefore, the H/C molar ratio for every peak could be directly obtained from the 18/44 area ratio using the H and C response factors obtained from a simple and generic alkane as an IS. Fig. S11 (ESI†) shows that the experimental and theoretical H/C ratios were in complete agreement (99%) for the different components of a mixture of 19 compounds whose H/C ratios vary as a function of unsaturation, ramification or the presence of N or S. This innovative feature opens the door to evaluate the individual (each peak) and total (sum of all peaks) unsaturation degree in complex samples.

Chromatograms for the mixtures of S- and N-containing compounds are shown in Fig. 2. The element selectivity is very high as demonstrated by the complete absence of $m/z$ 30 (NO) and 64 ($SO_2$) signals at the retention times of the compounds without heteroatoms in their structures. As can be seen, the peak shape was excellent for the N peaks while the S peaks were slightly tailing likely due to unspecific adsorptions of the $SO_2$. The DLs obtained were as low as 1 ng $g^{-1}$ for both N and S, which are in the same order of magnitude or even lower than those obtained with dedicated SCD and NCD.[1,9] The linear ranges for both elements cover more than 2 orders of magnitude (Fig. S12 and S13, ESI†). As previously stated, $CO_2$ can fragment to $^{12}C^{16}O$ ($m/z$ 28) in the EI source, whose minor isotopologue $^{12}C^{18}O$ is not detected at a C concentration below 5 μg C $g^{-1}$, as demonstrated in Fig. 2B by the absence of signals at $m/z$ 30 for the compounds containing C–H exclusively. However, the $^{12}C^{18}O$ contribution becomes significant at higher C concentrations. Fig. S14A (ESI†) shows unspecific signals at $m/z$ 30 for alkanes at 16 μg C $g^{-1}$. Interestingly, a selective nitrogen profile for the sample can be easily obtained just by point-by-point normalization of the $m/z$ 30 ($^{14}N^{16}O$ and $^{12}C^{18}O$) by the $m/z$ 28 ($^{12}C^{16}O$ exclusively). Fig. S14B (ESI†) shows the complete absence of unspecific signals in the resulting 30/28 profile.

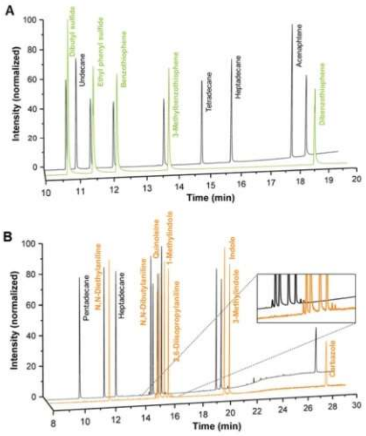

**Fig. 2** Chromatogram for a mixture of (A) S-containing (*ca.* 8 μg C $g^{-1}$) and (B) N-containing compounds (*ca.* 5 μg C $g^{-1}$), A and B, respectively, with organic compounds containing C–H exclusively: $m/z$ 44 (C: black line), 64 (S: green line) and 30 (N: orange line).

Once demonstrated that detection of C and H as $CO_2$ and $H_2O$ is compound-independent under selected conditions, we wanted to assess if N and S element selective detections such as NO and $SO_2$ could become equimolar as well. In that way, we could not only identify N and S compounds (fast screening) in complex samples (see Fig. 2), but determine their absolute concentration using simple generic standards as well. Additionally, the total amount of both the elements could be easily achieved after integration of their whole chromatograms. The formation of $SO_2$ and NO was assessed using temperatures and $O_2$/He flows assayed before. Individual S-specific response factors obtained for the 5 S-containing compounds are shown in Fig. S15 (ESI†). As can be seen, equimolar responses (6% RSD) were obtained at 850–925 °C with any $O_2$/He flows. It seems that the most volatile S compound, dibutyl sulfide, provides lower response factors at higher temperatures. The dispersion observed in the S response factors using this approach is very similar to the one obtained using the dedicated SCD (around 7% RSD).[9] The C, H and S selective quantification results obtained for the S-containing compounds using benzothiophene as a generic IS to provide the corresponding C, H and S response factors agreed well with the theoretical values (Table S3, ESI†). Again, the result of dibenzothiophene (88 ± 2%) became correct (101 ± 5%) when using the low film thickness column. Individual N-specific response factors obtained for the 8 N-containing compounds are shown in Fig. S16 (ESI†). An equimolar response (4–5% RSD) was obtained at 1150 °C with any $O_2$/He flow. Again, C, H and N selective quantification results obtained using 1-methylindole as a generic IS were excellent (Table S3, ESI†). The N results presented herein are remarkable as there is still significant ambiguity about the potential of the dedicated NCD to provide an equimolar response.[10] A critical comparison of the analytical features of the detection method presented here with other established detectors in GC is given in Table S4 (ESI†).

Finally, the applicability of the proposed approach to a real complex sample analysis was demonstrated with three different petroleum derivatives. A diesel sample, whose total sulfur content was previously determined to be 3900 ± 110 μg S $g^{-1}$ (95% confidence level) using the well-established X-ray fluorescence method (ASTM D2622), was injected after a dilution of 1 : 800 in hexane. The sulfur (selective) chromatographic profile is shown in Fig. 3 (generic Carbon profile is given in Fig. S17A, ESI†). In spite of the fact that the total sulfur content injected

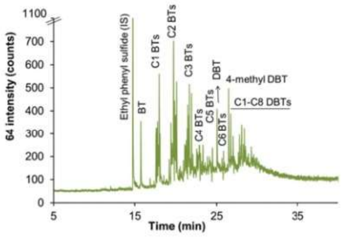

**Fig. 3** Sulfur chromatogram for the diesel sample diluted 1:800 and spiked with ethyl phenyl sulfide as an IS (BT: benzothiophene, DBT: dibenzothiophene).

after dilution was only 4.9 ppm S, tens of S-containing compounds were detected, demonstrating that the excellent DL calculated with standards applied for real complex samples as well. Ethyl phenyl sulfide was added as an IS. The total S content obtained after integration of the whole S chromatogram was found to be 3743 ± 216 μg S $g^{-1}$ (2 SD, $n = 3$), which is statistically undistinguishable from the reference value. Individual concentrations of some individual species and families were also determined and are given in Table S5 (ESI†). Next, another diesel sample with a total N content of 497 ± 10 μg $g^{-1}$ (95% confidence level), previously determined by the well-established chemiluminescence detection (similar to ASTM D4629), was injected after a dilution of 1:500 in hexane. In spite of the low total nitrogen injected (*ca.* 1 ppm), N-selective (normalized 30/28 profile) detection unveiled lots of N-containing compounds (Fig. S18, ESI†). *N*,*N*-Diethylaniline was spiked as an IS. The total N obtained (524 ± 22 μg N $g^{-1}$, 2 SD, $n = 3$) was again statistically undistinguishable from the reference value. Concentrations of the major families of N compounds (indoles and carbazoles) are given in Table S6 (ESI†).

Finally, H/C determination in a real petroleum sample was attempted because it is directly related to its unsaturation degree, which determines its properties.[11] A coker gas oil with a high content of unsaturated compounds[12] was selected. Previously, the original gas oil had been fractioned by polarity using SPE in 2 fractions, enriched with aromatic or aliphatic compounds, respectively. After a dilution of 1:500 in hexane, the overall 18/44 area ratio (whole chromatographic area under the 18 and 44 profiles) was computed in the three samples (Fig. S19A–C, ESI†) and transformed into H/C molar ratios (Table S7, ESI†) using generic standards. As expected, the H/C molar ratio obtained for the aliphatic fraction (1.99) was close to the typical alkane values (*ca.* 2.1, see Fig. S11, ESI†), which corroborated the enrichment. The value obtained for the aromatic fraction (1.32) suggested that the enrichment was not as high in this fraction as corroborated by the typical paraffin profile still observed in Fig. S19B (ESI†). The H/C molar ratio obtained (1.46) for the original gas oil was in between the ratios obtained for the two aliphatic fractions, but closer to the aromatic values, which is in agreement with the nature of the coker gas oil.

In summary, we present an innovative multipurpose GC detection system featuring element selective detection with generic quantification in complex samples, while maintaining the structural elucidation power of mass spectrometry. The approach, which requires a simple low-cost modification of a standard GC–MSD instrument, enables highly accurate and precise universal (C, H) and element specific (N, S, H) quantification of every species eluted from the GC column without the need for specific standards. It can also be considered as the first approach that enables online and simultaneously ultrasensitive elemental quantification (C, H, N, S) of individual organic compounds eluting from the GC column. Of course, integration of the whole chromatographic profile obtained provides the total elemental composition of a given sample. One downside factor is that oxygen can neither be detected nor quantified since it is externally added during the combustion process. Important application fields can be foreseen in the petroleum industry, and in pharmaceutical and environmental analysis, for which the variety and number of organic analytes is huge and continuously rising.

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## Conflicts of interest

There are no conflicts to declare.

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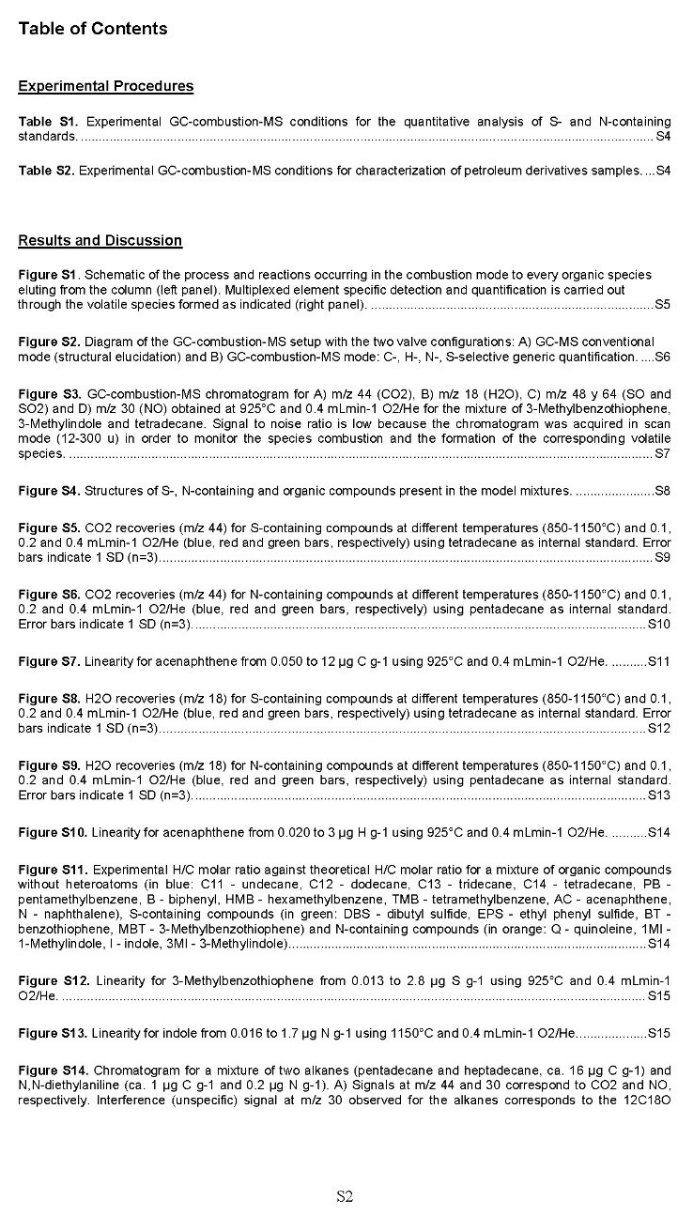
Electronic Supplementary Material (ESI) for Chemical Communications.

# Supplementary information

## Quantitative Multiplexed Elemental (C, H, N and S) Detection in Complex Mixtures using Gas Chromatography

Laura Freije-Carrelo, Javier García-Bellido, Laura Alonso Sobrado, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti, and Jorge Ruiz Encinar*



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## Experimental Procedures

All organic compounds injected, obtained pure from Sigma Aldrich, were diluted in hexane and dichloromethane. Combustion oven was obtained from Carbolite Hero (MFT 12/25/250). The valve employed was a two-position six-way stainless-steel valve with a 0.25 mm bore designed for operation at temperatures up to 350°C. All analyses were performed with a GC–MSD instrument (6890N GC coupled to a 5973Network quadrupole mass spectrometer, Agilent Technologies) equipped with an electron ionization source. Detailed conditions are given in Table S-1 and S-2.

**Table S1.** Experimental GC-combustion-MS conditions for the quantitative analysis of S- and N-containing standards.

| | |
|---|---|
| Column flow | 1.5 mLmin$^{-1}$ |
| Temperature gradient | 45°C (3 min), 15°Cmin$^{-1}$ to 120°C, 10°Cmin$^{-1}$ to 190°C, 15°Cmin$^{-1}$ to 250°C (1 min) |
| Column | Rxi-1ms (30 m x 0.32 mm x 1 µm) for S- compounds |
| | BD-EN14103, 30 m x 0.32 mm x 0.25 µm for N-compounds |
| Injection mode | Splitless |
| Inlet temperature | 250°C |
| Injection volume | 1 µL |
| Combustion reactor | 925°C, 0.4 mLmin$^{-1}$ O$_2$/He for S compounds |
| | 1150°C, 0.4 mLmin$^{-1}$ O$_2$/He for N compounds |
| Acquisition mode | *SIM*: m/z 18, 30, 44, 48, 64 |

**Table S2.** Experimental GC-combustion-MS conditions for characterization of petroleum derivatives samples.

| | |
|---|---|
| Column flow | 1.5 mLmin$^{-1}$ |
| Temperature gradient | 45°C (3 min), 8°Cmin$^{-1}$ to 250°C (20 min) |
| Column | Rxi-1ms (30 m x 0.32 mm x 1 µm) for sulfur quantification and H/C ratio |
| | BD-EN14103, 30 m x 0.32 mm x 0.25 µm for nitrogen quantification |
| Injection mode | Splitless |
| Inlet temperature | 250°C |
| Injection volume | 1 µL |
| Combustion reactor | 925°C, 0.4 mLmin$^{-1}$ O$_2$/He for S compounds |
| | 1150°C, 0.4 mLmin$^{-1}$ O$_2$/He for N compounds |
| Acquisition mode | *SIM*: m/z 18, 30, 44, 48, 64 |

## Results and Discussion

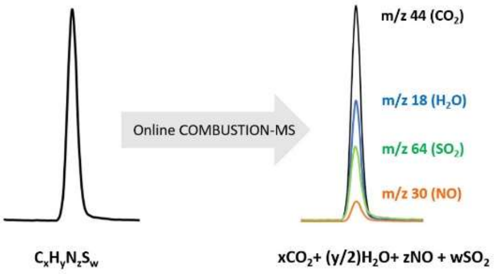


**Figure S1**. Schematic of the process and reactions occurring in the combustion mode to every organic species eluting from the column (left panel). Multiplexed element specific detection and quantification is carried out through the volatile species formed as indicated (right panel).

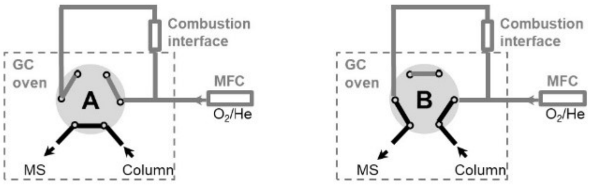


**Figure S2.** Diagram of the GC-combustion-MS setup with the two valve configurations: A) GC-MS conventional mode (structural elucidation) and B) GC-combustion-MS mode: C-, H-, N-, S-selective generic quantification.

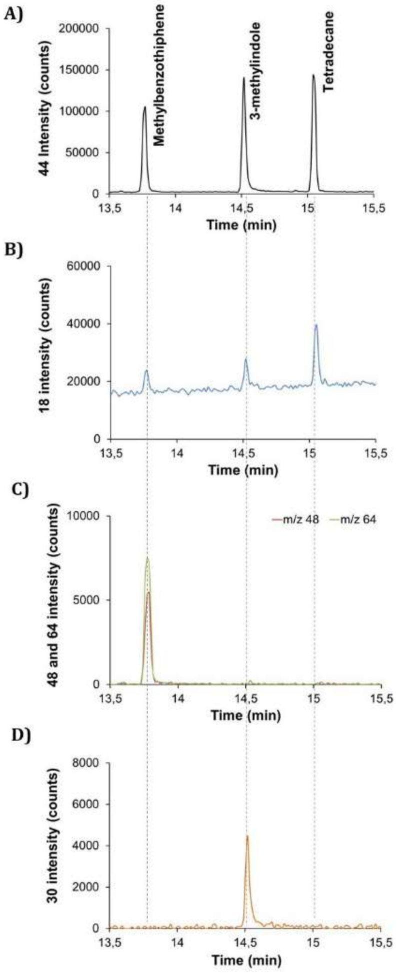


**Figure S3.** GC-combustion-MS chromatogram for A) **m/z** 44 ($CO_2$), B) m/z 18 ($H_2O$), C) **m/z** 48 y 64 (SO and $SO_2$) and D) m/z 30 (NO) obtained at 925°C and 0.4 mLmin$^{-1}$ $O_2$/He for the mixture of 3-Methylbenzothiophene, 3-Methylindole and tetradecane. Signal to noise ratio is low because the chromatogram was acquired in scan mode (12-300 u) in order to monitor the species combustion and the formation of the corresponding volatile species.

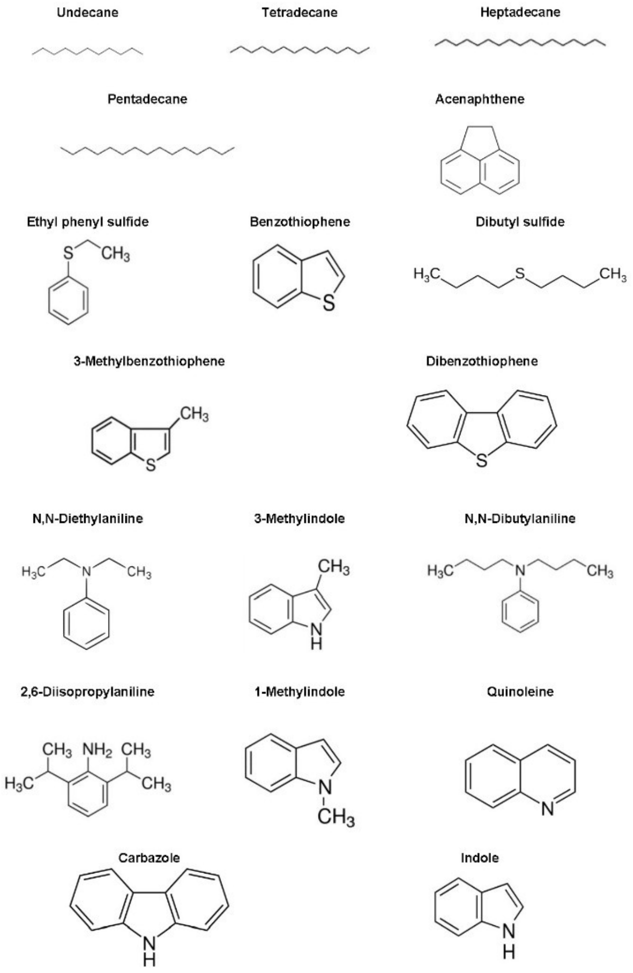


**Figure S4.** Structures of S-, N-containing and organic compounds present in the model mixtures.

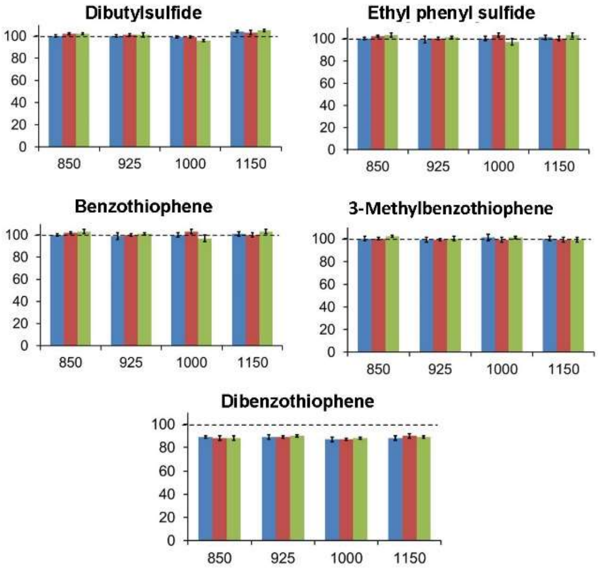


**Figure S5.** CO$_2$ recoveries (m/z 44) for S-containing compounds at different temperatures (850-1150°C) and 0.1, 0.2 and 0.4 mLmin$^{-1}$ O$_2$/He (blue, red and green bars, respectively) using tetradecane as internal standard. Error bars indicate 1 SD (n=3).

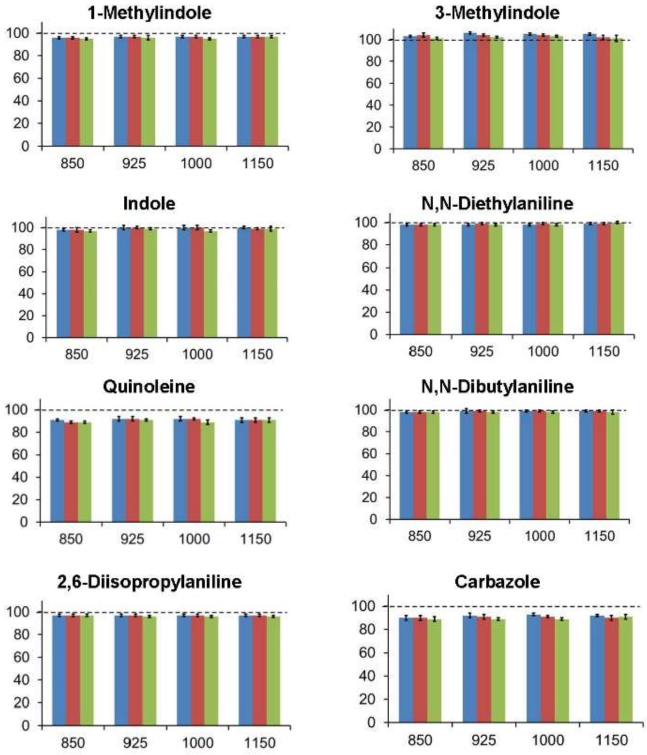


**Figure S6.** $CO_2$ recoveries (m/z 44) for N-containing compounds at different temperatures (850-1150°C) and 0.1, 0.2 and 0.4 mLmin$^{-1}$ $O_2$/He (blue, red and green bars, respectively) using pentadecane as internal standard. Error bars indicate 1 SD (n=3).

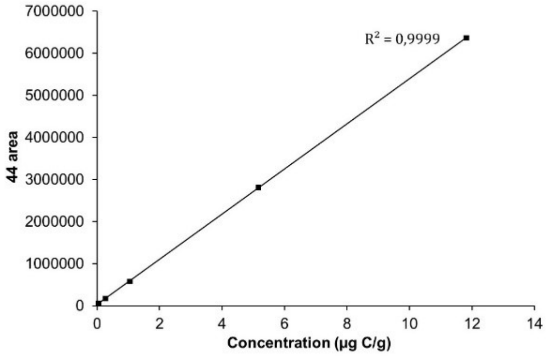


**Figure S7.** Linearity for acenaphthene from 0.050 to 12 µg C $g^{-1}$ using 925°C and 0.4 $mLmin^{-1}$ $O_2$/He.

S11

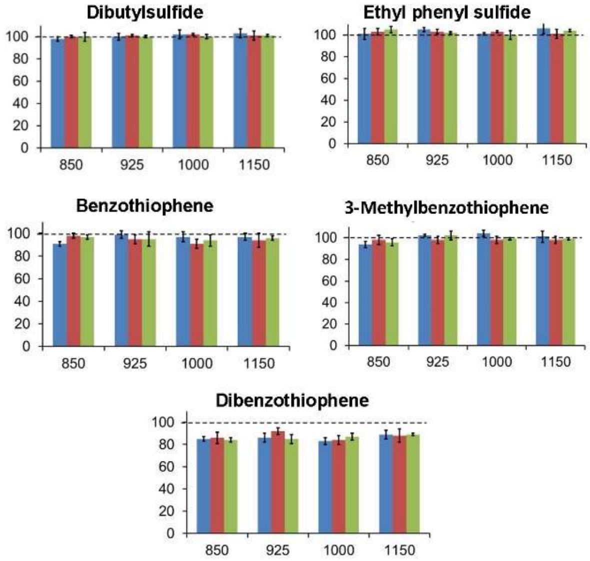


**Figure S8.** $H_2O$ recoveries (m/z 18) for S-containing compounds at different temperatures (850-1150°C) and 0.1, 0.2 and 0.4 mLmin$^{-1}$ $O_2$/He (blue, red and green bars, respectively) using tetradecane as internal standard. Error bars indicate 1 SD (n=3).

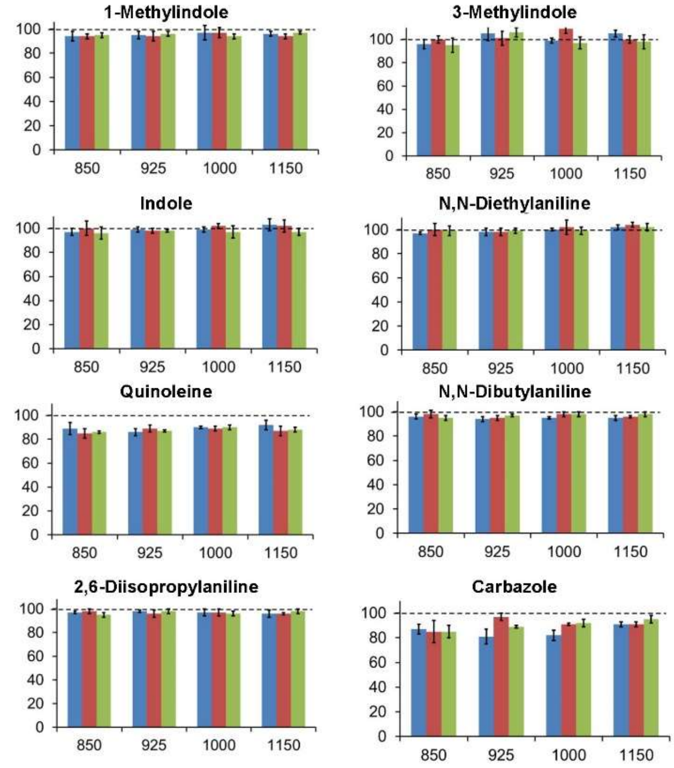


**Figure S9.** $H_2O$ recoveries (m/z 18) for N-containing compounds at different temperatures (850-1150°C) and 0.1, 0.2 and 0.4 mLmin$^{-1}$ $O_2$/He (blue, red and green bars, respectively) using pentadecane as internal standard. Error bars indicate 1 SD (n=3).

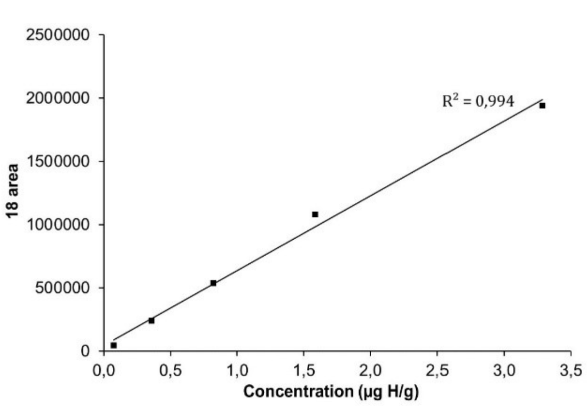


**Figure S10.** Linearity for acenaphthene from 0.020 to 3 µg H $g^{-1}$ using 925°C and 0.4 mLmin$^{-1}$ $O_2$/He.

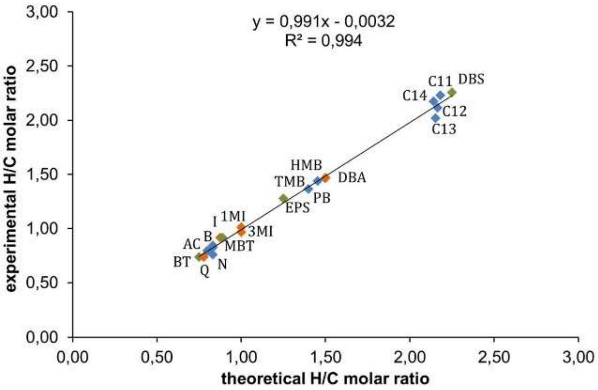


**Figure S11.** Experimental H/C molar ratio against theoretical H/C molar ratio for a mixture of organic compounds without heteroatoms (in blue: C11 - undecane, C12 - dodecane, C13 - tridecane, C14 - tetradecane, PB - pentamethylbenzene, B - biphenyl, HMB - hexamethylbenzene, TMB - tetramethylbenzene, AC - acenaphthene, N - naphthalene), *S*-containing compounds (in green: DBS - dibutyl sulfide, EPS - ethyl phenyl sulfide, BT - benzothiophene, MBT - 3-Methylbenzothiophene) and N-containing compounds (in orange: Q - quinoleine, 1MI - 1-Methylindole, I - indole, 3MI - 3-Methylindole).

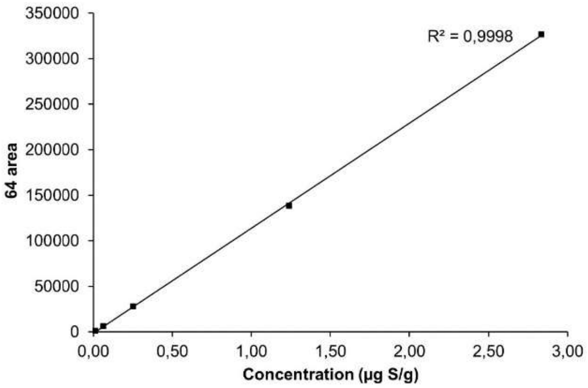


**Figure S12.** Linearity for 3-Methylbenzothiophene from 0.013 to 2.8 µg S $g^{-1}$ using 925°C and 0.4 $mLmin^{-1}$ $O_2/He$.

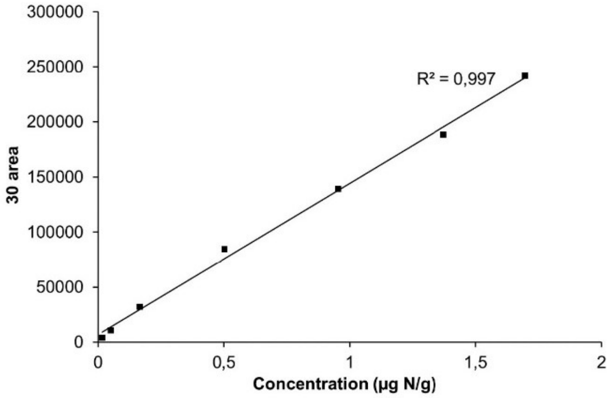


**Figure S13.** Linearity for indole from 0.016 to 1.7 µg N $g^{-1}$ using 1150°C and 0.4 $mLmin^{-1}$ $O_2/He$.

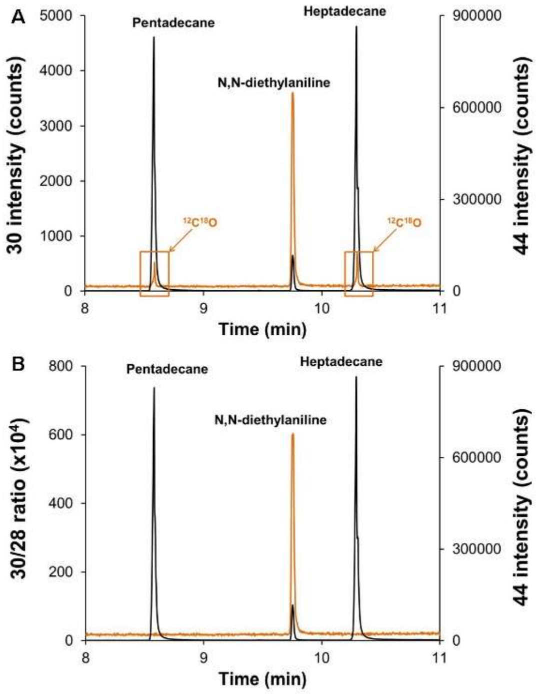


**Figure S14.** Chromatogram for a mixture of two alkanes (pentadecane and heptadecane, ca. 16 µg C $g^{-1}$) and N,N-diethylaniline (ca. 1 µg C $g^{-1}$ and 0.2 µg N $g^{-1}$). A) Signals at m/z 44 and 30 correspond to $CO_2$ and NO, respectively. Interference (unspecific) signal at m/z 30 observed for the alkanes corresponds to the $^{12}C^{18}O$ contribution due to the $CO_2$ degradation at the ion source. Using the response factor obtained from the N,N-diethylaniline, it can be estimated that such signals would correspond to a concentration of nitrogen of ca. 0.016 µg N $g^{-1}$. B) 30/28 and 44 signals. As can be seen, after normalization with the 28 signal (coming from the isotopologue $^{12}C^{16}O$ of the interference $^{12}C^{18}O$), the new 30/28 signal becomes specific of the N presence. As a matter of fact, the interference at 30 for the high concentrated alkanes disappears completely in the new 30/28 profile.

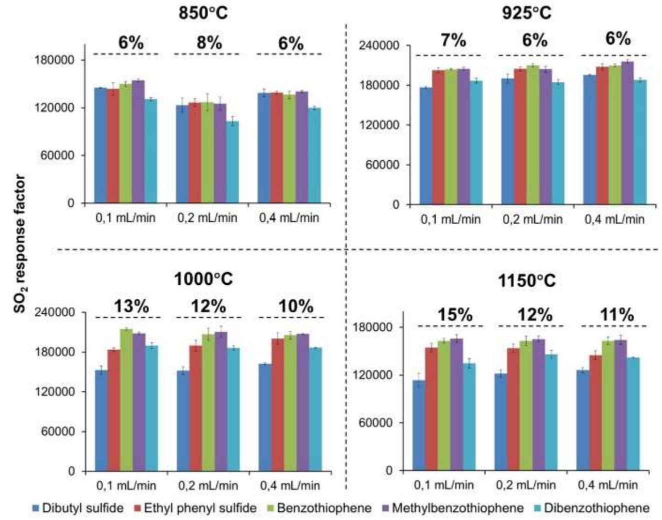


**Figure S15.** $SO_2$ response factors (peak area at m/z 64 divided by the S concentration in the corresponding compound) obtained for the mixture of S-containing compounds (2 µg S $g^{-1}$) at different temperatures (ranging from 850 to 1150°C) and $O_2$/He flows (0.1-0.4 $mLmin^{-1}$). Error bars indicate 1 SD (n=3). RSD of the different response factors obtained for the mixture of compounds under the different conditions assayed are given.

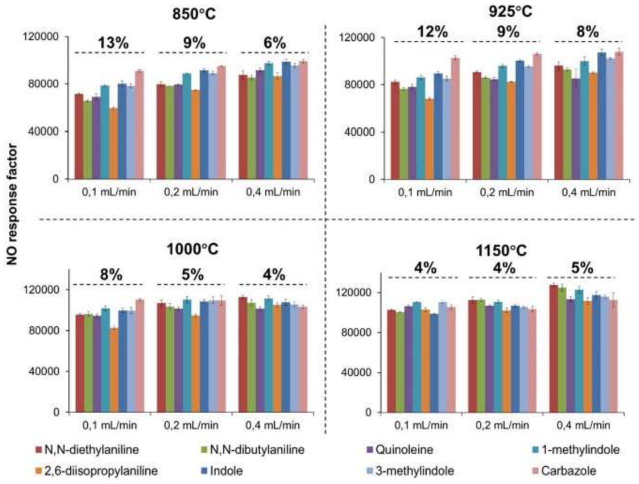


**Figure S16.** NO response factors (peak area at m/z 30 divided by the N concentration in the corresponding compound) obtained for the mixture of N-containing compounds (1 μg N $g^{-1}$) at different temperatures (ranging from 850 to 1150°C) and $O_2$/He flows (0.1-0.4 $mLmin^{-1}$). Error bars indicate 1 SD (n=3). RSD of the different response factors obtained for the mixture of compounds under the different conditions assayed are given.

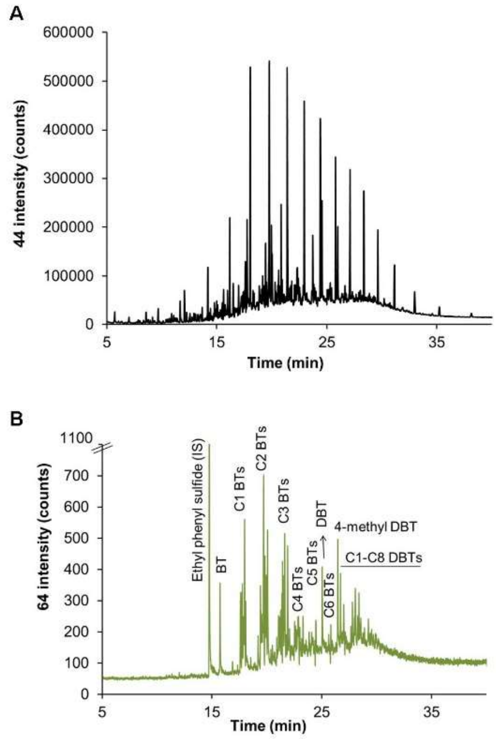


**Figure S17.** Chromatogram for the diesel sample (ca. 4000 µg S $g^{-1}$) diluted 1:800 and spiked with ethyl phenyl sulfide as internal standard; A) m/z 44 and B) m/z 64.

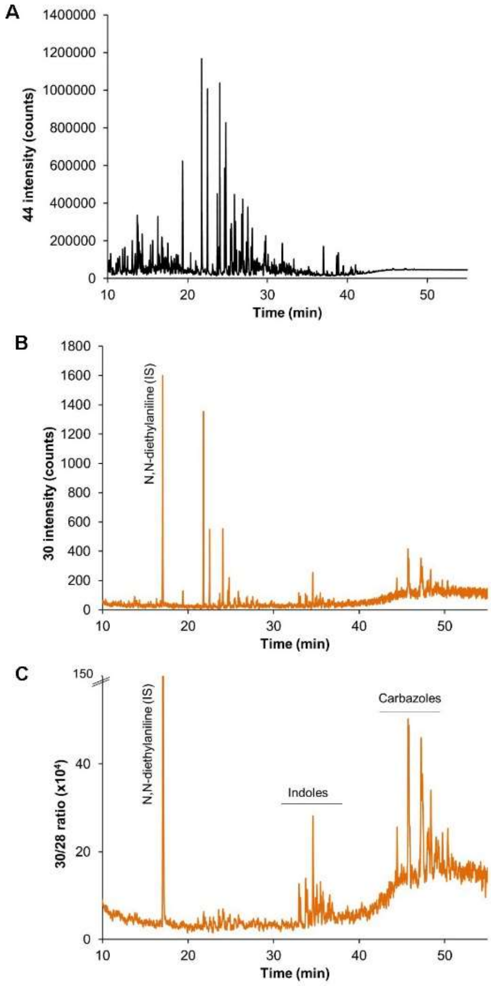


**Figure S18.** Chromatogram for the diesel sample (ca. 400 µg N g-1) diluted 1:500 with the internal standard (N,N-diethylaniline, 0.25 µg N $g^{-1}$) A) m/z **44**, B) m/z 30 (not corrected for $^{12}C^{18}O$ contribution), C) complete N-selective 30/28 profile (corrected for the unspecific $^{12}C^{18}O$ contribution, see Fig S13)

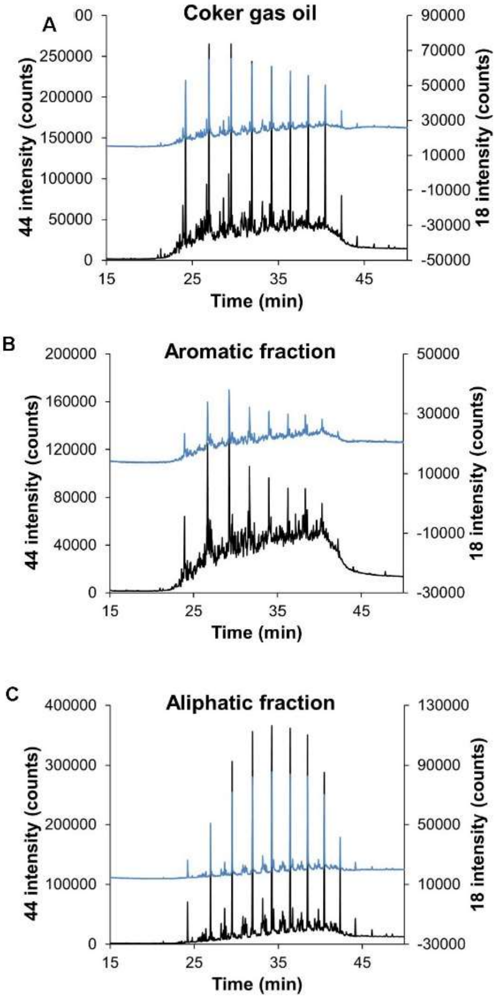


**Figure S19.** Chromatogram for m/z 44 (C, black line) and m/z 18 (H, blue line) for the original coker gas oil sample (A) and its aromatic (B) and aliphatic (C) fractions.

**Table S3.** Elemental composition (µg element g$^{-1}$, n=3) obtained for S- and N-containing compounds using benzothiophene or 1-Methylindole as corresponding generic IS, respectively. Uncertainty corresponds to 1 SD. Concentrations (µg compound g$^{-1}$) of the injected compounds were: Dibutyl sulfide: 9.6 µg g$^{-1}$; Ethyl phenyl sulfide: 10.8 µg g$^{-1}$; Methylbenzothiophene: 9.6 µg g$^{-1}$; Dibenzothiophene: 9.7 µg g$^{-1}$; N,N-Diethylaniline: 6.3 µg g$^{-1}$; N,N-Dibutylaniline: 6.6 µg g$^{-1}$; Quinoleine: 6.6 µg g$^{-1}$; 2,6-Diisopropylaniline: 7.2 µg g$^{-1}$; Indole: 6.8 µg g$^{-1}$; 3-Methylindole: 5.9 µg g$^{-1}$; Carbazole: 6.4 µg g$^{-1}$.

| Compound | Carbon | Hydrogen | Sulfur | Nitrogen |
|---|---|---|---|---|
| Dibutyl sulfide | 6.5 ± 0.2<br>(103±3%) | 1.22 ± 0.04<br>(104±4%) | 2.14 ± 0.08<br>(102±4%) | |
| Ethyl phenyl sulfide | 7.8 ± 0.2<br>(105±2%) | 0.85 ± 0.02<br>(109±2%) | 2.54 ± 0.09<br>(102±4%) | - |
| Methylbenzothiophene | 7.3 ± 0.1<br>(103±2%) | 0.52 ± 0.02<br>(99±3%) | 2.15 ± 0.10<br>(103±5%) | - |
| Dibenzothiophene | 6.7 ± 0.1<br>(89±2%)<br>(102±3%)* | 0.37 ± 0.01<br>(87±2%)<br>(99±5%)* | 1.48 ± 0.03<br>(88±2%)<br>(101±5%)* | - |
| N,N-Diethylaniline | 5.22 ± 0.06<br>(103±1%) | 0.67 ± 0.01<br>(106±2%) | - | 0.62 ± 0.01<br>(106±2%) |
| N,N-Dibutylaniline | 5.48 ± 0.03<br>(101±1%) | 0.75 ± 0.02<br>(102±1%) | - | 0.46 ± 0.01<br>(102±1%) |
| Quinoleine | 5.13 ± 0.04<br>(94±1%) | 0.32 ± 0.01<br>(91±2%) | - | 0.66 ± 0.01<br>(91±2%) |
| 2,6-Diisopropylaniline | 5.79 ± 0.01<br>(99±1%) | 0.78 ± 0.01<br>(101±1%) | - | 0.52 ± 0.01<br>(101±2%) |
| Indole | 5.70 ± 0.02<br>(102±1%) | 0.41 ± 0.01<br>(101±2%) | - | 0.78 ± 0.02<br>(101±1%) |
| 3-Methylindole | 5.06 ± 0.07<br>(104±1%) | 0.41 ± 0.01<br>(101±5%) | - | 0.60 ± 0.01<br>(101±5%) |
| Carbazole | 5.16 ± 0.06<br>(94±1%) | 0.34 ± 0.01<br>(98±4%) | - | 0.49 ± 0.02<br>(98±4%) |
| Mean | 101*% | 100*% | 102*% | 100*% |

*Values obtained when using BD-EN14103 column

**Table S4.** Critical comparison of the analytical features of the detection method presented here with other established detectors in GC for hydrogen, nitrogen and sulfur.

| Detector | DL for H | DL for N | DL for S | Reference |
|---|---|---|---|---|
| SCD | - | - | 3 pg | Anal. Methods, 2016, 8, 7014–7024 |
| NCD | - | 2 pg | - | J. Chromatogr. A, 2012, 1219, 180–187 |
| PFPD | - | - | 3 pg | J. Chromatogr. A, 2006, 1136, 89–98 |
| AED* | 11 pg | 12 pg | 3 pg | Fresenius J Anal Chem, 1997, 357, 1133—1141 |
| ICP-MS/MS | - | - | 0.3 pg | Anal. Chem. 2019, 91, 7019−7024 |
| GC-combustion-MS | 5 pg | 0.7 pg | 0.7 pg | This publication |

*Please note that AED suffers from significant quenching effects in the analysis of complex unresolved samples. Furthermore, simultaneous analysis of C, H, S and N cannot be performed with regular AED instrument becuase it allows multiplexed monitoring of several emission lines only when they fall within the spectral region covered by the photodiode-array spectrometer (typically a window of 20–25 nm). This is the case of H, whose detection wavelength (486 nm), falls out of the window created for simultaneous C, S and N detection (typically detected at 193, 181 and 174 nm, respectively).

**Table S5.** Quantification of total sulfur content and some major species and families in the diesel sample by GC-combustion-MS (2 SD, n=3). Concentrations are given in µg S g$^{-1}$. Total sulfur content determined by X-ray fluorescence method is also given.

| | |
|---|---|
| Total content by X-ray method | 3900 ± 110 |
| GC-combustion-MS | |
| Total analysis | 3743 ± 216 |
| Individual species | |
| Benzothiophene | 49 ± 2 |
| Dibenzothiophene | 83 ± 12 |
| 4-Methylbenzothiophene | 89 ± 15 |
| Families | |
| C1 benzothiophenes | 300 ± 26 |
| C2 benzothiophenes | 458 ± 60 |
| C3 benzothiophenes | 489 ± 50 |
| C4 benzothiophenes | 230 ± 14 |
| C5 benzothiophenes | 361 ± 26 |
| C6 benzothiophenes | 166 ± 26 |
| C1-C8 dibenzothiophenes | 1397 ± 176 |

**Table S6.** Quantification of total nitrogen content and some major families in the diesel sample by GC-combustion-MS (2 SD, n=3). Concentrations are given in µg N g$^{-1}$. Total nitrogen content determined by chemoluminiscence method is also given.

| | |
|---|---|
| Total content by chemoluminiscence | 497 ± 10 |
| GC-combustion-MS | |
| Total analysis | 524 ± 22 |
| Families | |
| Indoles | 111 ± 8 |
| Carbazoles | 368 ± 25 |

**Table S7.** H/C molar ratios for the coker gas oil sample and its corresponding aliphatic and aromatic fractions. Experimental $H_2O/CO_2$ ratios obtained which could be converted into H/C molar ratios using generic standards (given in Figure S9) are also given

| Sample | 18/44 experimental ratio | Calculated H/C molar ratio |
|---|---|---|
| Coker gas oil | 0.15 | 1.46 |
| Aromatic fraction | 0.13 | 1.32 |
| Aliphatic fraction | 0.20 | 1.99 |

## Experimentos asociados

Durante la estancia breve realizada en el laboratorio del Profesor Mondello, se llevó a cabo el análisis de las dos muestras analizadas durante este capítulo mediante los dos sistemas de GC×GC, modulador de flujo y criogénico. El objetivo de estos análisis era la identificación de los compuestos individuales de los perfiles selectivos obtenidos para compuestos de S y N en cada una de las muestras.

Como se ha comentado en el capítulo, la monitorización de compuestos con S y N en muestras de interés energético es relevante. Si bien en este capítulo se llevó a cabo la cuantificación de las especies de N y S de las muestras, la identificación no fue posible realizarla en el mismo prototipo en modo GC-MS dada la baja concentración a la que se encontraban. Esta limitación de los equipos de GC-MS de cuadrupolo simple ha quedado patente durante la introducción, siendo necesario aumentar la selectividad de las metodologías mediante, bien el uso de metodologías para extraer los analitos de la matriz, o el uso de analizadores de espectrometría de masas con mayores resoluciones que el cuadrupolo simple.

Una tercera alternativa es el uso de la cromatografía de gases bidimensional, como se hizo en este caso. Mediante una diferenciación cromatográfica de la matriz, hidrocarburos altamente concentrados, de los compuestos de nitrógeno a bajas concentraciones, un equipo con un analizador de cuadrupolo simple, sería capaz de identificar compuestos a concentraciones muy bajas.

La muestra LCO similar a un diésel se analizó mediante los dos sistema de GC×GC (Figura 19. *Total ion count* cromatograma en dos dimensiones de la muestra de LCO similar a un diésel obtenida con un modulador de flujo y Figura 20. *Total ion count* cromatograma de la muestra de LCO similar a un diésel obtenida con modulador criogénico.) pudiendo identificar los compuestos únicamente en el sistema criogénico. Esto puede deberse a, como se explicó en el experimental, la capacidad de preconcentración de este modulador.

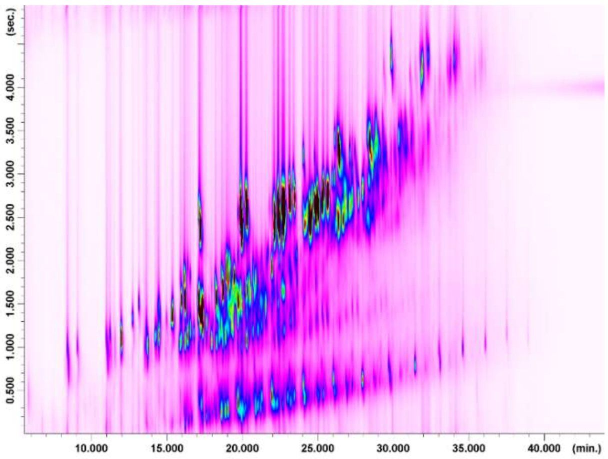


**Figura 19.** ***Total ion count*** **cromatograma en dos dimensiones de la muestra de LCO similar a un diésel obtenida con un modulador de flujo**

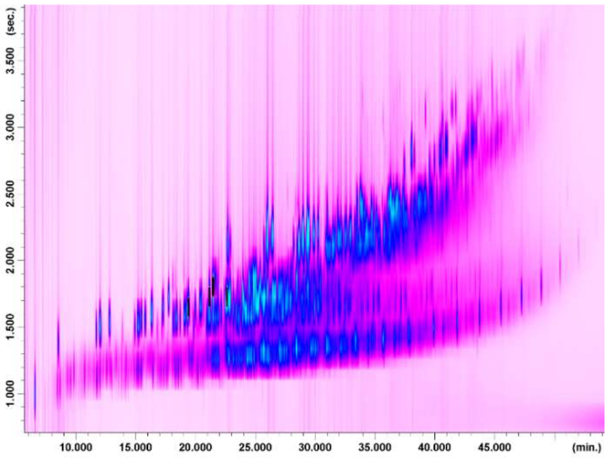
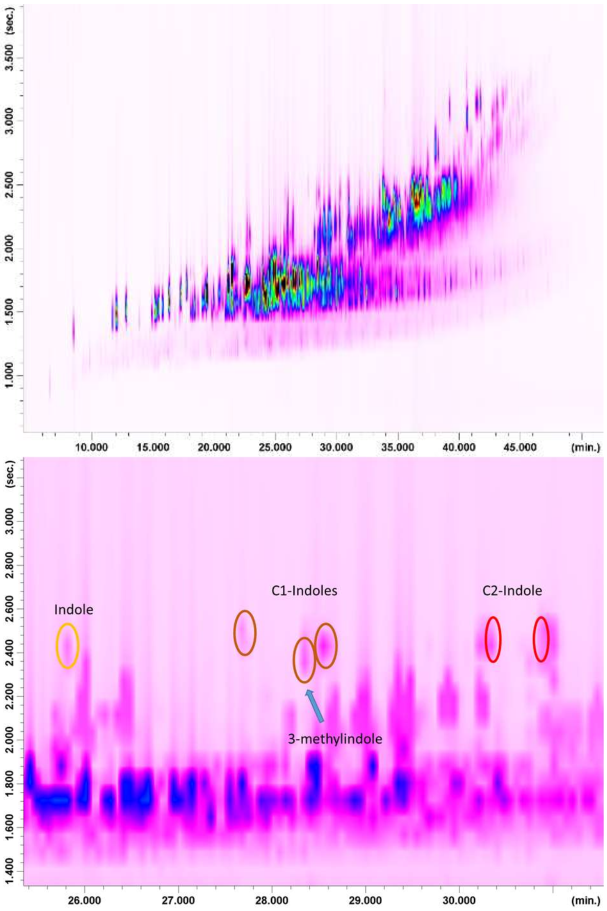


**Figura 20.** ***Total ion count*** **cromatograma de la muestra de LCO similar a un diésel obtenida con modulador criogénico.**

Para llevar a cabo la identificación, se seleccionaron iones característicos en la ionización electrónica basándose en datos obtenidos del NIST. De esta forma, se pueden detectar selectivamente los compuestos de interés, que posteriormente fueron identificados con esta misma biblioteca. En la Figura 21. A) Cromatograma general con iones seleccionados de los compuestos de interés (indoles y carbazoles) en la muestra de LCO Diésel para el modulador criogénico. B) y C) Zoom del cromatograma en las zonas de elución de los compuestos de interés, indoles y carbazoles, respectivamentese muestras las distintas familias de indoles y carbazoles identificadas en la muestra de diésel que concuerdan correctamente con el perfil selectivo obtenido por GC-combustión-MS



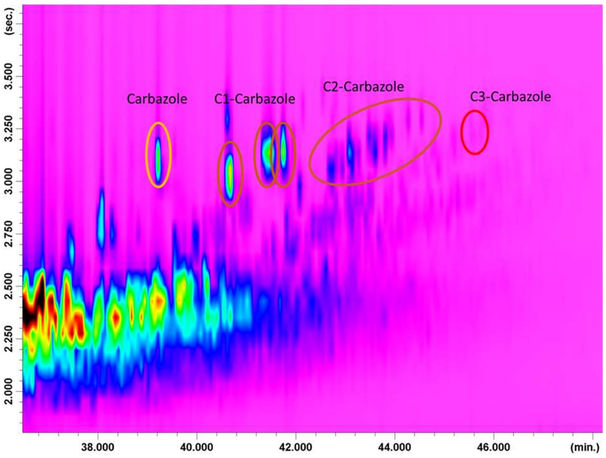


**Figura 21. A) Cromatograma general con iones seleccionados de los compuestos de interés (indoles y carbazoles) en la muestra de LCO Diésel para el modulador criogénico. B) y C) Zoom del cromatograma en las zonas de elución de los compuestos de interés, indoles y carbazoles, respectivamente**

En el caso del otro diésel analizado para el contenido de azufre por GC-combustión-MS, también se llevó a cabo su identificación haciendo uso de ambos moduladores (Figura 22, Figura 23).Se pudo identificar los compuestos de S con ambos moduladores, de nuevo, mediante la extracción de los iones más abundantes para el tipo de compuestos que se esperaba observar.

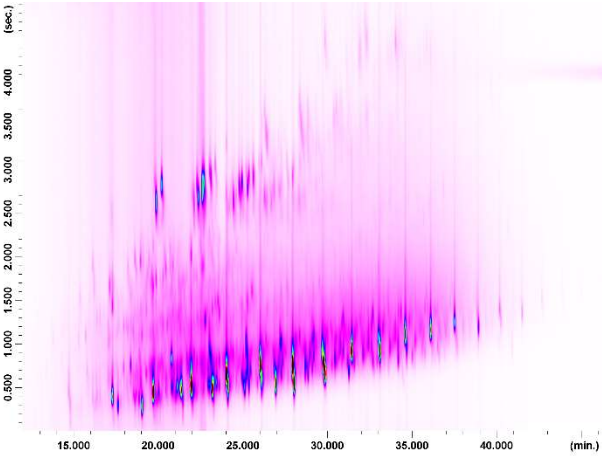


**Figura 22.** ***Total ion count*** **cromatograma de la muestra de diésel certificada en el contenido de azufre obtenida con modulador de flujo.**

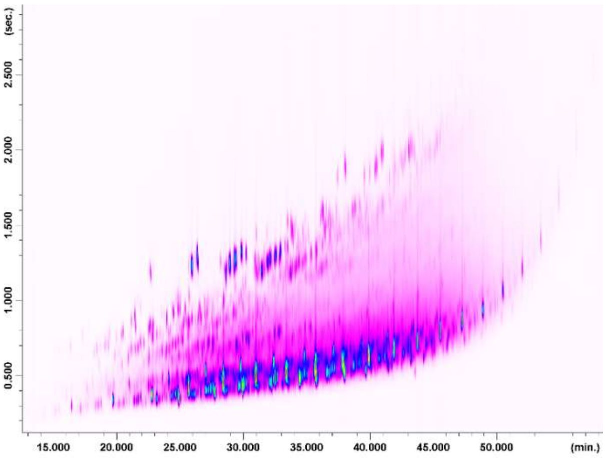


**Figura 23.** ***Total ion count*** **cromatograma de la muestra de diésel certificada en el contenido de azufre obtenida con modulador criogénico.**

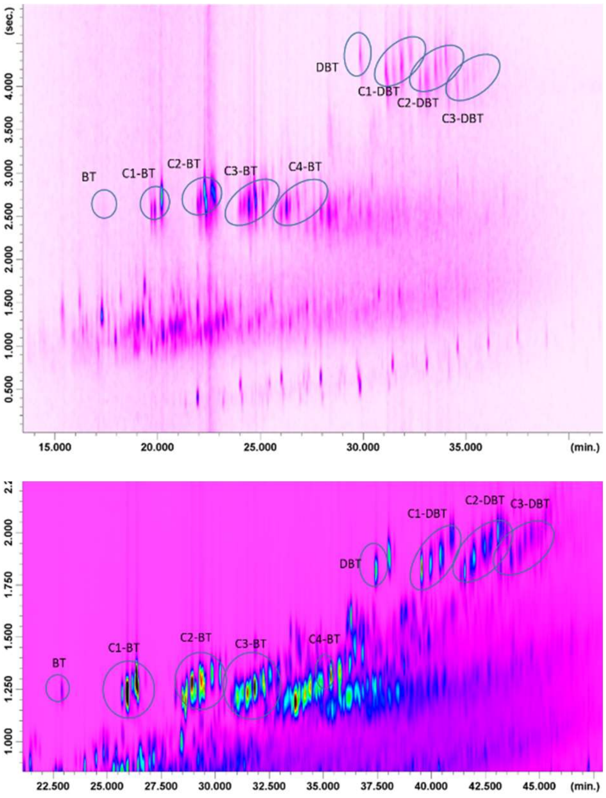


**Figura 24. A) Cromatograma general con iones seleccionados de los compuestos de interés (tiofenos y benzotiofenos) en la muestra de diésel certificada en el contenido de azufre para el modulador de flujo. B) Zoom del cromatograma con**

**iones seleccionados para los compuestos de interés en la muestra de diésel certificada en el contenido de azufre obtenido con el modulador de criogénico.**

Como conclusión para este apartado del capítulo, queda patente la necesidad de metodologías selectivas que permitan, por un lado, la cuantificación y, por otro, la identificación de las especies que se encuentran a concentraciones bajas y en matrices muy complejas. Las limitaciones en estas condiciones del sistema unidimensional de GC-MS queda presente, siendo necesario recurrir a la cromatografía bidimensional para llevar a cabo la identificación. Si bien, el sistema de GCxGC permitiría una cuantificación de las especies identificadas, el hecho de utilizar una fuente de ionización de impacto electrónico, impediría que fuera independiente de la especie y, por tanto, requeriría patrones específicos para cada compuesto identificado. Por otro lado, estos resultados ponen de manifiesto la alta selectividad del prototipo de GC-combustión-MS, siendo capaz de detectar concentraciones a nivel de trazas de los compuestos en matrices, además, muy complejas.

*Anexo. Condiciones de los análisis de cromatografía bidimensional*

| | |
|---|---|
| **Temperatura de inyector** | 300 ºC |
| **Modo de inyección** | Split 1/200 |
| **Volumen de inyección** | 1 µL |
| **Set de columnas** | 1ª dimensión:<br>SLB5-MS (20 m x 0.18 mm ID x 0.18 µm)<br>2ª dimensión:<br>SLB-35 (5 m x 0.32 m ID x 0.25 µm) |
| **Gradiente** | 45°C (3’) to 350 °C at 6°C/min |
| **Tiempo de modulación** | Modulador de flujo:<br>Tiempo de acumulación: 4.6 s<br>Tiempo de reinyección: 0.4 s<br>Modulador de criogénico:<br>Pulso caliente: 0.3 s<br>Pulso frío: 4 s |
| **Modo de adquisición** | SCAN (m/z: 45-450) |

# CAPÍTULO TRES

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## Potencial del detector GC-combustión-MS como detector selectivo de nitrógeno en cromatografía de gases.

## Introducción y contexto

El gran y continuado aumento de las industrias de combustibles en la sociedad actual, conlleva a su vez un aumento exponencial del número de compuestos de interés que analizar tanto cualitativamente como cuantitativamente. En este contexto, el desarrollo de combustibles de origen biológico o a partir de residuos de biomasa, de vertedero o plásticos, se consideran fuentes de energías renovables y sostenibles, lo que puede suponer un importante impulso de cara a producir nuevos combustibles o sustancias químicas a partir de ellos. Sin embargo, dado su origen desconocido, pueden contener cantidades importantes de compuestos que contienen heteroátomos que pueden derivar, tras los distintos procesos de combustión, en compuestos tóxicos como los óxidos de azufre o nitrógeno, que pueden tener un impacto tanto en términos de salud como en la calidad del producto. Desafortunadamente, estos productos se encuentran distribuidos entre multitud de compuestos a concentraciones mucho mayores derivados del complejo carácter de los procesos que influyen en su producción, y por tanto se requieren técnicas y metodologías que consigan detecciones selectivas a niveles ultrasensibles.

En el caso concreto de los compuestos de nitrógeno se conoce su extensa presencia en muestras basadas en crudos de petróleo y, en otras matrices complejas como los bioaceites o los aceites obtenidos a partir de la pirólisis de distintas materias. Durante el refinado de estos productos, los compuestos de nitrógeno pueden causar diversos problemas en el proceso, como es el envenenamiento de catalizadores, la corrosión de partes susceptibles de ello o la formación de materiales gomosos que interfieren en los procesos. Por ejemplo, los compuestos de nitrógeno son dañinos para los catalizadores usados durante el hidrotratamiento heterogéneo que tiene lugar en procesos de pirólisis, dependiendo este efecto del compuesto. Es por ello, que es crítico en el mundo de los combustibles, la determinación de especies individuales de compuestos de nitrógeno, tanto a nivel cualitativo, como cuantitativo de cara a optimizar los procesos de eliminación y mejora de cada producto. Además, la presencia de estos compuestos está directamente ligada con la emisión de especies contaminantes, como son los óxidos de nitrógeno, tras la combustión de los productos. Queda patente, por tanto, la necesidad de desarrollar metodologías capaces de monitorizar y cuantificar compuestos de nitrógeno a niveles de ultratraza en matrices complejas. Si a esto le sumamos el hecho de que existen miles de compuestos de interés, el desarrollo de estas metodologías se verá beneficiado si se usan cuantificaciones independientes de la especie que permitan usar un único patrón para todos los compuestos.

La técnica más ampliamente utilizada para la separación de compuestos volátiles y semivolátiles es la cromatografía de gases acoplada a diferentes detectores, como se ha explicado en la introducción de la presente Tesis Doctoral. Dada la relevancia de los compuestos de nitrógeno, el uso de detectores selectivos hacia este elemento se ha extendido en las últimas décadas, siendo como se ha comentado anteriormente, el AED, el NPD y el NCD los tres detectores desarrollados para una detección selectiva y más ampliamente usados en multitud de laboratorios. Dentro de estos, el NCD se ha establecido como el detector más relevante dada su alta sensibilidad, selectividad y equimolaridad relativa. Sin embargo, como se comentó en la introducción, cualquier detector espectroscópico puede sufrir limitaciones importantes debidas a la presencia de una gran cantidad de matriz, como efectos de matriz o de quenching. Una alternativa a los detectores señalados anteriormente es el uso de la espectrometría de masas como detector selectivo mediante cuando se usa en modo *single-ion monitoring* (SIM). Sin embargo, este modo de medida, está muy limitado para obtener información de familias específicas de compuestos, como las de N debido a las interferencias isobáricas en el MS debido a la coelución de la matriz. Además, el proceso de ionización en la MS es dependiente de la especie, siendo necesario el uso de patrones específicos para cuantificar cada compuesto individual de N.

Durante este capítulo, se extiende el detector de GC-combustión-MS desarrollado durante parte de esta tesis doctoral como detector selectivo de nitrógeno. Se detalla la optimización llevada a cabo para obtener una señal más reproducible de N. También se presenta el nuevo prototipo desarrollado junto con la empresa Shimadzu, con el que se llega a observar una mejora de la sensibilidad de más de un orden de magnitud, obteniendo los mejores límites de detección obtenidos hasta la fecha para un detector selectivo de nitrógeno. Además, de cara a llevar a cabo un estudio en profundidad de sus características, el detector se comparó críticamente con el NCD incluyendo su aplicabilidad para cuantificar especies de nitrógeno con patrones genéricos, en muestras de diésel y aceites de pirólisis de biomasa.

Al igual que en anteriores capítulos, se hizo uso de la cromatografía de gases bidimensional para llevar a cabo una identificación más exhaustiva de las muestras analizadas a lo largo del capítulo.

Artículo científico IV.

**Título**: *Potential of GC-Combustion-MS as Powerful and Versatile Nitrogen Selective Detector in Gas Chromatography*

**Autores**: Javier García-Bellido, Laura Freije-Carrelo, Montserrat Redondo-Velasco, Marco Piparo, Mariosimone Zoccali, Luigi Mondello, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti and Jorge Ruiz Encinar

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# Potential of GC-Combustion-MS as a Powerful and Versatile Nitrogen-Selective Detector in Gas Chromatography

Javier García-Bellido, Laura Freije-Carrelo, Montserrat Redondo-Velasco, Marco Piparo, Mariosimone Zoccali, Luigi Mondello, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti, and Jorge Ruiz Encinar*

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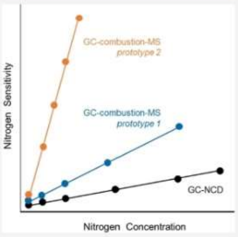
**ABSTRACT:** Here, we show the potential and applicability of the novel GC-combustion-MS approach as a nitrogen-selective GC detector. Operating requirements to achieve reproducible and compound-independent formation of volatile NO species as a selective N-signal during the combustion step are described. Specifically, high temperatures (≥1000 °C) and post-column $O_2$ flows (0.4 mL $min^{-1}$ of 0.3% $O_2$ in He) turned out to be necessary when using a vertical oven without makeup flow (prototype #1). In contrast, the use of a horizontal oven with 1.7 mL $min^{-1}$ He as an additional makeup flow (prototype #2) required milder conditions (850 °C and 0.2 mL $min^{-1}$). A detection limit of 0.02 pg of N injected was achieved, which is by far the lowest ever reported for any GC detector. Equimolarity, linearity, and peak shape were also adequate. Validation of the approach was performed by the analysis of a certified reference material obtaining accurate (2% error) and precise (2% RSD) results. Robustness was tested with the analysis of two complex samples with different matrices (diesel and biomass pyrolysis oil) and N concentration levels. Total N determined after the integration of the whole chromatograms (524 ± 22 and 11,140 ± 330 $\mu$g N $g^{-1}$, respectively) was in good agreement with the reference values (497 ± 10 and 11,000 ± 1200 $\mu$g N $g^{-1}$, respectively). In contrast, GC-NCD results were lower for the diesel sample (394 ± 42 $\mu$g N $g^{-1}$). Quantitative values for the individual and families of N species identified in the real samples by parallel GC−MS and additional GC × GC−MS analyses were also obtained using a single generic internal standard.



The great and continuous development of fuel and biofuel industries, among others, has brought an exponential growth in the number of target compounds to be detected and quantified. In this context, new feedstocks of biological origin or derived from waste (i.e., biomass, landfill waste, and plastics) are renewable and sustainable resources showing high potential to produce fuels and chemicals. Due to their uncontrolled origin, they may contain significant amounts of heteroatomic compounds[1] that lead to the formation of hazardous components (i.e., $NO_x$ and $SO_x$), which can have a negative impact in terms of safety and product quality. Unfortunately, such heteroatoms are split into a myriad of compounds due to the complex character of the chemical processes typically involved in their production (e.g., pyrolysis) and therefore require ultrasensitive and selective approaches for their characterization. In particular, nitrogen-containing compounds have been extensively reported in crude oil-based fuels[2,3] and other complex matrices such as bio-oil[4] and plastic-based pyrolysis oil.[5,6] During refining, N-compounds can cause catalyst poisoning, fouling, equipment corrosion, and gum formation.[7] For example, N-containing species are harmful for heterogeneous hydrotreatment catalysts during bio-oil pyrolysis, and this adverse effect is species-dependent. This is why it is critical to find out what individual N-species are present, and in which quantity, in order to select and optimize the industrial process for their removal to upgrade the product.[6] Moreover, their presence in fuels contributes to the environmental release of air pollutants NOx after their combustion.[8] It is therefore evident the need for analytical technologies able to monitor and quantify N-compounds at ultralow levels in such complex matrices without the need for specific standards due to the huge number of possible targets.

Gas chromatography (GC) is the technique of choice for the separation of volatile compounds that, when coupled with a suitable detector, can accomplish the analysis of a large number of organic compounds in several complex matrices. Given the relevance of N-compounds' analysis mentioned above, N-selective detectors such as the atomic emission,[9] nitrogen–phosphorus,[10] and nitrogen chemiluminescence (NCD)[11,12] detectors have been developed and are being extensively used in many laboratories. In recent years, the NCD has established

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as the most powerful N-selective detector due to its selectivity, sensitivity, and equimolarity.[13] Despite their technical capacities, all these spectroscopic detectors suffer however from some important limitations such as significant matrix[11] and quenching[14] effects when analyzing complex unresolved samples. Alternatively, mass spectrometry (MS) provides universal detection and structural identification as well as compound-selective detection when operated in single-ion monitoring (SIM). Unfortunately, this mode is highly limited in complex samples for the screening of specific families of compounds, such as N-containing ones, due to chromatographic coelutions and isobaric interferences in MS.[15] Additionally, the ionization process in MS is compound-dependent, which entails the need for specific standards to carry out the quantification of every individual N-containing target.[16]

A new detection system in GC able to provide generic carbon-based universal quantification of organic compounds while maintaining the structural elucidation capabilities of MS by simply actuating a switching valve was introduced in 2009.[17] A combustion interface was developed and installed in a GC–MS instrument for the quantitative conversion of each and every organic compound eluting from the column into $CO_2$ before the detection by MS, making their quantification truly compound-independent. Recently, such system was greatly improved based on the idea that the same way as the combustion of an organic at the exit of a GC column produces $CO_2$, other volatile species such as $H_2O$, $SO_x$, and $NO_x$ (if S and N are present) would be produced as well, opening the door to parallel S- and N-selective detection.[18] Therefore, the long-wished detection combining structural identification with compound-independent calibration, both universal (C, H) and element-selective (N, S), for every volatile organic compound separated by GC became within reach since then.

The focus of this work is to critically assess the potential of the innovative GC-combustion-MS approach as a powerful N-selective detector. We will describe in detail the system optimization and characterization, including the introduction of a new and greatly improved prototype that offers unsurpassed detection limits. Analytical validation will be performed by the analysis of a CRM (carbazole standard). Its analytical figures as a N-selective detector (detection limits, equimolarity, and complementary qualitative information) will be critically compared with those of the established and most widespread GC-NCD, including their applicability to the total and individual quantification of N species without specific standards in complex samples, such as diesel and biomass pyrolysis oil. In this case, the trueness of the results obtained was evaluated in comparison to the corresponding established ASTM methods.

## ■ EXPERIMENTAL SECTION

**Reagents, Solutions, and Materials.** Dichloromethane, hexane, pentadecane (C15), heptadecane (C17), *N,N*-diethylaniline (DEA, 99%), *N,N*-dibutylaniline (DBA, 99%), quinoleine (Q, 98%), 1-methylindole (1MI, 97%), 2,6-diisopropylaniline (DPA, 100%), indole (I, 99%), 3-methylindole (3MI, 98%), carbazole (C, 95%), 4-ethylpyridine (4EPy, 98%), diethylpropionamide (DEPA, 99%), aniline (A, 99.5%), nitrobenzene (NBz, 99%), 1,2-dimethyl-3-nitrobenzene (DMNBz, 97%), benzonitrile (BZN, 99.9%), capryloni-trilo (HpCN, 99%), and caprolactame (CAP, 100%) (see Figure S1) were purchased from Sigma-Aldrich. The Certified Reference Material D-4629-91-HB-CON, consisting of a nitrogen solution for high boiling solvents containing carbazole (998 μg N $g^{-1}$) in toluene/acetone (9:1), was acquired from AccuStandard. Helium and the mixture of 0.3% (v/v) $O_2$ in He were obtained from Air Liquide and Linde AG, respectively. Real samples, diesel and biomass pyrolysis oil, were provided by TotalEnergies Raffinage Chimie.

**Instrumentation.** *GC Separations.* Two different analytical columns, a BD-EN14103 (30 m × 0.32 mm ID × 0.25 μm) and a HP1-MS (50 m × 0.2 mm ID × 0.5 μm), both from Agilent J&W Scientific were used. Experimental conditions are summarized in Table S1.

*GC-Combustion-MS. Prototype #1.* Initially, an Agilent 6890 GC coupled with a 5973 Network quadrupole mass spectrometer equipped with an electron ionization source and a split/splitless inlet was used. A manually actuated high-temperature six-way valve (VICI Valco) was installed inside the GC oven to bypass the combustion furnace when necessary, allowing the setup to work under GC–MS (Figure S2A) or GC-combustion-MS (Figure S2B) configurations. The combustion interface, located on top of the GC, consisted of a combustion oven (Carbolite Gero) in which a ceramic tube (400 mm length × 3 mm width × 0.5 mm ID) (Elemental Microanalysis) containing two Pt wires was inserted and connected to the six-way valve by means of a metallic inert tubing and a reducing union (VICI Valco). A flow of $O_2$ diluted in He (0.3% v/v) provided by a Mass Flow Controller (MFC, Bronkhorst) was mixed online with the eluting flow from the column before entering the ceramic tube using a capillary flow "Tee" (Capillary Flow inert Tee, Agilent) located inside the GC oven. **Prototype #2**: Later, a much modern instrument (Shimadzu GC-MS-QP-2020NX), equipped with an electron ionization source and a split/splitless inlet, was modified based on the GC-combustion-MS prototype previously described. An automatically actuated high-temperature six-way valve was installed inside the GC oven, allowing the setup to work under GC–MS (Figure S3A) or GC-combustion-MS (Figure S3B) configurations. In this case, the combustion interface was horizontally placed on the right side of the GC oven and connected by means of a metallic block heated at 250 °C, which had two inlets. The first one was used to introduce an additional He makeup flow (ca. 1.7 mL $min^{-1}$) to protect the capillary interface and reduce peak broadening. The second one was used to introduce the $O_2$ diluted in He (0.3% v/v). The total flow was then introduced into the same ceramic tube (containing two Pt wires) for combustion.

*GC-NCD Instrument.* The system consisted of a GC Agilent 6890 N equipped with a 255 Agilent NCD detector. A nonpolar HP-1 (50 m × 0.2 mm ID × 0.5 μm) column was used. The system was operated under the optimum conditions indicated by the manufacturer. The combustion temperature was 950 °C with 6.0 mL/min hydrogen and 9.0 mL/min oxygen flow rates.

*GC × GC–MS Instrument.* Additional identification of the N-compounds present in the real samples was carried out using a Shimadzu GC × GC-QqQ MS instrument (operated in SCAN mode), consisting of a GC-2010 with a split/splitless injector and an AOC-20i autosampler, coupled with a TQ8040 MS. SLB-5ms (20 m × 0.18 mm ID × 0.18 μm) and SLB-35 (5 m × 0.32 mm ID × 0.25 μm) columns (Merck Life Science) were used as first and second dimension, respectively. The modulation was performed every 5 s (an accumulation time of 4.6 s and a re-injection period of 0.4 s) by using a flow modulator consisting of a 7-port wafer with an accumulation loop with a dimension of 20 cm × 0.51 $\mu$m developed by Chromaleont and Trajan (Trajan Scientific and Medical).[19] The system was operated in the constant flow mode in both the first and the second dimensions at 0.4 and 8 mL min$^{-1}$, respectively.

## ■ RESULTS AND DISCUSSION

**Assessment of the Combustion Efficiency.** We assessed first the impact of key parameters on the complete combustion of the target compounds using the prototype #1, such as the temperature of the combustion oven and the $O_2$/He flow added online post-column. For that purpose, we evaluated the formed $^{12}C^{16}O_2$ (measured at $m/z$ 44), originated from the carbon present in each compound. A mixture of eight N-compounds (DEA, DBA, DPA, I, 1MI, 3MI, Q, and C; ca. 5−6 $\mu$g C g$^{-1}$) and two alkanes (C15 and C17; ca. 6 $\mu$g C g$^{-1}$) was prepared in hexane and injected in triplicate for each condition using splitless mode. Four different temperatures (850, 925, 1000, and 1150 °C) combined with three $O_2$/He flows (0.1, 0.2, and 0.4 mL min$^{-1}$) were evaluated. As an example, the black line in Figure 1 shows the GC-combustion-MS chromatogram obtained at $m/z$ 44 for the eight N-compounds and two alkanes at 1150 °C and 0.4 mL min$^{-1}$. Pentadecane was selected as an internal standard (IS), since its quantitative combustion was already proved,[17] to compute the recoveries of the rest of the nine eluting peaks of the chromatogram. As can be seen in Figure S4, the average of the nine recoveries computed under each condition assayed was always quantitative, ranging from 96 ± 4 to 99 ± 4% ($n$ = 9). These results clearly demonstrated the completeness of the combustion reaction regardless of the temperature and $O_2$/He flow used.

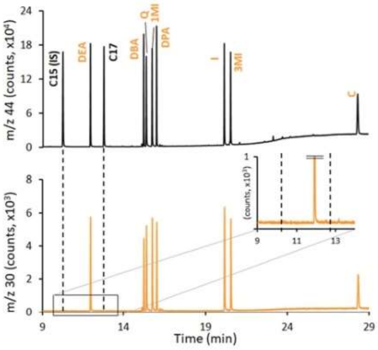

Figure 1. GC-combustion-MS chromatogram of a mixture of eight N-compounds and two alkanes (ca. 5−6 $\mu$g C g$^{-1}$ each compound). Compounds' abbreviations are written out in the Experimental Section. Black and orange lines correspond to $m/z$ 44 (C) and $m/z$ 30 (N) signals, respectively. Inset shows the absence of N-signal at the retention times of the two alkanes.

**Assessment of the Nitrogen-Selective Detection.** The previous mixture was also used to assess the potential of the formation of $^{14}N^{16}O$ ($m/z$ 30) as an N-selective signal under different experimental conditions using prototype #1 (orange line in Figure 1). It is difficult to study $NO_2$ formation because the $^{14}N^{16}O_2$ ($m/z$ 46) signal is highly interfered by the formation of the $CO_2$ isotopologue, $^{12}C^{16}O^{18}O$ ($m/z$ 46). However, the very similar isotope ratios 44/46 measured in N-compounds (230 ± 3, $n$ = 10) and alkanes (235 ± 9, $n$ = 10) suggest negligible $NO_2$ formation. On the other hand, the absence of signal at $m/z$ 30 for pentadecane and heptadecane clearly indicates the selective N-detection as NO. The N concentration ranged from 0.45 to 0.82 $\mu$g N g$^{-1}$, depending on the species. In order to assess the NO formation under the different instrumental conditions assayed and make it independent of the sensitivity changes intra- and inter-chromatograms performed, we normalized each N response factor (peak area at 30 per N concentration unit) by the corresponding and already demonstrated quantitative C response factor (peak area at 44 per C concentration unit). Figure 2A shows the average of the normalized NO response factors obtained for the eight N-containing compounds at the four different temperatures (850, 925, 1000, and 1150 °C) and three $O_2$/He flows (0.1, 0.2, and 0.4 mL min$^{-1}$). It is clear that the observed N response factors increased both with temperature and the $O_2$ flow within each temperature. It seems that even though the combustion process is complete already at 850 °C and using the lowest $O_2$ flow as indicated by the $CO_2$ recoveries (Figure S4), efficient NO formation

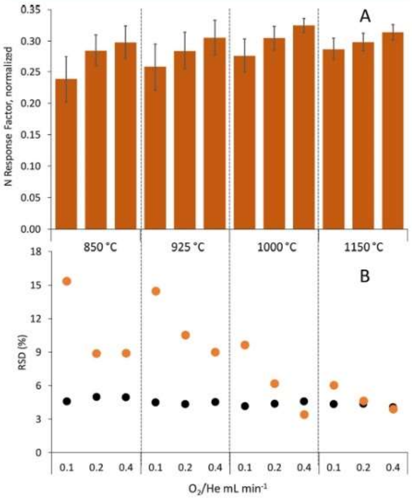

Figure 2. (A) Average N response factor ($n$ = 8), expressed as formed NO ($m/z$ 30) normalized by C response factor at ($m/z$ 44), obtained at different combustion oven temperatures (850, 925, 1000, and 1150 °C) and $O_2$/He flows (0.1, 0.2, and 0.4 mL/min) using the prototype #1. Uncertainty corresponds to one standard deviation ($n$ = 3). (B) Relative standard deviation (%) obtained at each temperature and flow for the response factors of C and N (black and orange, respectively).

requires more drastic conditions, what makes sense according to their standard enthalpies of formation at high temperatures (−393 and +90 kJ mol$^{-1}$ for $CO_2$ and NO, respectively). In particular, average NO normalized factors were statistically lower (0.24 ± 0.04 and 0.26 ± 0.04) when using the lower $O_2$ flow rate at the two lower temperatures. This effect will determine the sensitivity of the N-detection. Notably, while average NO response factors were stable (0.30, 0.31, 0.32, and 0.31) when using the highest $O_2$ flow (0.4 mL min$^{-1}$) regardless of the temperature used, their variability increases significantly when moving from higher (3−4% RSD) to lower temperatures (8−9% RSD), as clearly shown in Figure 2B. In fact, the precision of the NO response factors obtained for the eight different N-compounds evolves from values above 15% RSD at 850 and 925 °C and 0.1 mL min$^{-1}$ $O_2$ to a plateau around 3−6% RSD obtained at both, 1150 °C regardless the $O_2$ flow and 1000 °C only at the highest flows (0.2−0.4 mL min$^{-1}$ $O_2$). This effect will determine the equimolarity of the N-detection. In fact, Figures 2B and S4 show that the universal C-detection at $m/z$ 44 is species-independent always, which suggests that combustion is complete, regardless of the temperature-$O_2$ flow combination assayed. In contrast, Figure 2A,B seems to indicate that NO formation is strongly favored, becoming species-independent, only at high (0.4 mL min$^{-1}$) $O_2$ flow rates, easing this requirement at higher temperatures. Finally, a temperature ≥1000 °C and a 0.4 mL min$^{-1}$ $O_2$/He flow were selected as optimum for N-selective detection when using prototype #1.

Surprisingly, the results obtained for the same mixture were quite different when using the prototype #2. As can be seen in Figure S5, the average of the nine carbon recoveries (using pentadecane as reference) computed using the three $O_2$/He flows (0.1, 0.2, and 0.4 mL min$^{-1}$) at different temperatures (850, 925, and 1000 °C) was always close to 100% (ranging from 96 ± 5 to 104 ± 4%), indicating again the completeness of the combustion reaction regardless the experimental conditions used. However, the behavior of the average of the eight NO normalized factors was different. Figure 3 clearly shows that, except for the lowest $O_2$ flow at 850 (0.57 ± 0.03) and 925 °C (0.57 ± 0.03), the rest of the experimental conditions assayed led to consistent average NO normalized factors with values ranging from 0.61 ± 0.01 to 0.65 ± 0.02. Additionally, the precision of the normalized NO response factors obtained for the eight different N-compounds was always excellent (around 3% RSD) and very similar to the precision of the C response factors, regardless the experimental conditions used. Therefore, it was not necessary to assay higher temperatures (1150 °C) for prototype #2.

However, the selectivity of the proposed N detection using the NO signal should be evaluated under stringent conditions where the concentration of other organic but noncontaining N-compounds is very high simulating the actual conditions in real complex samples. In this regard, a challenge to the proposed selective N-detection is the in-source fragmentation of $CO_2$ to CO. This is generally established and shown in $CO_2$ NIST spectra with an abundance close to 10% (28/44 ratio of 0.098). Of course, its very minor isotopologue $^{12}C^{18}O$, detected as $m/z$ 30, is also produced and could lead to false positives when searching for N-containing compounds using the $^{14}N^{16}O$ signal in the sample. The very low abundance of $^{18}O$ (0.2%) makes that this interference is not detected at all in solutions injected at concentrations below 5 $\mu$g C g$^{-1}$, as clearly shown in Figure 1. A mixture containing pentadecane and heptadecane at high concentrations (14 and 19 $\mu$g C g$^{-1}$, respectively) and two low concentrated N-containing compounds *N*,*N*-diethylaniline and *N*,*N*-dibutylaniline (ca. 2 $\mu$g C g$^{-1}$ and 0.2 $\mu$g N g$^{-1}$) was injected. As illustrated in Figure 4 (red trace), under these conditions, a tiny but still detectable unspecific signal is observed at $m/z$ 30 for both alkanes, which therefore requires an appropriate correction. As we can assume that within the ion source, the formation of $^{12}C^{18}O^+$ ($m/z$ 30) is isotopically related to the formation of $^{12}C^{16}O^+$ ($m/z$ 28), a

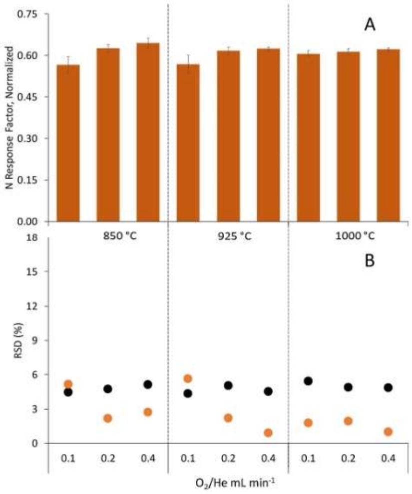

**Figure 3.** (A) Average N-compounds response factor ($n$ = 8), expressed as formed NO ($m/z$ 30) normalized by C response factor at ($m/z$ 44), obtained at different combustion oven temperatures (850, 925, and 1000 °C) and $O_2$/He flows (0.1, 0.2, and 0.4 mL/min) using the prototype #2. Uncertainty corresponds to one standard deviation ($n$ = 3). (B) Relative standard deviation (%) obtained at each temperature and flow for the response factors of C and N (black and orange, respectively).

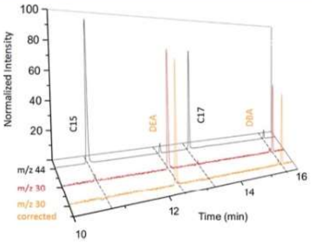

**Figure 4.** Chromatogram of a mixture of C15 and C17 (ca. 14 and 19 $\mu$g C g$^{-1}$, respectively), *N*,*N*-diethylaniline (DEA), and *N*,*N*-dibutylaniline (DBA) (ca. 2 $\mu$g C g$^{-1}$ and 0.2 $\mu$g N g$^{-1}$): $m/z$ 44 (black line), $m/z$ 30 (red line), and corrected $m/z$ 30 (orange line).

correction for the unspecific signal at $m/z$ 30 can be performed based on the $^{18}O/^{16}O$ abundance ratio (isotopic abundances of $^{16}O$ and $^{18}O$ are 99.76% and 0.2%, respectively) by measuring in parallel the signal at $m/z$ 28. In fact, after application of this correction point by point (orange trace), the unspecific $m/z$ 30 signal for the alkanes is eliminated. Notably, as can be seen in Figure 4, the correction applied did not lead to signal-to-noise ratio deterioration, being the chromatographic profiles of the raw (30) and corrected NO (30/28) very similar. For that reason, we recommend to perform the correction always in the analysis of real samples.

**Analytical Characteristics.** First, in order to study peak broadening, a solution containing approximately 2 $\mu$g compound $g^{-1}$ of indole and 3-methylindole in *n*-hexane was injected both in the qualitative (GC−MS) and quantitative (GC-combustion-MS) modes. The TIC chromatogram obtained is shown in Figure S6A, whereas the chromatogram obtained at masses 44 (C) and 30 (N) after combustion is shown in Figure S6B. As can be observed, no significant peak broadening due to the combustion unit or to the different connections was observed, being the peak width measured at the half height (0.030 min) only slightly higher to that found when operating the GC−MS in the conventional way (0.025 min). An increase in retention times was observed (∼40 s) because of the combustion furnace.

Then, in order to study the GC-combustion-MS equimolar response, we resorted again to the same mixture of eight N-compounds covering most of the relevant N-compounds families. Since compound concentration could play a role in the response factors obtained, calibration graphs ($n$ = 7) covering more than 2 orders of magnitude (for instance, from 0.016 to 1.7 $\mu$g N $g^{-1}$ for indole) were built to check for such concentration-dependent effects. The calibration slopes obtained for every individual N-compound were very similar with excellent linearities. In fact, as shown in Figure 5, a "multispecies" generic calibration plot containing every calibration point of each of the eight N-compounds under study (total $n$ = 56) can be built with excellent linearity ($r^2$ = 0.9944). The most striking conclusion to emerge from Figure 5 is that full species-independent response can be obtained when using GC-combustion-MS as a N-selective detector. This equimolarity adds to that already observed for the GC-combustion-MS system when working as a universal detector through C signal ($CO_2$, $m/z$ 44).[17,20] Of course, this feature translates into the possibility to carry out accurate quantifications of the diverse N-containing compounds present using a simple N-containing compound as an internal standard. Such possibility was assessed with an extended mixture containing up to 16 different N-compounds including different new N-functionalities such as open and closed amides (*N,N*,-diethylpropionamide and caprolactam), pyridines, nitriles (caprylo and benzonitrile), and nitro derivates. In order to avoid coelutions, the different N-compounds were distributed in three different mixtures and 2,6-diisopropylaniline was chosen always as an internal standard. Figure S7 displays the different chromatograms obtained, while quantitative results are given in Table S2. As can be observed, this methodology allowed us to quantify a full series of N-compounds with a broad range of boiling points (from 168 to 355 °C), with acceptable precision (an average value of 2% RSD, $n$ = 3) and accuracy (a mean recovery of 98 ± 5%, 2 SD, $n$ = 15), without the need for specific standards. For comparison purposes, we wanted to evaluate the equimolar response of the N-selective detector of reference, GC-NCD by analyzing a mixture of N-compounds covering different organic chemical structures and in a range of concentrations from ca. 4 to 100 $\mu$g N $g^{-1}$. Such higher concentration range was selected due to the much lower sensitivity observed of the GC-NCD compared to the GC-combustion-MS. As it can be seen in Figure S8, a "multi-species" generic calibration plot (total $n$ = 42) could be built with excellent linearity ($r^2$ = 0.994) as well.

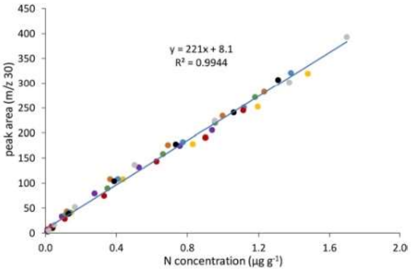

**Figure 5.** Multispecies generic calibration curve obtained using GC-combustion-MS (prototype #1) for a mixture of N-containing compounds. Color code: *N,N*-diethylaniline (orange), indole (grey), 2,6-diisopropylaniline (green), carbazole (red), *N,N*-dibutylaniline (violet), 1-methylindole (blue), quinoleine (yellow), and 3-methylindole (black).

Detection limit (DL) was then calculated for the prototype #1 based on three times the standard deviation of the baseline and turned out to be 0.7 pg of N injected. This is already better than the DL of the well-established N-selective GC-NCD instrument (typically ranging from 2 to 20 pg N).[21,22] Nevertheless, the great improvement was observed when using the prototype #2, which provided an impressive DL of 0.02 pg of N injected, at least 2 orders of magnitude lower than NCD and, to the best of our knowledge, by far the lowest detection limit for N ever published for a GC detector. Such much higher sensitivity is borne out by the calibration plots obtained for indole using prototype #2 (0.0013 to 0.13 $\mu$g N $g^{-1}$), prototype #1 (0.02 to 1.7 $\mu$g N $g^{-1}$), and NCD (3 to 84 $\mu$g N $g^{-1}$) as shown in Figure S9. It must be noted that the great sensitivity improvement in N-detection using the prototype #2 in comparison to the prototype #1 is most likely due to the brut sensitivity difference between a modern (#2) and an old (#1) MS instrument.

**Validation.** A certified reference material, carbazole (998 ± 24 $\mu$g N $mL^{-1}$) was spiked with 3-methylindole as an internal standard and analyzed by GC-combustion-MS. Figure S10 shows the chromatogram obtained. Experimental results obtained, 977 ± 39 $\mu$g N $mL^{-1}$ (uncertainty corresponds to 95% confidence interval, $n$ = 5), was within the 95% confidence interval of the CRM. In addition, precision was as low as 2% RSD. Such results validate our approach and demonstrate its potential for the accurate and precise quantification of N-containing compounds using a single generic standard.

**Analysis of Real Samples.** Finally, the applicability of the proposed approach to real sample analysis was tested with two different matrices, a biomass pyrolysis oil and a diesel. The

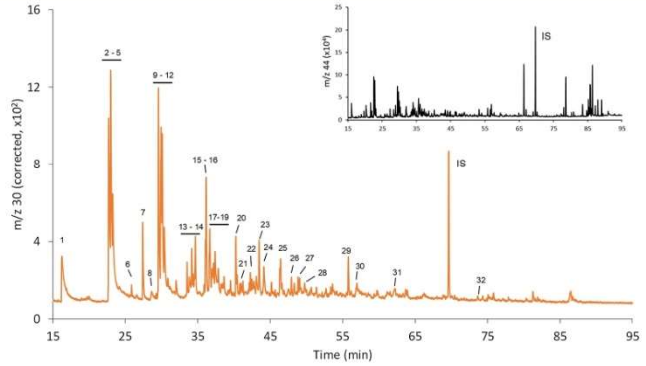

**Figure 6.** N-selective ($m/z$ 30 corrected, orange line) and universal (C, $m/z$ 44, inset, black line) GC-combustion-MS analysis of biomass-based pyrolysis oil using *N,N*-diethylaniline as an internal standard (IS). Identification and individual absolute quantification ($\mu$g N g$^{-1}$) of the numbered chromatographic peaks are listed in Table 1.

biomass pyrolysis oil was diluted 1:300 in hexane and spiked with *N,N*-diethylaniline as an internal standard. Figure 6 presents the N-selective (corrected $m/z$ 30) GC-combustion-MS profile, while its inset shows the universal (carbon, $m/z$ 44) profile that matches pretty well with the universal GC−MS profile given in Figure S11. Most of the N-containing peaks were detected at the beginning of the gradient (20−55 min) and corresponded to low abundant compounds in the sample as clearly shown in the inset of Figure 6 where most abundant organic compounds eluted later (65−90 min). The correction using the signal at $m/z$ 28 was applied, although no significant differences were observed between the raw and corrected as the C concentration of the most intense peaks was below 7 $\mu$g C g$^{-1}$. The total N content obtained after integration of the whole N chromatogram and using response factor of the spiked *N,N*-diethylaniline was found to be 11,140 ± 330 $\mu$g N g$^{-1}$ (2 SD, $n = 3$), which is statistically undistinguishable from the reference value obtained using the well-established total chemiluminescence detection (11,000 ± 1200 $\mu$g N g$^{-1}$, similar to the ASTM 4629 method), and very similar to the GC-NCD results (10,640 ± 320 $\mu$g N g$^{-1}$, Figure S12). The selective N-chromatographic profile obtained was then used to help in the identification (MS similarity, NIST library) of the different N-containing species present using first the GC−MS configuration of our system. However, due to the sample complexity, only few compounds (16) could be identified. We resorted then to the resolving power of the multidimensional GC × GC−MS to boost species identification. For that purpose, we first used the 16 N-compounds previously detected and identified by our approach to establish a correlation between the retention times in the GC-combustion-MS and GC × GC-MS instruments that could be later used to translate the identification of another 16 compounds achieved by GC × GC-MS to the GC-combustion-MS chromatogram. The result of this multi-technique approach was the identification and quantification of up to 32 N-containing compounds in the complex biomass pyrolysis oil sample. Table 1 summarizes the qualitative and quantitative information obtained to provide an in-depth quantitative characterization of the N speciation in the biomass pyrolysis oil. The sum of the successful individual quantifications accounted for 70% of the total N quantified in the sample because some N-compounds could not be reliably identified.

Next, in order to validate further our approach with a more challenging real sample, we selected a diesel with much lower total N content, 497 ± 10 $\mu$g g$^{-1}$ (95% confidence level, previously determined by the well-established total chemiluminescence detection). It was injected after a dilution of ca. 1:450 in hexane (Figure S13). Despite the low total nitrogen injected (ca. 1 $\mu$g N g$^{-1}$), N-selective detection unveiled many N-containing compounds. Due to the much more complex matrix and much lower concentration of the N-compounds, correction of the $m/z$ 30 signal was mandatory. In fact, elution of highly concentrated (>10 $\mu$g C g$^{-1}$) paraffin species at the beginning of the gradient (19−30 min, clearly shown in the $m/z$ 44 profile given in the inset to Figure S13A and in the GC−MS profile given in Figure S14), resulted in significant interference signals at $m/z$ 30 (Figure S13A) due to the already commented $^{12}C^{18}O$ formation in the ion source. However, after application of the isotopic correction using the signal at $m/z$ 28 ($^{12}C^{16}O$), such interference peaks were turned out negligible and the corrected N-profile shown in Figure S13B became dominated by the elution of the major families of N compounds at longer elution times (indoles at 32−37 min and carbazoles at 45−50 min). *N,N*-Diethylaniline was spiked as an internal standard, and the total N obtained (524 ± 22 $\mu$g N g$^{-1}$, 2 SD, $n = 3$) was again statistically undistinguishable from the reference value. The concentrations of the major families of N compounds are given in Table S3. For further internal validation purposes, the diesel sample was also injected in a longer (50 m) and more apolar (HP-1) column using another internal standard (Quinoleine) and the total N obtained (493 ± 47 $\mu$g N g$^{-1}$, 2 SD, $n = 3$) was again in excellent agreement. Interestingly, the GC-NCD (Figure S15) results obtained in this case (394 ± 42 $\mu$g N g$^{-1}$, 2 SD, $n = 3$) were significantly lower than both the reference value (ASTM 4629 method) and our approach, likely due to the significant

**Table 1. List of N-Containing Compounds Identified in the Biomass-Based Pyrolysis Oil (Figure 6)**[a]

| peak | compound | $\mu$g N g$^{-1}$ |
|---|---|---|
| 1[b] | methylpyrazine | 593 ± 78 |
| 2[b] | 2,6-dimethylpyrazine | 2346 ± 76 |
| 3 | 4,6-dimethylpyrimidine | |
| 4 | 5-methyl-2-pyridinamine | |
| 5[b] | 2-ethylpyrazine | |
| 6[b] | 1-ethylpyrrole | 22 ± 6 |
| 7[b] | isopropylpyrazine | 213 ± 28 |
| 8 | aniline | 49 ± 8 |
| 9[b] | 2-ethyl-6-methylpyrazine | 2219 ± 366 |
| 10[b] | 2-ethyl-5-methylpyrazine | |
| 11[b] | 2-ethyl-3-methylpyrazine | |
| 12[b] | Propylpyrazine | |
| 13[b] | *N*-methylsuccinimide | 568 ± 64 |
| 14[b] | 1-pentylpyrrole | |
| 15[b] | 1-ethyl-2,5-dimethylpyrazine | 352 ± 56 |
| 16 | 2,6-diethylpyrazine | |
| 17[b] | 2-methyl-5-propylpyrazine | 164 ± 12 |
| 18 | *N*-ethylsuccinimide | |
| 19 | 2-isobutyl-3-methylpyrazine | |
| 20[b] | 5*H*-5-methyl-6,7-dihydrocyclopentapyrazine | 167 ± 54 |
| 21 | 3,5-diethyl-2-methylpyrazine | 34 ± 8 |
| 22 | 2,5-dimethyl-3-propylpyrazine | 61 ± 6 |
| 23[b] | 2-pentylpyrazine | 174 ± 32 |
| 24[b] | 2-methyl-5-trans-propenylpyrazine | 145 ± 38 |
| 25 | 5*H*-2,5-dimethyl-6,7-dihydrocyclopentapyrazine | 164 ± 30 |
| 26 | 3-ethyl-4-methyl-1*H*-Pyrrole-2,5-dione | 53 ± 22 |
| 27 | 2-butyl-3-methylpyrazine | 52 ± 16 |
| 28 | 2-methyl-3-propylpyrazine | 57 ± 12 |
| 29 | 3-methylquinoline | 90 ± 22 |
| 30 | 5-methylindole | 86 ± 44 |
| 31 | 1-(2′-phenylethyl)-pyrrole | 64 ± 2 |
| 32 | 5,6,7-trimethyl-1*H*-indole | 16 ± 2 |

[a] Individual compound independent quantification was carried out using *N*,*N*-diethylaniline as an internal standard. Uncertainty corresponds to two standard deviations ($n = 3$). [b] Identified using the GC−MS mode. Rest of the peaks were identified by GC × GC−MS.

and well-known quenching effects suffered by this spectroscopic detector when analyzing complex samples.[11,14]

## ■ CONCLUSIONS

This work describes in detail the capabilities of the GC-combustion-MS approach as a powerful and unique nitrogen selective detector. We have herein demonstrated that every N-containing species produces $^{14}N^{16}O$ that can be used for their highly selective and sensitive detection and species-independent quantification. The successive and improved prototypes presented and evaluated offer several advantages over established N-selective detectors. First, the ultimate GC-combustion-MS prototype provides by far the lowest detection limit ever reported for N-containing species (0.02 pg N injected). Second, the additional detection at $m/z$ 44 of the $CO_2$ generated for any C-containing compound provides simultaneous universal and compound-independent detection. Third, the structural elucidation capabilities of conventional EI-MS are maintained intact and available after a parallel injection in the same instrument. Fourth, the system showed excellent robustness and reliability in the analysis of two real samples (biomass pyrolysis oil and diesel) with different matrix complexity and N concentration level.

We have proved that the N-selective profile greatly helps in digging into the MS data in the search for the identity of the N-species detected. However, such improved identification capabilities are still severely constrained in complex samples, which obliged us to resort to parallel multidimensional GC × GC−MS analysis to expand the list of N-compounds individually quantified. In fact, future implementation of the proposed instrumental setup in a standard GC × GC−MS instrument could bring together in the same instrument the excellent quantitative (universal, C and element-selective, N) features and powerful identification capabilities of both approaches.

Applications can be foreseen in a wide variety of fields from the petroleum and chemical (polymer, plastic) industries to quantitative metabolomics where the determination of the great and rising variety of N-containing compounds is increasingly important. Finally, it is worth mentioning that the element-selective detection capabilities of the GC-combustion-MS setup is not limited to N-compounds but could also be applied to S-containing compounds through the measurement of the volatile $SO_x$ species generated after combustion. Of course, such complementary information (still not investigated in detail) would extend even further its potential niche applications.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c01943.

List of compounds used, schemes of both prototypes, recoveries and reproducibility studies, chromatograms of mixtures of N-compounds, calibration curves, GC−MS, GC-NCD and GC-combustion-MS chromatograms of samples, chromatographic conditions, and quantitative results of mixtures and real samples (PDF).

## ■ AUTHOR INFORMATION

### Corresponding Author

**Jorge Ruiz Encinar** − *Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain;* orcid.org/0000-0001-6245-5770; Email: ruizjorge@uniovi.es

### Authors

**Javier García-Bellido** − *Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain*

**Laura Freije-Carrelo** − *TotalEnergies One Tech Belgium, 7181 Feluy, Belgium; International Joint Laboratory—iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France*

**Montserrat Redondo-Velasco** − *Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain*

**Marco Piparo** − *International Joint Laboratory—iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France; TotalEnergies, TotalEnergies Research & Technology Gonfreville, 76700 Harfleur, France;* orcid.org/0000-0003-4404-442X

**Mariosimone Zoccali** – *Department of Mathematical and Computer Science, Physical Sciences and Earth Sciences, University of Messina, 98168 Messina, Italy;* orcid.org/0000-0002-7469-7408

**Luigi Mondello** – *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences and Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy*

**Mariella Moldovan** – *Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain;* orcid.org/0000-0001-6697-4252

**Brice Bouyssiere** – *International Joint Laboratory—iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France; Universite de Pau et des Pay de l'Adour, E2S UPPA CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux UMR5254, 64053 Pau, France;* orcid.org/0000-0001-5878-6067

**Pierre Giusti** – *International Joint Laboratory—iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France; TotalEnergies, TotalEnergies Research & Technology Gonfreville, 76700 Harfleur, France;* orcid.org/0000-0002-9569-3158

Complete contact information is available at:
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### Notes

The authors declare no competing financial interest.

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## Supporting Information

# Potential of GC-Combustion-MS as powerful and versatile Nitrogen Selective Detector in Gas Chromatography

Javier García-Bellido,[a,‡] Laura Freije-Carrelo,[b,c,‡] Montserrat Redondo-Velasco,[a] Marco Piparo,[c,d] Mariosimone Zoccali,[e] Luigi Mondello,[f,g] Mariella Moldovan,[a] Brice Bouyssiere,[ch] Pierre Giusti,[c,d] and Jorge Ruiz Encinar[a]*

[a] Department of Physical and Analytical Chemistry, University of Oviedo, 33006, Oviedo, Spain

[b] TotalEnergies One Tech Belgium, Zone Industrielle C, 7181 Feluy, Belgium

[c] International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France

[d] TotalEnergies, TotalEnergies Research& Technology Gonfreville, 76700 Harfleur, France

[e] Department of Mathematical and Computer Science, Physical Sciences and Earth Sciences, University of Messina, 98168 Messina, Italy

[f] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy

[g] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy

[h] Universite de Pau et des Pay de l'Adour, E2S UPPA CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux UMR5254, 64053 Pau, France

* Jorge Ruiz Encinar ruizjorge@uniovi.es

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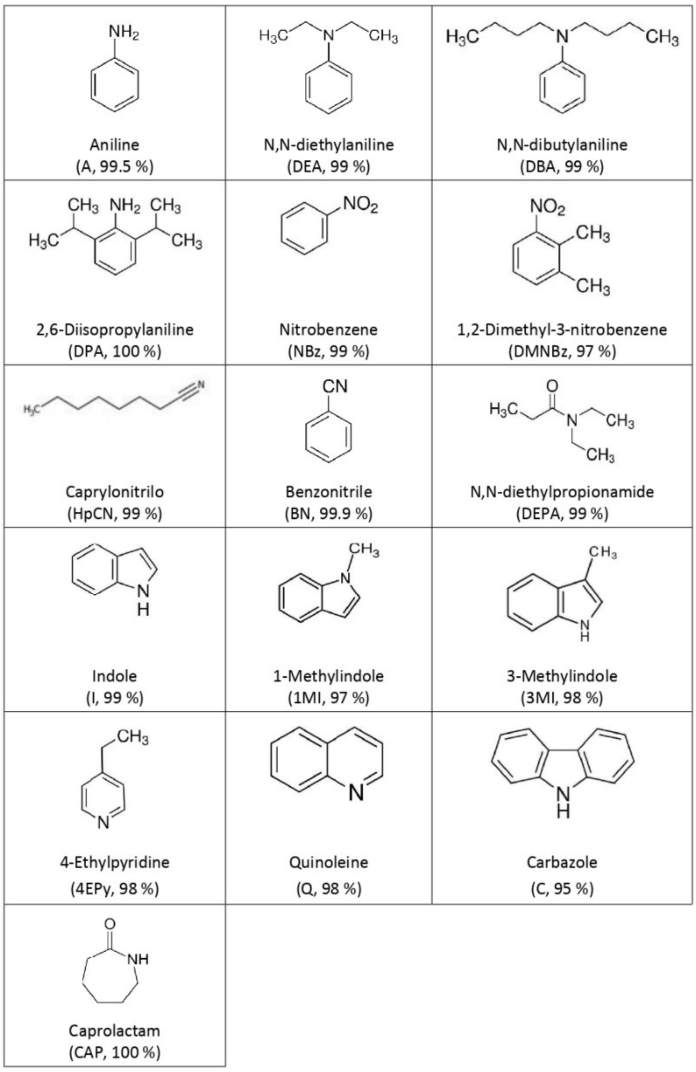


**Figure S1**. List, chemical structure, and purity of the N-containing compounds used.

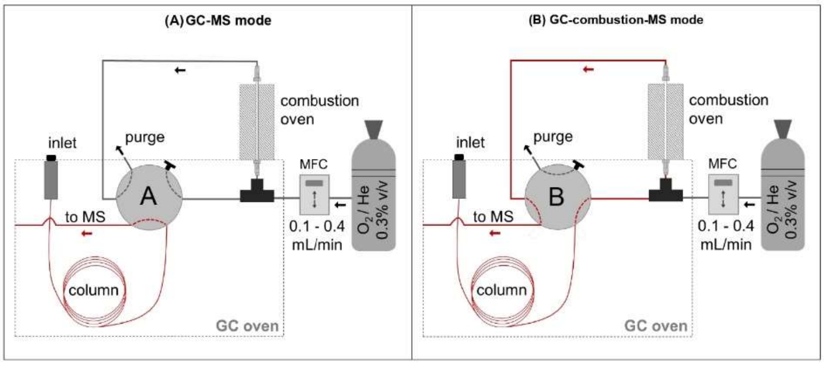


**Figure S2.** Scheme of the six-way valve and its connections within GC-combustion-MS **prototype #1**. Position A (GC-MS mode): GC effluent is directly sent to the MS. Position B (GC-combustion-MS mode): GC effluent is first mixed online with the $O_2$/He combustion gas and brought to the combustion furnace before being directed to the MS.

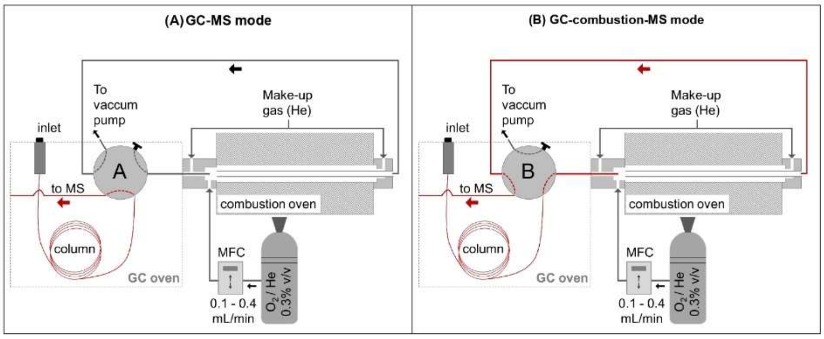


**Figure S3.** Scheme of the six-way valve and its connections within GC-combustion-MS **prototype #2**. Position A (GC-MS mode): GC effluent is directly sent to the MS. Position B (GC-combustion-MS mode): GC effluent is first mixed online with the $O_2$/He combustion gas and the He makeup-flow and finally brought to the combustion furnace before being directed to the MS.

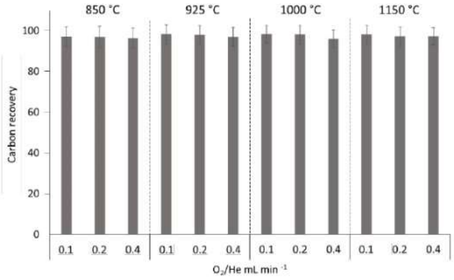


**Figure S4.** Average of the recoveries for heptadecane (C17) and eight N-containing compounds (DEA, DBA, Q, 1MI, DPA, I, 3MI, see Figure S1) obtained from the $CO_2$ signals (m/z 44) under different combustion oven temperatures (850, 925, 100 and 1150 °C) and $O_2$/He flows (0.1, 0.2 and 0.4 mL min$^{-1}$) and using prototype #1 and pentadecane (C15) as internal standard. Error bars correspond to one standard deviation (n=9).

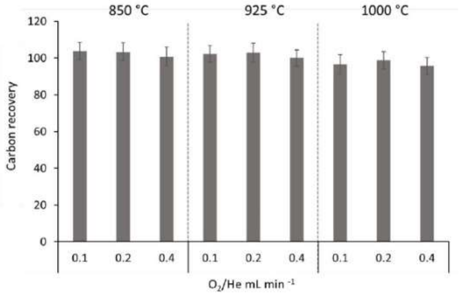


**Figure S5.** Average of the recoveries for heptadecane (C17) and eight N-containing compounds (DEA, DBA, Q, 1MI, DPA, I, 3MI, see Figure S1) obtained from the CO2 signals (m/z 44) under different combustion oven temperatures (850, 925, and 1000 °C) and $O_2$/He flows (0.1, 0.2 and 0.4 mL min$^{-1}$) and using prototype #2 and pentadecane (C15) as internal standard. Error bars correspond to one standard deviation (n=9).

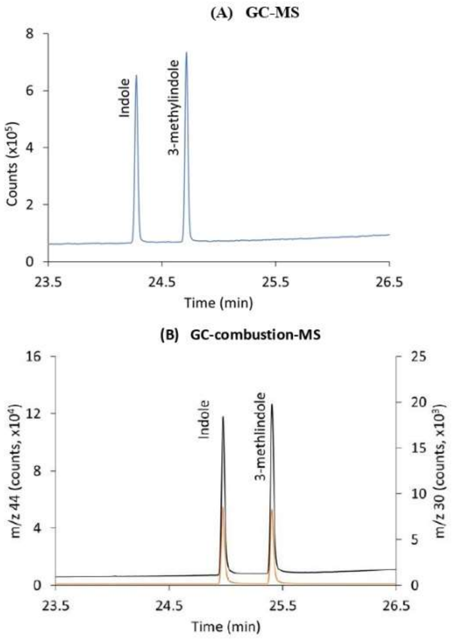


**Figure S6.** Peak shape comparison of two N-containing compounds, indole (2.1 µg compound/g; 1.7 µg C/g; 0.25 µg N/g) and 3-methylindole (2.2 µg compound/g; 1.8 µg C/g; 0.23 µg N/g) analyzed under: **(A)** GC-MS mode; **(B)** GC-combustion-MS mode (prototype #2) with universal C detection (m/z 44, black) and N-selective detection (m/z 30, orange).

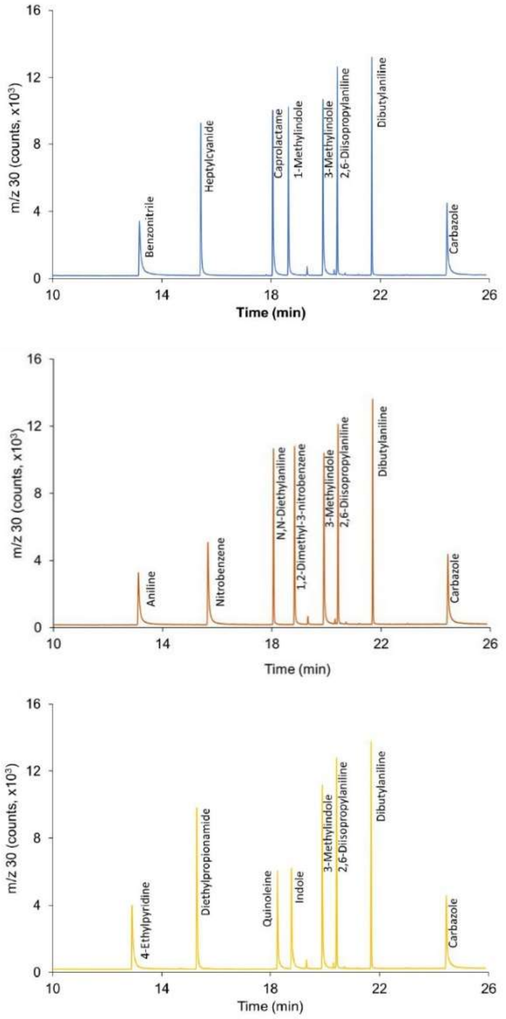


**Figure S7**. GC-combustion-MS chromatograms (prototype #1) of the extended mixtures of nitrogen-containing compounds. Concentrations in µg N·g$^{-1}$ are: 4EPy=0.61, DEPA=0.65, Q=0.53, I=0.55, 3MI=0.72, DPA=0.59, DBA=0.65, C=0.62, A=0.57, NBz=0.63, DEA=0.56, DMNBz=0.55, BN=0.72, HpCN=0.75, CAP=0.65, 1MI=0.64.

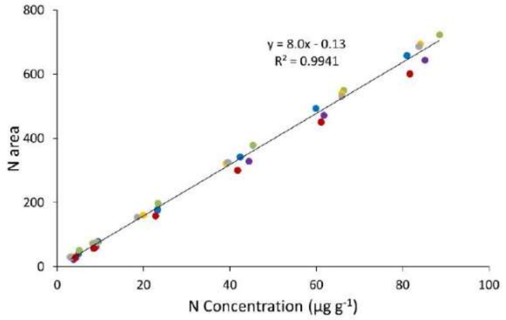


**Figure S8.** Multispecies generic calibration curve obtained using GC-NCD for six N-compounds in a range of concentrations up to 90 μg N g$^{-1}$. Color code: Propionitrile (violet), 4-ethylpyridine (blue), Aniline (green), Quinoleine (yellow) Indole (grey), Carbazole (red)

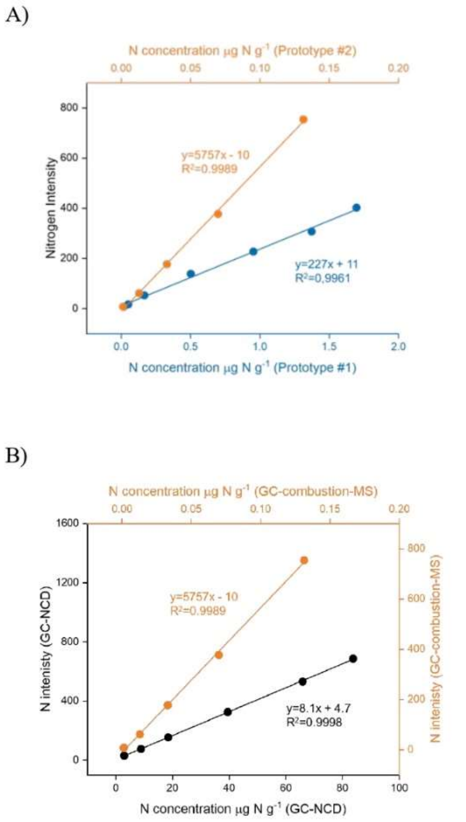


**Figure S9. A)** Calibration plots obtained for indole using prototype #1 (blue) and #2 (orange). 25-fold increase in sensitivity (slope) was observed for prototype #2. **B)** Calibration plots obtained for indole using prototype #2 (orange) and GC-NCD (black). A 700-fold difference was observed between both systems, what matches well with the difference observed in the limits of detection obtained (0.02 pg of N for GC-combustion-MS vs 2-20 pg N for GC-NCD). Please note that individual chromatograms (raw data, csv files) for the three instruments (prototypes #1 and #2 and NCD) were processed (peak integration) using the same software (OriginPro). In addition, different X-axis (N-concentration) are used for each instrument to allow comparison.

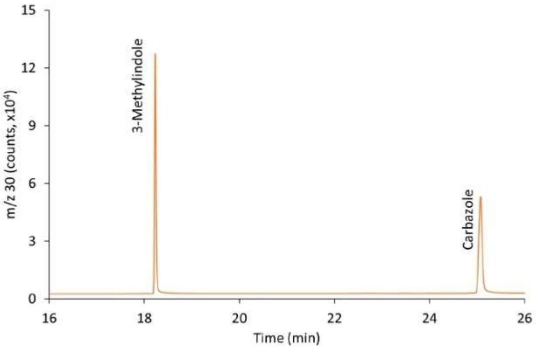


**Figure S10.** GC-combustion-MS chromatogram of the aliquot of the CRM D-4629-91-HB-CON (carbazole in toluene:acetone, 9:1) spiked with 3-methylindole (c.a. 0.5 µg N $g^{-1}$) as internal standard.

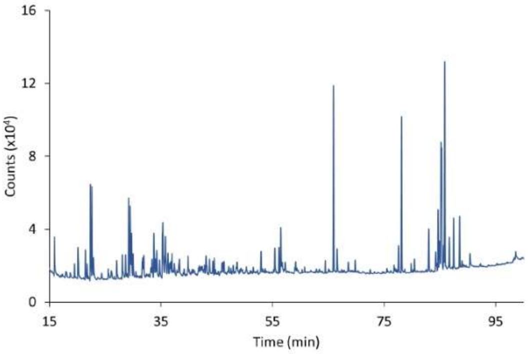


**Figure S11.** GC-MS (TIC) chromatogram of biomass pyrolysis oil.

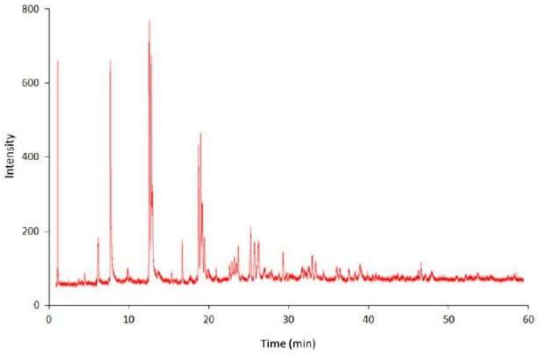


**Figure S12.** GC-NCD chromatogram of biomass pyrolysis oil.

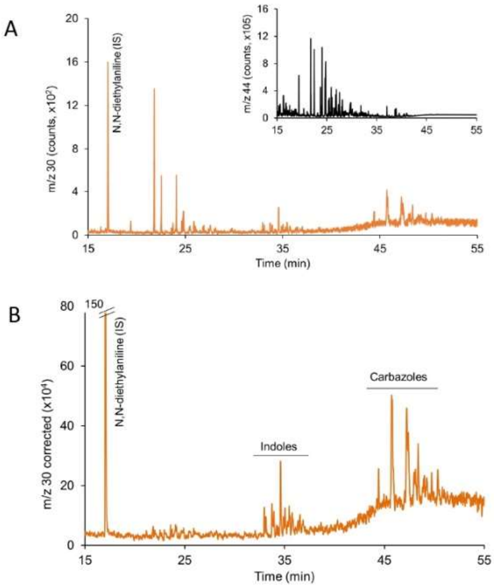


**Figure S13.** GC-combustion-MS chromatogram for diesel. A) Orange profile corresponds to non-corrected nitrogen and black profile (inset) corresponds to carbon profile obtained in the same analysis (column: BD-EN14103). B) Corrected N-profile

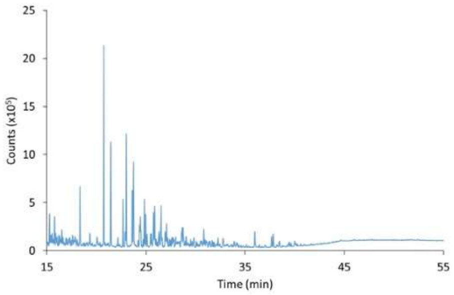


**Figure S14.** GC-MS (TIC) chromatogram for diesel (column: BD-EN14103)

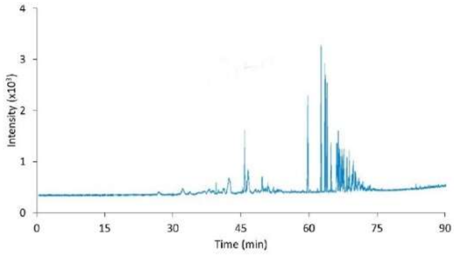


**Figure S15.** GC-NCD chromatogram for diesel (column: HP-1)

**Table S1**. Experimental conditions used for the quantitative analysis of N-containing compounds by GC-combustion-MS and GC

| | |
|---|---|
| Inlet temperature | 250 ºC |
| Injection mode | splitless |
| Injection volume | 1 µL |
| Column | BD-EN14103 (30 m x 0.32 m x 0.25 µm)<br>HP1-MS (50 m x 0.2 mm x 0.5 µm) |
| Carrier gas | He (1.5 mL/min) |
| GC Oven temperature | Standards: 45 ºC (3 min) - 15 ºC/min to 100 (5 min) - 15 ºC/min to 250 (5 min)<br>Diesel: 45 ºC (3 min) - 5 ºC/min to 250 ºC (20 min)<br>Biomass Pyrolysis Oil: 40 ºC (3 min) – 2 ºC/min to 250 ºC (20 min) |
| Acquisition mode | SIM: m/z 28, m/z 30 and m/z 44 |
| Combustion oven temperature | 850-1150 ºC |
| $O_2$/He flow | 0.1-0.4 mL/min |

Additional information of the columns used in the GC×GC-QqQ MS experiments:

- **First dimension:** SLB-5ms (20 m × 0.18 mm ID × 0.18 µm df), silphenylene polymer virtually equivalent in polarity to poly (5% diphenyl/95% methyl siloxane).

- **Second dimension:** SLB-35 (5 m × 0.32 mm ID × 0.25 µm df), bonded and highly crosslinked; proprietary polymer virtually equivalent in polarity to poly (35% diphenyl/65% dimethyl siloxane).

**Table S2.** Compound-independent quantification of a mixture of 15 different N-compounds using 2,6-diisopropylaninline as internal standard (Figure S7). Added and found N-concentrations and corresponding recoveries (%) are given. Uncertainty corresponds to 2 standard deviations (n=3).

| **Compound** | **Added (µg N g$^{-1}$)** | **Found (µg N g$^{-1}$)** | **Recovery (%)** |
|---|---|---|---|
| 4-Ethylpyridine | 0.61 | 0.55 ± 0.04 | 90 ± 6 |
| N,N-Diethylpropionamide | 0.65 | 0.68 ± 0.01 | 105 ± 2 |
| Quinoleine | 0.53 | 0.47 ± 0.03 | 89 ± 6 |
| Indole | 0.55 | 0.49 ± 0.03 | 90 ± 6 |
| Aniline | 0.57 | 0.56 ± 0.03 | 98 ± 6 |
| Nitrobenzene | 0.63 | 0.64 ± 0.03 | 102 ± 5 |
| N,N-Diethylaniline | 0.56 | 0.58 ± 0.01 | 103 ± 3 |
| 1,2-Dimethyl-3-nitrobenzene | 0.55 | 0.60 ± 0.02 | 111 ± 4 |
| Benzonitrile | 0.72 | 0.71 ± 0.04 | 99 ± 6 |
| Heptylcyanide | 0.75 | 0.72 ± 0.01 | 96 ± 2 |
| Caprolactam | 0.65 | 0.65 ± 0.01 | 100 ± 3 |
| 1-Methylindole | 0.64 | 0.62 ± 0.02 | 97 ± 3 |
| 3-Methylindole | 0.73 | 0.69 ± 0.04 | 96 ± 6 |
| 2,6-Diisopropylaniline | 0.59 | IS | IS |
| N,N-Dibutylaniline | 0.65 | 0.61 ± 0.01 | 94 ± 2 |
| Carbazole | 0.63 | 0.61 ± 0.04 | 97 ± 7 |

**Table S3.** Quantification of total nitrogen content and major families in diesel sample by GC-combustion-MS (Figure S13B) compared with the reference value and GC-NCD. Uncertainty corresponds to 2 standard deviations (n=3).

| | **µg N g$^{-1}$** |
|---|---|
| Total content by chemiluminiscence (ASTM 4629) | **497 ± 10** |
| GC-combustion-MS: | |
| Total | **524 ± 22** |
| Indoles | 111 ± 6 |
| Carbazoles | 368 ± 25 |
| GC-NCD | **394 ± 42** |

S15

## Experimentos asociados

Aunque finalmente quedaron fuera del trabajo de este capítulo, durante la presente tesis doctoral, con la idea de extender el sistema de GC-combustión-MS hacia un detector selectivo de nitrógeno, se llevó a cabo el análisis de muestras de distintos orígenes. Por ejemplo, como se puede ver en Figura 25. Cromatograma selectivo de N obtenido mediante el sistema de GC-combustión-MS para una muestra real de aceite de pirólisis obtenida de plástico., una muestra real de aceite de pirólisis se analizó para obtener el perfil selectivo de nitrógeno. Mayoritariamente se observaron dos compuestos sin contar con el patrón interno. Estos compuestos fueron identificados mediante GC×GC-MS como benzonitrilo y caprolactama en función de su espectro de masas y comparando con la librería NIST:

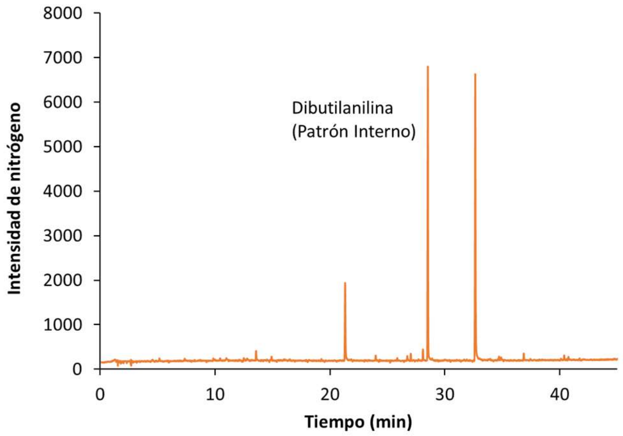


**Figura 25. Cromatograma selectivo de N obtenido mediante el sistema de GC-combustión-MS para una muestra real de aceite de pirólisis obtenida de plástico.**

Al igual que en el capítulo anterior, las muestras usadas en este, se caracterizaron por GC×GC con modulador de flujo. Dada que la concentración de los compuestos de nitrógeno en la muestra de aceite de pirólisis obtenida de biomasa era mayor que en la muestra de diésel, la identificación de estos y la posterior correlación con el perfil selectivo obtenido por GC-combustión-MS fue más sencilla. En este caso se analizó la muestra únicamente en el sistema con modulador de flujo, obteniendo el siguiente cromatograma.

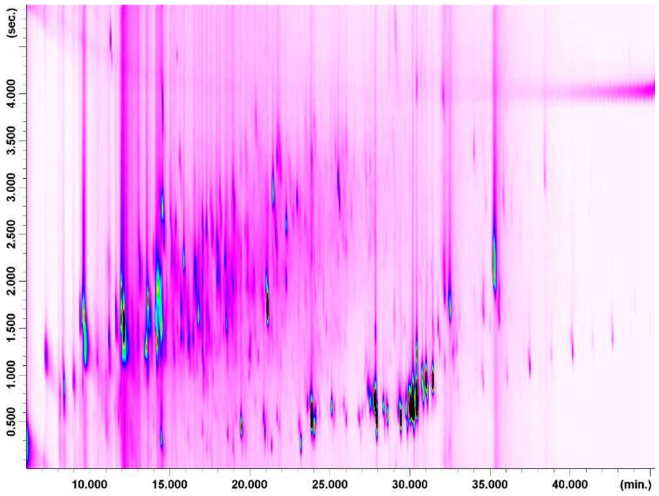


**Figura 26. Cromatograma en dos dimensiones de la muestra de aceite de pirólisis del artículo IV obtenido de biomasa obtenida con el equipo de GC×GC con modulador de flujo**

Los compuestos fueron identificados automáticamente mediante el software *Chromeleont* consiguiendo identificar un número mayor de compuestos de nitrógeno que en GC-MS unidimensional.

*Anexo. Condiciones de los análisis de cromatografía bidimensional*

| | |
|---|---|
| **Temperatura de inyector** | 300 ºC |
| **Modo de inyección** | Split 1/200 |
| **Volumen de inyección** | 1 µL |
| **Set de columnas** | 1ª dimensión:<br>SLB5-MS (20 m x 0.18 mm ID x 0.18 µm)<br>2ª dimensión:<br>SLB-35 (5 m x 0.32 m ID x 0.25 µm) |
| **Gradiente** | 45°C (3’) to 350 °C at 6°C/min |
| **Tiempo de modulación** | Modulador de flujo:<br>Tiempo de acumulación: 4.6 s<br>Tiempo de reinyección: 0.4 s |
| **Modo de adquisición** | SCAN (m/z: 45-450) |

# CAPÍTULO CUATRO

## Aplicación de la Cromatografía de Gases-Combustión- Espectrometría de masas a la detección selectiva de oxígeno

## Introducción y contexto

El oxígeno es uno de los elementos más comunes en compuestos orgánicos y la determinación de los compuestos que lo contienen es hoy en día de suma importancia en multitud de disciplinas científicas, como puede ser la metabolómica, la petroleómica o la clínica y, en diferentes aplicaciones industriales como son el procesado de hidrocarburos, el desarrollo de nuevos (bio)combustibles, la síntesis de gas natural y biogás, la farmacéutica o la síntesis de diferentes productos químicos o aditivos. Es importante en estos procesos la determinación, no solo del contenido total de oxígeno, sino también la especiación de los diferentes compuestos en los que se encuentra y su concentración individual.

En concreto, la cuantificación de compuestos individuales que contienen oxígeno es de vital importancia en procesos relacionados con nuevas materias primas y combustibles de cara a evaluar su potencial y optimizar el procesado de hidrotratamiento que requieren estos productos de cara a disminuir la concentración de los heteroátomos, entre ellos el oxígeno, presentes. La importancia de este proceso se refleja, por ejemplo, en el control de ácidos carboxílicos, cuya capacidad corrosiva puede afectar a las distintas partes de mecánicas de los procesos que sufren estos materiales, o los aldehídos y cetonas y su capacidad de formar sustancias gomosas. Esta caracterización cuantitativa de los compuestos de oxígeno permitirá un mejor entendimiento del proceso de combustión, lo que se verá reflejado en mejores herramientas para escoger los mejores combustibles y desarrollar rutas catalíticas de producción de estos mucho más eficientes. Otro campo en el que la determinación de compuestos de oxígeno tiene una importancia importante es el de la metabolómica, en el que ciertas enfermedades derivan en la producción de especies reactivas de oxígeno que pueden producir una degradación de las membranas celulares.

Como se ha venido comentando a lo largo de la presente Tesis Doctoral, la cromatografía de gases ha sido la técnica más ampliamente utilizada en la caracterización de matrices complejas históricamente. En el caso de compuestos de oxígeno, en numerosas ocasiones estos coeluyen con compuestos de la matriz impidiendo la detección y la cuantificación. Algunas de las estrategias que se han seguido para superar estas limitaciones es el uso de procesos de preconcentracion o extracción (SPME, SPFE) para enriquecer la fracción inyectada en el cromatógrafo previo a su inyección en el GC. Otra metodología que también está ampliamente establecida en la separación de compuestos de oxígeno de la matriz en muestras complejas es el uso de la cromatografía de gases bidimensional. Sin embargo, el uso de estas técnicas, aunque permiten obtener resultados satisfactorios, implican tiempos de análisis largos y detectores adecuados para su cuantificación.

En este contexto, otra aproximación a la caracterización de compuestos de oxígeno es el uso de detectores selectivos para este elemento. Como se comentó en la Introducción General, el detector de ionización de llama de oxígeno (O-FID) basado en la transformación cuantitativa de las especies que contienen este elemento, en metano, para después detectarlo por FID. El límite de detección absoluto de este detector está en torno a los 300 pg, cuando se aplica a compuestos muy volátiles. Selectividad ($>10^5$) y linealidad ($10^3$) son adecuadas, pero desafortunadamente, la tolerancia a la matriz y la equimolaridad son bajas ya que como se comentó previamente, el detector FID requiere el cálculo del factor de respuesta relativo para cada compuesto individual. El detector de transformada de Fourier (FTIR), que mide la absorción en el infrarrojo de grupos funcionales específicos, también se ha usado en la caracterización de compuestos de oxígeno. Sin embargo, la selectividad es demasiado baja debido al solapamiento de bandas y el límite de detección es relativamente alto para ciertas aplicaciones (100 ng). Otro de los detectores que permite cierta caracterización de compuestos de oxígeno es el detector de emisión atómica (AED), sin embargo, la capacidad de un análisis multielemental se pierda cuando el oxígeno es monitorizado, ya que se requieren condiciones de flujo muy específicas, requiriendo el uso de He como fase móvil con una altísima pureza para evitar contaminaciones que pudieran afectar a la selectividad y sensibilidad. Aunque el límite de detección es adecuado (1ng O) esta técnica sufre de efectos de matriz y efectos de quenching cuando se analizan muestras complejas, además de estar limitado de cara a la cuantificación dada su baja equimolaridad, especialmente en compuestos aromáticos. Finalmente, la espectrometría de masas queda como una de las herramientas más potentes para analizar estas muestras complejas mediante el uso de analizadores de alta resolución. Sin embargo, el proceso de ionziación es altamente dependiente del compuesto lo que limita la cuantificación requiriendo patrones específicos para la cuantificación de cada compuesto individual.

Siguiendo el desarrollo del detector de GC-combustión-MS que se ha llevado a cabo durante la última década en el grupo de investigación, en este trabajo se ha pretendido exportar la detección selectiva y universal que se ha conseguido en capítulos anteriores, para distintos elementos como C, H, N y S, al O. En este caso, el equipo sufre una modificación, que es el uso como gas de combustión de $^{18}O_2$, en lugar de $^{16}O_2$. En este capítulo se presenta la prueba de concepto para la detección selectiva de compuestos de O con respecto a una matriz compleja, además de llevar a cabo una cuantificación independiente de la especie. Se usó un material de referencia (SRM 2772) para validar la metodología, además del análisis de una muestra de diésel adicionada con concentraciones variables de compuestos de oxígeno.

Artículo científico V.

**Título**: *Sensitive and Selective Determinatino of Oxygenated Compounds in complex samples using GC-combustion-MS with isotopic oxygen as oxidizing gas*

**Autores**: Javier García-Bellido, Montserrat Redondo-Velasco, Laura Freije-Carrelo, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti and Jorge Ruiz Encinar

**Referencia**: *A la espera de ser enviado*

# Sensitive and Selective Determination of Oxygenated Compounds in complex samples using GC-combustion-MS with isotopic oxygen as oxidizing gas

*Javier García-Bellido,*[a,‡] *Montserrat Redondo-Velasco,*[a,‡] *Laura Freije-Carrelo,*[bc, ??] *Mariella Moldovan,*[a] *Brice Bouyssiere,*[c,d] *Pierre Giusti*[c,e] *and Jorge Ruiz Encinar*[a]*

[a] Department of Physical and Analytical Chemistry, University of Oviedo, 33006, Oviedo, Spain

[b] TotalEnergies One Tech Belgium, Zone Industrielle C, 7181 Feluy, Belgium

[c] International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France

[d] Universite de Pau et des Pay de l'Adour, E2S UPPA CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux UMR5254, 64053 Pau, France

[e] TotalEnergies, TotalEnergies Research& Technology Gonfreville, 76700 Harfleur, France

* Jorge Ruiz Encinar ruizjorge@uniovi.es

‡ These authors contributed equally to this work

## ABSTRACT

This paper presents a new element selective Gas Chromatography (GC) detector for the detection and accurate quantification of traces of volatile Oxygen containing compounds in complex samples without the need of specific standards. The use of isotopically labeled oxygen (highly enriched in $^{18}O$) as oxydizing gas in a combustion unit heated at 800 °C and located between the GC oven and the mass spectrometer allows to directly and unambiguously detect the natural oxygen present in the separated compounds through its incorporation into the volatile species formed after their combustion and their subsequent degradation to $^{16}O$ in the ion source. Unspecific signal due to the low $^{16}O$ abundance in the oxidizing gas (and residual air contamination) could be compensated measuring the m/z 12 that comes as well from the $CO_2$ degradation in the ion source and is directly related to the size of the compound. A detection limit in the order of 110 pg of O injected was achieved, which is the lowest ever reported for any GC detector. Equimolarity, linearity, and peak shape were also adequate. Validation of the approach was performed by the analysis of a standard reference material obtaining accurate (99-103% recoveries) and precise (1-4% RSD) results. Robustness was tested after spiking a hydrotreated diesel with ten O-compounds at the low ppm level. In spite of the sample complexity, every O-compound could be easily discriminated from the matrix crowd and quantified with a mean recovery of 102 ± 9%.

## INTRODUCTION

Oxygen is one of the most commonly occurring constituents of organic compounds and the direct determination of oxygen-containing compounds is nowadays crucial in a wide variety of scientific disciplines (e.g. petroleomics, metabolomics, clinical) and various industrial applications (e.g. hydrocarbon processing, new combustibles, natural gas and biogas, pharmaceuticals, chemicals and additives manufacturing).[1] All these processes requires for the knowledge not only of the total oxygen present in the sample but of the characterization of the different oxygen compounds[2] and harderstill, their individual concentration. In particular, determination of the individual amounts of the different oxygen-containing compounds present in new feedstocks and sustainable biofuels is of paramount importance nowadays to assess their potential uses and/or optimizing the hydro-treatment necessary for controlling the amount of oxygen present[3] because, for example, carboxylic acids can be corrosive while aldehydes and ketones can lead to the formation of gums.[4] Such detailed quantitative characterization together with a deep understanding of the combustion process will be critical to select better fuel candidates and develop more efficient catalytic production routes.[5] Another consideration is the attention and importance of oxygen-containing compounds in clinical samples. In fact, current GC-MS-based metabolomics approaches could benefit enormously from the selective detection and quantification of oxygen-metabolites in target biological samples, such as body fluids (e.g plasma or urine).[6]

Gas Chromatography (GC) is typically employed to separate the huge number of sample volatilizable constituents in complex samples. Unfortunately, chromatographic peaks corresponding to oxygenated compounds frequently overlap with those from the matrix constituents in complex matrices hampering their detection and quantification. One conventional solution to this problem is resorting to selective preconcentration or extraction (SPME, SPFE) methodologies to enrich oxygenates in purified fractions prior to the GC analysis.[4,7] Similarly, recent works have shown that comprehensive two-dimensional gas chromatography is very efficient for the separation of most of the oxygenates[8,9] in complex samples. While these methods are certainly reliable, they involve long analysis times and still require for adequate detectors for identification and accurate quantification. In this context, another complementary approach to measuring concentrations of oxygenated compounds in complex samples is to resort to selective GC detectors that responds only to compounds that contain oxygen. Four main types of oxygen-selective GC detectors have been described so far. The oxygen-flame ionization detector (O-FID) converts oxygenated compounds in a catalytic reactor, first into carbon monoxide and then into methane, which is then detectable by conventional FID. The absolute detection limit can be as low as 3OO pg when applied to the very volatile oxygen compounds eluting below 120°C.[10] Selectivity and linearity are very adequate (>$10^5$ and $10^3$, respectively) [ASTM D5599-22]; however matrix tolerance and equimolarity are low as relative response factors for every individual compound must be computed in advance as in regular FID. The Fourier transform infrared (FTIR) detector measures the absorption of IR energy by specific functional groups on the GC analyte. For oxygenated compounds, the C-0 stretching region is monitored.[11,12] Unfortunately, selectivity is low (< 1000), presumably owing to broad overlapping gas-phase IR absorption bands and the detection limit achievable (100 ng) restricts its application fields. The Atomic Emission detector (AED) for GC has many of the characteristics of an ideal oxygen-selective detector. Microwave-induced plasmas atomize the analyte and excite the constituent atoms producing atomic emission characteristic of the compounds eluting from the GC column. Unfortunately, capability of multi-element characterization is lost when oxygen is monitored since it demands for very specific gas flow conditions.[13] In fact, oxygen analysis requires for extremely high purity He as minute quantities of air and water produce a background emission signal that can severely decrease oxygen selectivity and sensitivity. Detection limit is adequate (1 ng O);[14] however, it suffers from significant matrix and quenching effects when analyzing complex unresolved samples and equimolarity is not as good as it should be expected since deviations as high as 40% are possible, especially for aromatics.[15] Finally, it is clear that Mass Spectrometry is a potent technique for analyzing mixtures especially when using high resolution instruments. Unfortunately, its identifying capability is highly limited in complex samples for the screening of O-containing compounds due to chromatographic coelutions and isobaric interferences.[13] Moreover, the ionization process is extremely compound dependent making necessary the use of specific standards to perform the quantification of every individual O-containing compound.[16] Therefore, in spite of the pressing need for the accurate determination of oxygen-containing compounds at trace levels in complex samples, no generally satisfactory method for this very complicate challenge exits at present.

In this work, we have developed a highly sensitive instrumental approach to detect selectively and quantify O-containing compounds in complex samples without the need to resort to specific standards. Our approach makes use of isotopically enriched $^{18}O_2$ during the combustion step that takes place in between the GC separation and the ionization in the MS instrument. The proof of concept strategy to stand out the O-containing compounds from the organic crowd and perform their accurate quantification is presented and demonstrated. Excellent agreement with the certified values of a soy-based biodiesel (SRM 2772) with the use of simple generic internal standards demonstrates its accuracy and precision. Moreover, we successfully applied this approach to detect and quantify up to 10 different oxygenated compounds previously spiked at the low (7-50) ppm range to a complex diesel sample.

## EXPERIMENTAL SECTION

### Reagents, Solutions and Materials

Dodecane (C12; 99%), tetradecane (C14; 99%), nonadecane (C19;98.5%), eneicosane (C20; 99.8%), butylbencene (BB;99%), butanol (C4OL;100%), pentyl butyrate (PB; 99.3%), acenapthene (AC; 98.5%), cyclohexanone (Cy6ONE; 99%), 2-ethoxyethylacetate (EtO; 99%), hexyl butyrate (HB; 97%), 1-heptanol (C7OL; 98%), 1-octanol (C8OL; 99%), acetophenone (A; 99.8%), benzaldehyde (B; 99%), dimethylmaleate (DiMAL; 98.7%), benzothiophene (BT; 97%), methylbenzothiophene (MBT, 96%), phenetylacetate (PhA; 97%), dibutilanilina (DBA; 99%), dibutyl sulfide (DS; 98%), dibenzofurane (DBF; 100%), dimethylphtalate (DPh; 99.5%), indole (I, 99%), 1-methylindole (1MI, 97%), 3-methylindole (3MI, 98%), dibenzothiophene (DBT, 98%) were purchased from Merck. A certified reference material (CRM) SRM 2772 soy-based B100 biodiesel (NIST, Gaithersburg, MD, USA) was used to validate the methodology. Helium of purity 99.999% purchased from Air Liquide was used in the analysis. Two different gas mixtures were used as combustion gas, $^{16}O_2$:He (0.3%, v/v) from Linde and $^{18}O_2$:He (1% v/v, 97% $^{18}O_2$ enriched) from Westfalen (Münster, Germany). Real samples (diesel) were provided by TotalEnergies Raffinage Chimie.

### Instrumentation

GC separations. Experimental conditions are summarized in Table S1.

GC combustion-MS instrument. A Shimadzu GC-combustion-MS instrument, based on a GC-MS QP-2020NX as described in Figure S1, was used. The instrument was configured with a split/splitless inlet, and an electron ionization source operated at 70 eV. The modification consisted on a combustion oven that allows the complete combustion of the analytes by using an alumina tube (400 mm length × 3 mm width × 0.5 mm ID; Elemental Microanalysis) with 2 Pt wires as catalyzers. The installation of an automatic 6-way valve allows the system to work as a standard GC-MS system. An additional He makeup flow (ca. 1.7 mL $min^{-1}$) was introduced to protect the capillary interface and reduce peak broadening..

## RESULTS AND DISCUSSION

The guiding principle is the GC detector introduced in 2009[17] able to provide generic universal quantification of organic compounds while maintaining the inherent structural elucidation capabilities of MS by simply actuating a switching valve. The combustion interface developed and installed in a regular GC−MS instrument allowed for the quantitative conversion of each and every organic compound eluting from the column, and mixed on-line with an oxygen flow, into $CO_2$ before the ionization.[18] The system was considerably improved over time based on the idea that other volatile species such as $H_2O$, $SO_x$, and $NO_x$ (if S and N are present) would be produced as well in the combustion oven together with $CO_2$, opening the gate to parallel H-, S- and N-selective detection.[19] Figure S1 shows the detailed schematics of the system. Therefore, oxygen detection became the one stumbling block to the long-wished GC detector combining structural identification (MS) with compound-independent calibration, both universal (C, H) and element-selective (N, S, O). As it has been already pointed out before, oxygen detection is already a very challenging task and, to make matter worse, our strategy uses an on line flow of 0.4 mL $min^{-1}$ of oxygen diluted in He (0.3% v/v) to produce the combustion of the organic compounds previously separated in the GC column. In order to avoid peak broadening such combustion is on line produced inside a narrow ceramic tube (400 mm length × 3 mm width × 0.5 mm ID) containing two Pt wires (catalyst) and heated at $\geq$ 800 °C. The resulting volatile combustion species are then brought to a manually actuated high temperature six-way valve installed inside the GC, which in turn allows to direct them to the ion source of the MS instrument. Such valve allows also bypassing the combustion furnace when necessary, allowing the setup to work under GC−MS (Figure S1A) or GC-combustion-MS (Figure S1B) configurations.[20] The expected products for a complete oxidation of organic compounds[21] with $O_2$ as the oxidant would then comprise:

$$C_aH_bO_c + \left(a + {}^{b}/_{4} - {}^{c}/_{2}\right)O_2 \xrightarrow{850°C} aCO_2 + {}^{b}/_{2}\,H_2O \qquad (1)$$

Of course, other N- and S-containing volatile species would be formed (i.e $NO_x$ or $SO_x$) if present in the compound. Therefore, in principle, it should be expected that the O present in the original organic compounds would be distributed between the oxidized volatile species formed together with the O used in the combustion (in excess). This scenario makes O detection impossible unless the oxygen used in the combustion and the target O present in the compounds are different. In order to check this starting hypothesis, we resorted to one compound isotopically enriched in $^{18}$Oxygen (Ab$^{18}$O=97.1%), benzaldehyde. For comparison purposes, we mixed it with two alkanes (C14 and C19) and two compounds containing natural oxygen (Ab$^{16}$O=99.76% and Ab$^{18}$O=0.20%), acetophenone and phenethyl acetate. The mixture was then injected into the GC-combustion-MS system described above. The resulting chromatogram is shown in Figure 1. As can be clearly observed, the intensity profiles at m/z 44 (corresponding exclusively to $^{12}C^{16}O_2$) and m/z 46 (corresponding mostly to $^{12}C^{16}O^{18}O$, assuming that Ab$^{17}$O is negligible in both the natural and enriched oxygens) differed significantly in benzaldehyde where the intensity for m/z

46 was much higher in comparison to the other four compounds (please note that signal profile at m/z 48 was too low for being properly measured). The 44/46 ratios measured (n=5) for the alkanes (241±2 and 241±4), where oxygen had been incorporated exclusively from the combustion gas, and those for the natural O-containing acetophenone and phenethyl acetate (239±4 and 242±4) matched perfectly the theoretical natural 44/46 ratio computed using the natural oxygen and carbon abundances (243±1). In contrast, the 44/46 ratio decreased to 63±1 in the case of the benzaldehyde due to the incorporation of the enriched $^{18}O$ originally present into the $CO_2$ molecules formed after combustion increasing significantly the signal at m/z 46 as clearly shown in Figure 1. Notably, the chromatogram obtained for the same mixture containing instead benzaldehyde with natural oxygen led to the same 44 and 46 profiles for all the compounds (Figure S2), being the 44/46 ratio obtained for natural Benzaldehyde 247±4 in this case.

Once demonstrated that isotopically labeled oxygen compounds can be detected when using natural oxygen as combustion gas, we decided to reverse the reasoning and explore the use of isotopically labeled oxygen (97% abundance $^{18}O$) as combustion gas to detect compounds that contain natural oxygen. Initially, a mixture of twelve O-compounds (including alcohols, aldehydes, esters, ethers and carbonils with saturated and aromatic structures), two alkanes and one aromatic compound was prepared in hexane and injected in triplicate. As shown in Figure S3 and detailed in Table S2, O-containing compounds could be distinguished from the noncontaining O-compounds as their 44 and 46 profiles were higher and so the corresponding 46/48 ratios due to the additional contribution of natural $^{16}O$ in the compounds with regards to the residual presence in the rest of the noncontaining O-compounds coming the low (3% and likely higher due to air contamination in the system) but still significant abundance of $^{16}O$ observed in the enriched combustion gas. Unfortunately, neither the 44 or 46 peak areas nor the 44/46 – 44/48 ratios followed any clear relationship with the O concentration present in each species. On the other hand, traditional equations of isotope dilution are also difficult to use in this case as they apply when the amount of isotopically enriched (natural or radioactive) tracer ($^{18}O_2$ in our case) is accurately known and controlled[22] and an equilibrium is established between the natural element and the known amounts of isotopic element within the blend that is analyzed. On the contrary, the isotopically-labelled species ($^{18}O_2$ diluted in He) is used here[23] as a reagent added on line in huge excess before the combustion furnace to react with the organic compounds of the sample eluting from the GC for their complete oxidation. Therefore, the mass flow of the sample typically computed in on line isotope dilution applications, where the enriched spike is not reacting with the sample and is simply added as quantification standard,[24,25,26] cannot be computed here. Both the natural oxygen originally present in the organic compounds of the sample and the isotopic-$^{18}O_2$ are distributed in the volatile species formed ($CO_2$ and $H_2O$). In fact, the amount of enriched spike incorporated to the volatile species formed after combustion for each eluting compound depends on the amount of natural oxygen originally present in each detected compound and their corresponding elemental composition. This is why oxygen detection in the target compounds is only possible after comparing the signals obtained for the noncontaining O-compounds used as internal standards and for the targets O-compounds.

Notably, the in-source fragmentation of $CO_2$ to O, generally established and shown in the $CO_2$ NIST reference spectrum with an abundance close to 10% (16/44 ratio of 0.096), generates an analytical signal directly related to oxygen. Notably, in-source fragmentation of the other volatile species generated after combustion where oxygen is incorporated (e.g. $H_2O$ and NO with 0.9 and 1.5% abundance, respectively) also produces signal at $^{16}O$. Therefore, the intensity at m/z 16 could be directly related to the amount of $^{16}O$ originally present in every organic compound eluting from the GC column and could be used for quantification purposes. This is clearly observed in Figure 2 that shows the profile at m/z 16 of the chromatogram obtained for the mixture under study. Notably, it is apparent from Figure S4A that peak areas at m/z 16 followed already a quite linear trend ($R^2$=0.97) with the O concentration for each compound. As expected, the m/z 16 peaks obtained for the two alkanes and the aromatic are significantly lower but still significant because the low abundance of $^{16}O$ (3%) in the enriched combustion gas that could be even increased by small air leaks within the instrumental system. This unspecific contribution is responsible for the significant intercept of the m/z 16 calibration (Fig. S4A) and is ruled by the size of every compound since the higher the number of C, the higher the number of $CO_2$ molecules produced and the higher the amount of residual $^{16}O$ incorporated. Notably, the in-source fragmentation also brings about the production of a C signal at m/z 12 (shown in NIST reference spectrum with an abundance of 8.7 %) that reflects compound size and could be used as an internal standard to estimate such unspecific contribution. Profile at m/z 12 is also given in Figure 2. As expected, every compound containing Oxygen or not, provides a signal at m/z 12, depending exclusively on their C concentration. In fact, as shown in Figure S4B, there is a good linearity ($R^2$=0.99) between the peak areas for all the compounds present in the mixture and their corresponding C concentration.

In the search for an analytical strategy to selectively screen for O-compounds in mixtures, we decided to plot the peak intensity ratios (easily provided by the software of the instrument) 46/48 vs 16/12 (m/z 44 intensity was too low to be measured properly for lower oxygen concentrations). Figure 3A, where the results for the three replicates are plotted (n=45), demonstrates that noncontaining O-compounds are well discriminated gathered in close formation in the lower left corner of the graphic (red shaded circle). In contrast, the O-compounds are classified along the two-axis depending on their O to C ratio. Notably, to our knowledge, this is the first time that molar ratios O/C are measured in separated GC peaks through their corresponding elemental signals (m/z 12 and 16).

Before starting to develop the quantitative strategy, we needed to assure that the enrichment of the oxidizing gas used for combustion was constant along the gradient. Initially, a set of experiments were conducted using a mixture containing eleven alkanes from C12 to C20. Chromatogram is shown in Figure S5. Peak areas ratios at 46, 47 and 48 obtained for each alkane were used to compute for the corresponding oxygen abundances (considering carbon natural isotope abundances). It is apparent from Table S3A that $^{18}$O-enrichment was slightly decreasing along gradient from 92.60% (C12) to 92.17% (C20) while the $^{16}$O-abundance increased from 6.21% to 6.64%, likely due to a slight change in the residual air leaks in the system along gradient. Interestingly we observed that the 32/34 ratio (measured simultaneously at their corresponding m/z), that could be related somehow to the $^{16}$O/$^{18}$O ratio, was also slightly increasing along the gradient. In fact, as can be seen in Table S3B, after application of the 32/34 increasing trend to correct for the $CO_2$ peak area ratios measured for the alkanes, both the $^{18}$O and $^{16}$O abundances remained stable (from 92.60 to 92.58% and from 6.21 to 6.23%, respectively). Therefore, we decided to apply this correction in our quantitative strategy, as the peak areas measured at m/z 16 should also follow the same trend because they come from the in-source degradation of $CO_2$.

Next step was to correct our analytical signal at m/z 16 for the unspecific contribution of the low $^{16}$O abundance (that finally amounts to aprox. 6%, see above) in the $^{18}$O-enriched combustion gas. For that purpose, the mean 16/12 peak area ratio measured in the noncontaining O-compounds, taken as Internal Standards of C (IS-C), present in the mixture was used to estimate the unspecific contribution at 16 for each O-compound (oxy), taken into account their corresponding signals (peak areas) at 12:

$$(Int16)_{oxy}^{cor} - (Int12)_{oxy} \cdot \left(\frac{Int16}{Int12}\right)_{IS-C}^{cor} = (Net16)_{oxy}^{cor} \qquad (2)$$

where superscript “cor” refers to the peak area at 16 previously corrected using the 32/34 trend as explained above and “Net16” corresponds to the ultimate 16 signal that comes exclusively from the natural Oxygen (99.8% $^{16}$O abundance) originally present in the target compound. We could then plot the $(Net16)_{oxy}^{cor}$ for each present in the mixture against their corresponding O concentration and the resulting calibration graph is given in Figure 3B. As can be seen, the developed quantitative strategy provides a clear advance on the linearity achievable that now rises up to $R^2$=0.990. Another striking benefit to emerge from the Figure 3B is that the approach proposed is fully species-independent (ca. equimolar) since eleven very different O-compounds in size, functional groups and aromaticity provides very similar response factor (i.e. calibration slope). This feature opens the door to compound independent calibration, especially interesting in complex samples with lots of unknown O-compounds.

In order to explore further the selectivity of the approach proposed, we created another set of mixtures containing additionally N- (Dibutilanilina, Indol, 1- and 3-Methyl Indol) and S- (Benzothiophene, Dibenzothiophene, Methylbenzothiophene and Dibutylsulfide) compounds. We also included another alkane (nonadecane), aromatic (Butylbenzene) and O-containing compound (1-heptanol). These new mixtures were also analyzed in triplicate. In total, 200 chromatographic peaks were processed along different working days (5), including 93 noncontaining and 107 O-containing compounds (with an average and lowest concentration of 14 and 4.1 ppm O, respectively). As can be seen in Figure S6, the 46/48 vs 16/12 plot allowed us to distinguish clearly the O-compounds. In fact, after considering all the noncontaining compounds we could estimate the mean and standard deviation for each ratio. Then, according to the criteria of the 99% confidence interval (means + 2.6σ, n=93), any compound providing ratios above the corresponding limits (red dotted lines in Figure S6) was classified as O-compound. Interestingly, as can be clearly seen in the inset to the Figure, N-compounds provided the higher 16/12 values, likely due to the formation of Nitrogen hydride species during electron ionization (as observed in the NIST mass spectra of NO). Surprisingly, although analyses were carried out in different working days and the dispersion of the data is higher, the 16/12 ratio still allowed complete discrimination (O% false positives or negatives). However, under such stringent conditions, the 46/48 ratio failed to classify one triplicate of 1-butanol as O-compound, likely due to its poor peak shape (see Figure 2) and classified one triplicate of dibutylaniline just in the border line. This result with a large population measured in different days further strengthened our confidence in the selectivity of the approach proposed.

Detection limit (DL) was then calculated based on three times the standard deviation of the unspecific signal calculated using the signals at m/z 12 for different O-compounds and the factor $\left(\frac{Int16}{Int12}\right)_{IS-C}^{cor}$ calculated for noncontaining O-compounds and turned out to range from 80 to 150 pg of O injected, depending on the peak/compound. To the best of our knowledge, this is the lowest detection limit for O ever published for a GC detector.

For validation purposes, we resorted to a Standard Reference Material (NIST, SRM 2772) consisting of a soy-based B100 biodiesel with certified and reference values for several Fatty

Acid Methyl Esters (FAMEs). After adequate dilutions with hexane, the SRM sample was spiked with Nonadecane and Dimethylphtalate as Internal Standards of C (IS-C) and O (IS-O), respectively, and analyzed in quintuplicate. Figure S7A shows a representative GC-combustion-MS chromatogram obtained at masses 16 and 12. The five main FAMEs, C16:0, C18:0, C18:1, C18:2 and C18:3 were detected. Unfortunately, position isomers C18:1(n-9) and C18:1(n-7) could not be chromatographically resolved so they were quantified together. As expected, the 46/48 vs 16/12 plot allowed us to distinguish clearly the O-compounds, both the FAMEs and IS-O, from the IS-C (Figure S7B). The oxygen concentration determined for each FAME was translated into compound concentration for comparison purposes with the SRM values. Table 1 shows the excellent agreement between the concentrations found and the certified values for every quantified FAME with recoveries ranging from 99 to 103%. In addition, precision ranged from 0.9 to 4.3% RSD, depending on the concentration level. Such results validate our approach and demonstrate its potential for the accurate and precise quantification of O-containing compounds using simple generic standards.

Finally, the applicability of the proposed approach to real sample analysis was tested with a diesel sample that was previously hydrotreated to remove completely heteroatoms below the ppm level. Diesel sample was diluted 1:100 in hexane and spiked with ten O-compounds to be determined using our approach. In parallel, 2-Ethoxyethyl acetate (EtO) was spiked as IS-O. In addition, three noncontaining O-compounds (C12, C20 and AC) were spiked to serve as IS-C. Figure 4A shows the GC-combustion-MS chromatogram obtained at masses 16 and 48, that matches pretty well with the universal GC–MS profile. Up to 25 significant peaks were detected at m/z 16. It is interesting to note that only those matrix compounds with concentrations higher than 37 µg C $g^{-1}$ in the injected sample produced a significant unspecific signal at m/z 16. After application of the plot 46/48 vs 16/12 as discrimination strategy (figure 4B), the spiked eleven O-containing compounds were unambiguously classified as O-containing compounds (labeled with an asterisk in Fig. 4A) and clearly distinguished from the three noncontaining O-compounds spiked and the other components of the matrix. Quantitative results are given in Table 2. In spite of the sample complexity, recoveries obtained were adequate ranging from 82 to 112% with a mean recovery value of 102%.

## CONCLUSIONS

In conclusion, a sensitive and robust strategy was developed to stand out oxygen-containing compounds from the matrix compounds and accurately quantify them without resorting to specific standards. The strategy involves the use of a GC to separate the sample compounds, isotopically enriched, $^{18}O_2$ as combustion gas and further analysis by mass spectrometry. The usage of a distinct oxygen to produce compounds combustion allows the detection of the natural oxygen originally present, in spite that both are incorporated into the volatile species formed (i.e. $CO_2$, $H_2O$). In fact, the approach allows to directly monitor our target analyte, the natural oxygen ($^{16}O$) present in the compounds, through the well-known and established degradation that suffer their corresponding $CO_2$ molecules formed (and other minor volatile species) at the ion source of the MS instrument. Notably, the limitation due to the low unspecific m/z 16 observed for highly concentrated noncontaining O-compounds because of the residual abundance (ca. 6%) of $^{16}O$ in the isotopic $^{18}O$-combustion gas used can be accurately corrected by measuring as well the $^{12}C$ signal (also coming from in-source degradation). The measurement of the 16/12 ratio in a noncontaining O-compound, that is used as IS, provides the tool to compute the unspecific contribution of such residual $^{16}O$ in the O-compounds. In fact, the measurement of the 16/12 ratios in every detected GC peak turned out be an easy to compute and perfect discriminating factor (0% of false positives and negatives) to accurately highlight O-compounds in all the samples analyzed, including biodiesel and diesel, and despite the low oxygen concentrations (low ppm range) assayed. The accuracy of the method, tested in standards, SRM and complex real samples, is comparable to that of other established element-selective detectors in GC, such as O-FID, NCD or SCD.[13]

Applications can be foreseen in a wide variety of fields from the petroleum and chemical (polymer, plastic) industries to quantitative metabolomics where the determination of the great and rising variety of O-containing compounds is increasingly important. Finally, it is worth mentioning that this work is the last step in the development of an innovative multipurpose GC detection system featuring element selective detection with generic quantification in complex samples, while maintaining the structural elucidation power of mass spectrometry.[19,20] The system, which requires a simple low-cost modification of a standard GC–MS instrument and the use of isotopic oxygen as combustion gas (whose consumption is negligible, 0.4 mL min$^{-1}$), can be regarded as the first approach that enables online and simultaneously ultrasensitive elemental quantification (C, H, N, S, O) of every individual volatizable (GC) organic compounds present in complex samples.

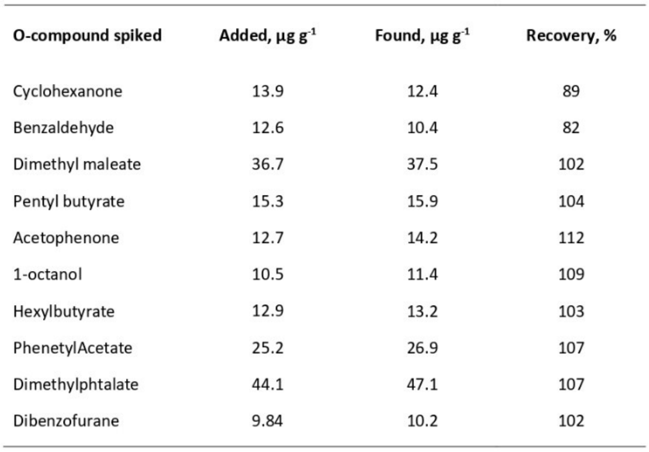
**TABLE 1.** Quantitative recoveries obtained for the FAMEs determination in SRM 2772 using Nonadecane and Dimethylphtalate as generic Internal Standards of C and O. Concentrations are referred to the original SRM sample and are given in mg $g^{-1}$ of compound. Uncertainty corresponds to the 95% confidence interval (n=5)

| **FAME compound** | **SRM 2772** | **GC-Combustion-MS** | |
|---|---|---|---|
| | **Certified, mg $g^{-1}$** | **Found, mg $g^{-1}$** | **Recovery, %** |
| Methyl palmitate (C16:0) | 107 ± 2 | 110 ± 2 | 103 |
| Methyl stearate (C18:0) | 43.0 ± 2.7 | 43.9 ± 3.8 | 102 |
| Methyl oleate (C18:1, n-9) | 233 ± 6 | 249 ± 10** | 101** |
| Methyl vaccinate (C18:1, n-7) | 14.3 ± 1.5 | ----- | ----- |
| Methyl linoleate (C18:2, n-6) | 523 ± 17 | 521 ± 7 | 100 |
| Methyl linolenate (C18:3, n-3) | 69.3 ±2.6* | 68.6 ± 3.3 | 99 |

* reference value

** sum of C18:1(n-9) and C18:1(n-7)

**TABLE 2.** Quantitative recoveries obtained for the ten O-compounds spiked to the hydrotreated diesel. 2-Ethoxyethyl acetate (EtO) was spiked as Internal quantification Standard (IS-O).



| O-compound spiked | Added, $\mu g\ g^{-1}$ | Found, $\mu g\ g^{-1}$ | Recovery, % |
|---|---|---|---|
| Cyclohexanone | 13.9 | 12.4 | 89 |
| Benzaldehyde | 12.6 | 10.4 | 82 |
| Dimethyl maleate | 36.7 | 37.5 | 102 |
| Pentyl butyrate | 15.3 | 15.9 | 104 |
| Acetophenone | 12.7 | 14.2 | 112 |
| 1-octanol | 10.5 | 11.4 | 109 |
| Hexylbutyrate | 12.9 | 13.2 | 103 |
| PhenetylAcetate | 25.2 | 26.9 | 107 |
| Dimethylphtalate | 44.1 | 47.1 | 107 |
| Dibenzofurane | 9.84 | 10.2 | 102 |

**FIGURE 1**

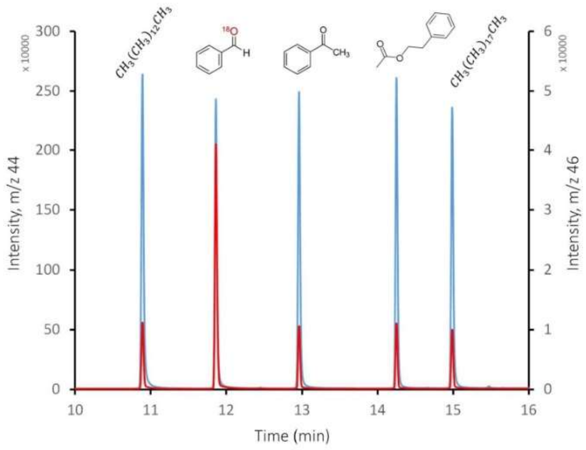


**Figure 1**. GC-combustion-MS chromatogram obtained with natural oxygen as combustion gas for a mixture of benzaldehyde containing isotopically enriched oxygen (Ab$^{18}$O= 97.1%) with two alkanes (tetradecane and and nonadecane) and two compounds containing natural oxygen as well, acetophenone and phenethyl acetate. Red and blue profiles correspond to 46 and 44, respectively.

FIGURE 2

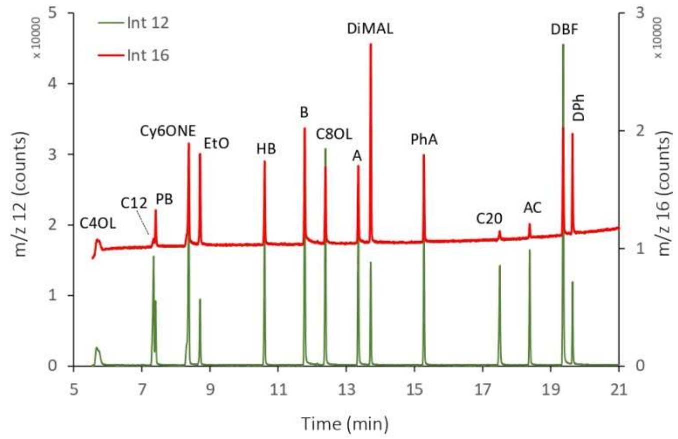


**Figure 2.** GC-combustion-MS chromatogram obtained for a mixture of three noncontaining (C12, C20 and AC) and twelve O-containing (C4OL, PB, Cy6ONE, EtO, HB, B, C8OL, A, DiMAL, PhA, DBF, DPh) compounds using $^{18}$O-enriched (Ab$^{18}$O= 95%) oxygen (1% in He) as combustion gas. Compounds' abbreviations and compound concentrations are given in the Experimental Section. Oxygen concentration ranged from 7.1 to 34.2 ppm with an average value of 17 ppm.

FIGURE 3

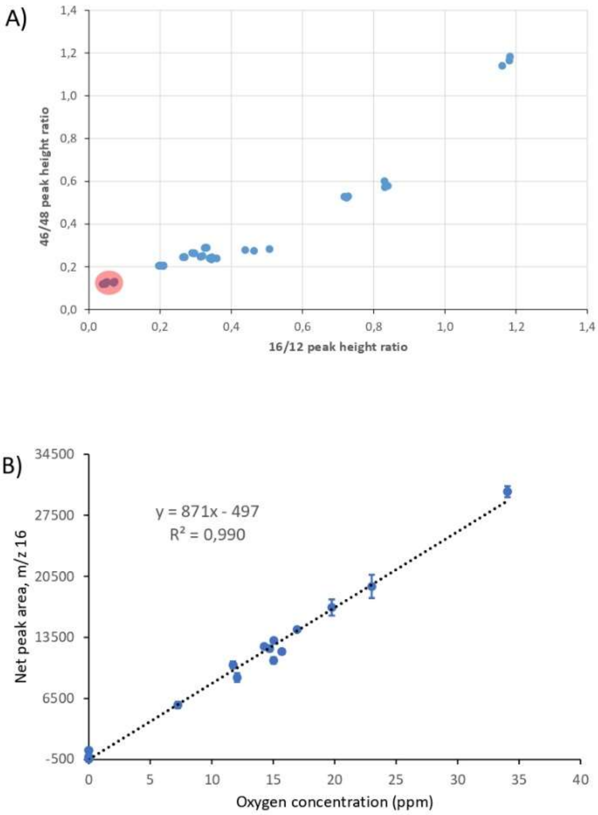
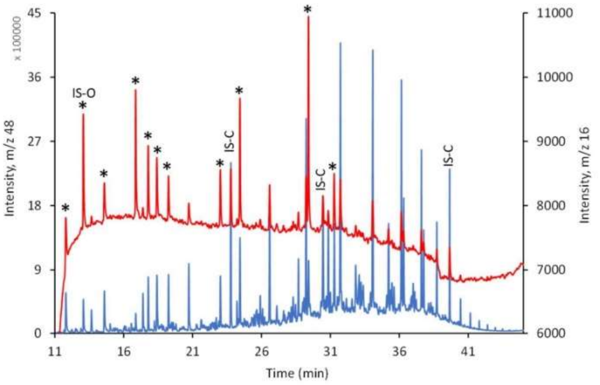


**Figure 3.** A) Plot of the ratios 16/12 vs 46/48 measured for each chromatographic peak detected in the spiked diesel sample. Color code: IS-C added (blue), C-peaks detected (yellow) and spiked O-compounds (grey).

**FIGURE 4**

**A)**



B)

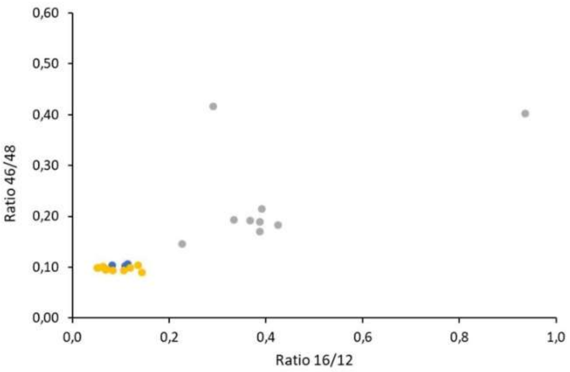


**Figure 4.** A) GC-combustion-MS chromatogram of the hydrotreated diesel spiked with ten O-compounds (labeled with an asterisk). 2-Ethoxyethyl acetate (EtO) was used as Internal Standard (IS-O). B) Plot of the ratios 16/12 vs 46/48 measured for each chromatographic peak detected in the spiked diesel sample. Color code: IS-C added (blue), C-matrix peaks detected (yellow) and spiked O-compounds (grey).

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## Supporting Information

# Sensitive and Selective Determination of Oxygenated Compounds in complex samples using GC-combustion-MS with isotopic oxygen as oxidizing gas

*Javier García-Bellido,*[a,‡] *Montserrat Redondo-Velasco,*[a,‡] *Laura Freije-Carrelo,*[b,c] *¿?, Mariella Moldovan,*[a] *Brice Bouyssiere,*[c,d] *Pierre Giusti,*[c,e] *and Jorge Ruiz Encinar*[a]*

[a] Department of Physical and Analytical Chemistry, University of Oviedo, 33006, Oviedo, Spain

[b] TotalEnergies One Tech Belgium, Zone Industrielle C, 7181 Feluy, Belgium

[c] International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization, TRTG, 76700 Harfleur, France

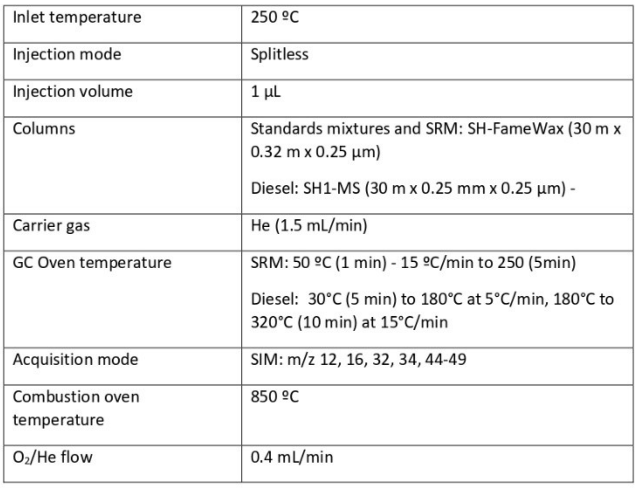
[d] Universite de Pau et des Pay de l'Adour, E2S UPPA CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux UMR5254, 64053 Pau, France

[e] TotalEnergies, TotalEnergies Research& Technology Gonfreville, 76700 Harfleur, France

* Jorge Ruiz Encinar ruizjorge@uniovi.es

‡ These authors contributed equally to this work

**Table S1.** Experimental conditions used for the quantitative analysis of N-containing compounds by GC-combustion-MS and GC



| | |
|---|---|
| Inlet temperature | 250 ºC |
| Injection mode | Splitless |
| Injection volume | 1 µL |
| Columns | Standards mixtures and SRM: SH-FameWax (30 m x 0.32 m x 0.25 µm)<br>Diesel: SH1-MS (30 m x 0.25 mm x 0.25 µm) - |
| Carrier gas | He (1.5 mL/min) |
| GC Oven temperature | SRM: 50 ºC (1 min) - 15 ºC/min to 250 (5min)<br>Diesel: 30°C (5 min) to 180°C at 5°C/min, 180°C to 320°C (10 min) at 15°C/min |
| Acquisition mode | SIM: m/z 12, 16, 32, 34, 44-49 |
| Combustion oven temperature | 850 ºC |
| $O_2$/He flow | 0.4 mL/min |

**Table S2.** Oxygen concentration (µg O·mL$^{-1}$) and 46 ($^{12}C^{16}O^{18}O$) to 48 ($^{12}C^{18}O_2$) peak area ratios for the mixture of twelve O-compounds (including alcohols, aldehydes, esters, ethers and carbonils with saturated and aromatic structures) with three noncontaining O compounds (two alkanes and one aromatic compound, labelled in red) shown in Figure S3. Uncertainty corresponds to 1 SD (n=3).

| Compound | O concentration (µg O·mL$^{-1}$) | Peak area ratio, 46/48 |
|---|---|---|
| 1-butanol | 12.1 | 0.2787 ± 0.0042 |
| Dodecane | 0.0 | 0.1201 ± 0.0016 |
| Pentyl butyrate | 7.24 | 0.2385 ± 0.0026 |
| Cyclohexanone | 23.0 | 0.2891 ± 0.0006 |
| 2-Ethoxyethyl acetate | 16.9 | 0.584 ± 0.015 |
| Hexylbutyrate | 15.7 | 0.2487 ± 0.0017 |
| Benzaldehyde | 19.8 | 0.2644 ± 0.0007 |
| 1-octanol | 15.0 | 0.2051 ± 0.0012 |
| Acetophenone | 11.7 | 0.2459 ± 0.0011 |
| Dimethyl maleate | 34.1 | 1.163 ± 0.022 |
| PhenetylAcetate | 14.7 | 0.2415 ± 0.0026 |
| Icosane | 0.0 | 0.1265 ± 0.0018 |
| Acenapthene | 0.0 | 0.1276 ± 0.0026 |
| Dibenzofurane | 14.3 | 0.2059 ± 0.0008 |
| Dimethylphtalate | 15.0 | 0.5269 ± 0.0029 |

**Table S3.** Oxygen isotope abundances computed for a mixture of eleven alkanes (Figure S5) based on the 46, 47 and 48 peak area ratios. Uncertainty corresponds to 1 SD (n=3).

**A)**

| Compound | Without correction | | |
|---|---|---|---|
| | Ab $^{16}O$ | Ab $^{17}O$ | Ab $^{18}O$ |
| C12 | 6.21 ± 0.02 | 1.19 ± 0.01 | 92.60 ± 0.01 |
| C13 | 6.27 ± 0.04 | 1.20 ± 0.001 | 92.54 ± 0.04 |
| C14 | 6.31 ± 0.05 | 1.20± 0.006 | 92.49 ± 0.05 |
| C15 | 6.33 ± 0.05 | 1.20± 0.003 | 92.48 ± 0.05 |
| C16 | 6.40 ± 0.04 | 1.19± 0.004 | 92.41 ± 0.04 |
| C17 | 6.45 ± 0.06 | 1.20± 0.005 | 92.35 ± 0.05 |
| C18 | 6.51 ± 0.04 | 1.19± 0.004 | 92.30 ± 0.04 |
| C19 | 6,56 ± 0.06 | 1.19± 0.004 | 92.25 ± 0.05 |
| C20 | 6.64 ± 0.03 | 1.19± 0.007 | 92.17 ± 0.03 |
| mean | 6.41 | 1.19 | 92.40 |
| SD | 0.14 | 0.005 | 0.1 |
| RSD (%) | 2 | 0.4 | 0.2 |

**B)**

| Compound | After correction using the 32/34 trend | | |
|---|---|---|---|
| | Ab $^{16}O$ | Ab $^{17}O$ | Ab $^{18}O$ |
| C12 | 6.21 ± 0.01 | 1.19 ± 0.01 | 92.60 ± 0.003 |
| C13 | 6.25 ± 0.02 | 1.20 ± 0.001 | 92.56 ± 0.02 |
| C14 | 6.26 ± 0.04 | 1.20 ± 0.006 | 92.53 ± 0.04 |
| C15 | 6.25 ± 0.04 | 1.20 ± 0.003 | 92.55 ± 0.03 |
| C16 | 6.27 ± 0.03 | 1.20 ± 0.003 | 92.53 ± 0.02 |
| C17 | 6.27 ± 0.04 | 1.20 ± 0.004 | 92.53 ± 0.04 |
| C18 | 6.26 ± 0.03 | 1.20 ± 0.003 | 92.54 ± 0.02 |
| C19 | 6,24 ± 0.04 | 1.20 ± 0.004 | 92.56 ± 0.04 |
| C20 | 6.23 ± 0.02 | 1.20 ± 0.007 | 92.58 ± 0.02 |
| mean | 6.25 | 1.19 | 92.40 |
| SD | 0.02 | 0.005 | 0.1 |
| RSD (%) | 0.3 | 0.4 | 0.2 |

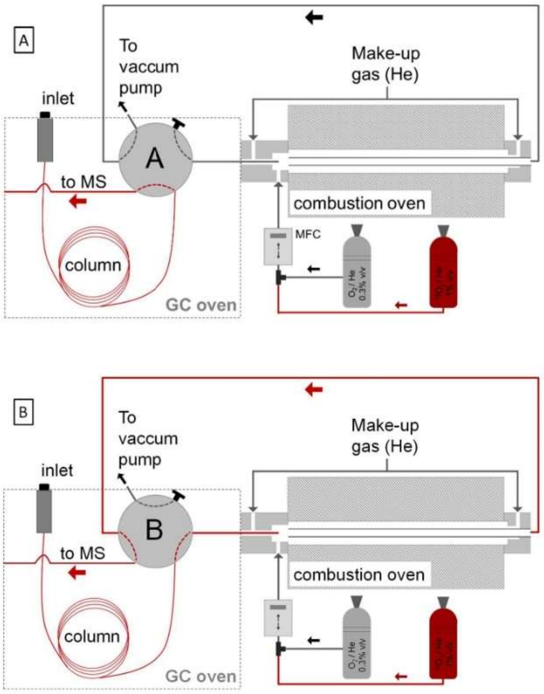


**Figure S1.** Scheme of the six-way valve and its connections within GC-combustion-MS. Position A (GC-MS mode): GC effluent is directly sent to the MS. Position B (GC-combustion-MS mode): GC effluent is first mixed online with the $O_2$/He combustion gas and the He makeup-flow and finally brought to the combustion furnace before being directed to the MS.

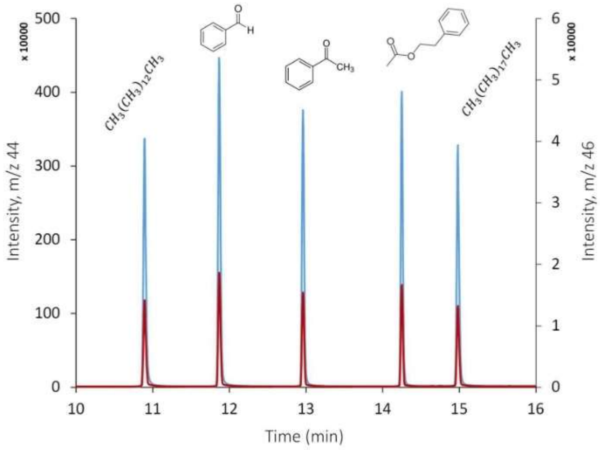


**Figure S2.** GC-combustion-MS chromatogram obtained for a mixture of benzaldehyde containing natural oxygen with two alkanes (tetradecane and and nonadecane) and two compounds containing natural oxygen as well, acetophenone and phenethyl acetate (concentration range from 26 to 37 µg C g$^{-1}$). Red and blue profiles correspond to 46 and 44, respectively.

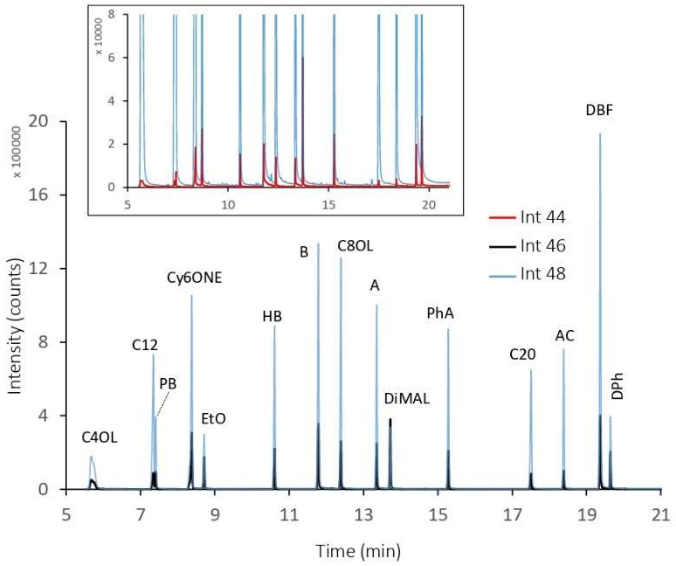


**Figure S3.** GC-combustion-MS chromatogram obtained for a mixture of three noncontaining (C12, C20 and AC) and twelve O-containing (C4OL, PB, Cy6ONE, EtO, HB, B, C8OL, A, DiMAL, PhA, DBF, DPh) compounds using $^{18}$O-enriched oxygen (1% in He) as combustion gas. Compounds' abbreviations are given in the Experimental Section. Oxygen concentration ranged from 7.1 to 34.2 ppm with an average value of 17 ppm.

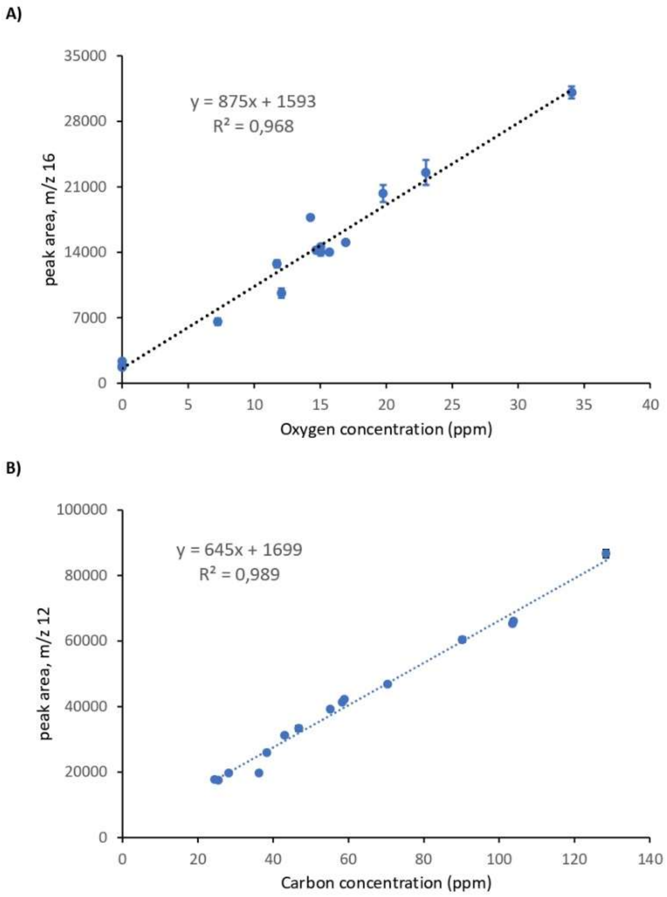


**Figure S4.** Oxygen (A) and Carbon (B) calibration curves obtained from the GC-combustion-MS chromatogram shown in Figure S3 which consists of a mixture of three noncontaining (C12, C20 and AC) and twelve O-containing (C4OL, PB, Cy6ONE, EtO, HB, B, C8OL, A, DiMAL, PhA, DBF, DPh) compounds using $^{18}$O-enriched oxygen (1% in He) as combustion gas. Uncertainty bars correspond to 1 SD (n=3). Compounds' abbreviations and compound concentrations are given in the Experimental Section.

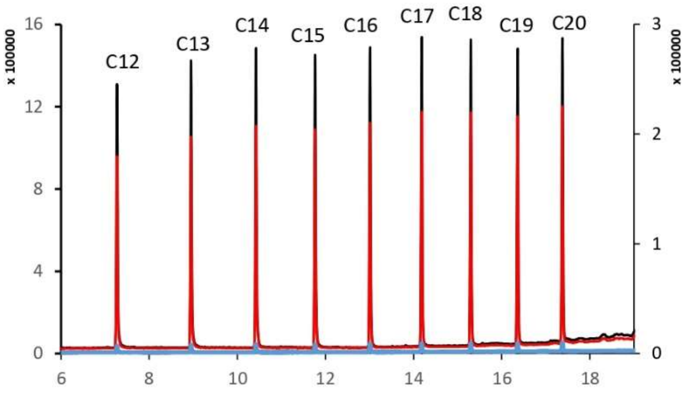


**Figure S5.** GC-combustion-MS chromatogram obtained for a mixture of eleven alkane compounds (C12-C20) using 18O-enriched oxygen (1% in He) as combustion gas. Blue, red and black profiles correspond to m/z 44, 45 and 46, respectively. Compounds' abbreviations and compound concentrations are given in the Experimental Section.

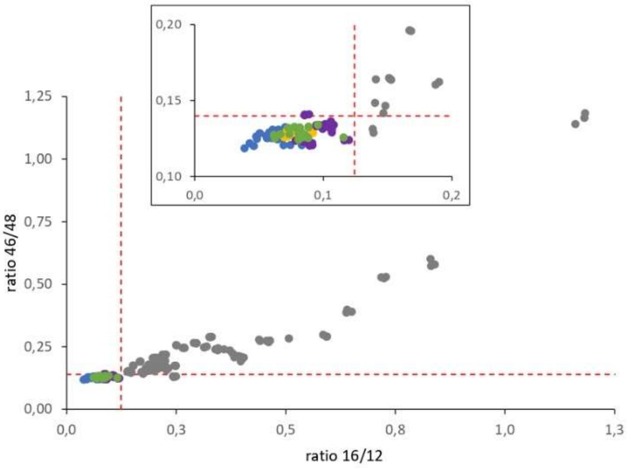


**Figure S6.** Plot of the ratios 46/48 vs 16/12 measured for each chromatographic peak detected in several mixtures of noncontaining and O-containing compounds and analyzed in triplicate on different working days. In total, 200 chromatographic peaks were processed, including 93 noncontaining (3 alkenes, 1 aromatic, 4 N-compounds, 4 S-compounds) and 107 O-containing peaks (13 O-compounds). Oxygen concentrations ranged from 4 to 37 ppm, with an average value of 14 ppm. See experimental section for details. Color code: alkanes (blue), aromatics (yellow), N-compounds (purple), S-compounds (green) and O-compounds (grey).

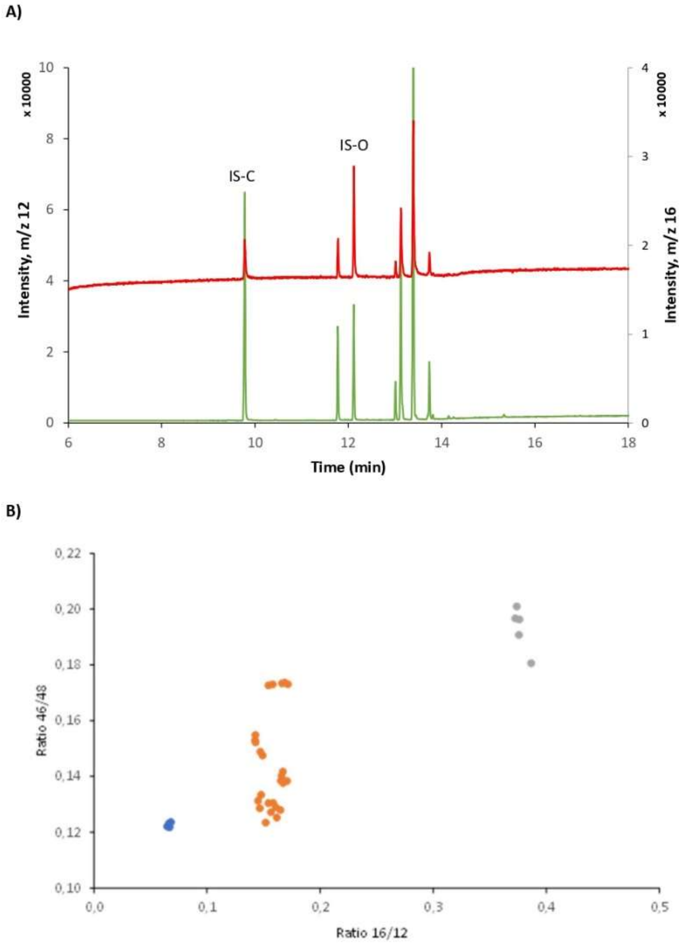


**Figure S7.** A) GC-combustion-MS chromatogram of an aliquot of the Biodiesel SRM 2772 previously spiked with Nonadecane and Dimethylphtalate as generic Internal Standards of C (IS-C) and O (IS-O). B) Plot of the ratios 46/48 vs 16/12 measured for each chromatographic peak detected in the quintuplicate analysis. Color code: IS-C (blue), IS-O (orange) and FAMEs (grey, O-compounds).

# CONCLUSIONES - CONCLUSIONS

# Conclusiones

La presente Tesis Doctoral pretendía una evaluación crítica de metodologías para el análisis selectivo de distintos elementos en muestras de tipo energético, haciendo uso para ello de metodologías basadas en la cromatografía de gases acoplada a distintos detectores de espectrometría de masas, como son el GC-ICP-MS/MS y el nuevo detector de combustión-MS.

La técnica de GC-ICP-MS/MS, es posiblemente una de las más sensibles en cuanto a análisis de especiación de heteroátomos se refiere, sin embargo, está muy limitada a un número importante de elementos dado que su fuente de ionización, es decir, el plasma, se encuentra a presión atmosférica. Esto impide que cualquier elemento que se encuentre de forma natural en el aire que la rodea no pueda ser medido sin obtener una ingente señal de fondo, que, por un lado, impediría la detección del elemento a niveles bajos de concentración y, por otro, saturaría el detector rápidamente. De esta forma, con el GC-ICP-MS quedan limitados o directamente prohibidos, elementos tales como C, H, N u O.

Durante la presente tesis doctoral se han abordado una serie de objetivos ya enumerados acometidos principalmente con dos detectores acoplados la GC. el ICP-MS/MS, permitió en el Capítulo 1 la caracterización de compuestos de Cl en muestras de gran complejidad, como son los aceites de pirólisis obtenidos a través de plásticos. En este capítulo se consiguió desarrollar una metodología selectiva para la detección de Cl obteniendo una respuesta equimolar, que permitió el uso de patrones genéricos para llevar a cabo la cuantificación de las especies. Se desarrolló una metodología que permitió el análisis por especiación, así como el análisis del contenido total de Cl sin separación cromatográfica. La metodología fue validada con el uso de un material certificado de referencia.

Durante los siguientes capítulos, la técnica usada fue la de GC-combustión-MS. Esta metodología fue desarrollada durante la tesis doctoral en colaboración con la empresa de instrumental, Shimadzu. En el capítulo 2, se extendió el primer prototipo diseñado hacia la detección de compuestos imposibles de detectar por ICP-MS/MS como son el C, H, N además del S. Mediante la modificación instrumental de un equipo comercial de GC-MS, la instalación de un horno de combustión entre la columna y el MS, permitió la combustión cuantitativa de los compuestos que eluyen de la columna a la misma especie, el correspondiente óxido de cada elemento (C-$CO_2$, H-$H_2O$, N-NO, S-$SO_2$). Esto permite posteriormente una ionización independiente de la especie en el MS, permitiendo en última estancia el uso de patrones genéricos para llevar a cabo la cuantificación de especies desconocidas. La técnica fue optimizada para distintos parámetros de temperatura y flujo. Además, la técnica se validó mediante el análisis de varios tipos de diésel cuyo contenido de S y N era conocido.

En este contexto, durante el capítulo 3, se evaluó el prototipo como detector selectivo de N. En este capítulo, además, entró en juego el nuevo equipo desarrollado por la empresa Shimadzu. Así, se compararon las características de ambos equipos, en términos de sensibilidad y reproducibilidad de los resultados. También se comparó críticamente el equipo con un detector dedicado exclusivamente a la detección selectiva de N, el NCD. En términos analíticos de linealidad y equimolaridad se obtuvieron resultados similares para ambos, sin embargo, se observó una mejora considerable en el límite de detección de hasta dos órdenes de magnitud con respecto al NCD y de un orden con respecto al prototipo de Agilent. Varias muestras fueron analizadas consiguiendo resultados similares en cuanto al perfil cromatográfico con ambos detectores, además de obtener resultados cuantitativos estadísticamente indistinguibles.

Durante la presente Tesis Doctoral, se realizó una estancia breve con el objetivo de caracterizar las muestras que se fueron analizando durante los capítulos 1, 2 y 3 mediante cromatografía de gases bidimensional. Se consiguió correlacionar los perfiles selectivos obtenidos en los detectores de ICP-MS y combustión-MS con los cromatogramas en dos dimensiones obtenidos durante la estancia para la mayoría de las muestras. Si bien es cierto, que los compuestos de cloro obtenidos durante el capítulo 1 tuvieron cierta dificultad de cara a identificarse y, solamente uno de ellos pudo confirmarse con la fiabilidad adecuada.

Por último, durante el capítulo 4, el desarrollo del detector de GC-combustión-MS llegó a uno de los puntos álgidos, permitiendo el desarrollo de una metodología para la detección selectiva de compuestos de O, para los cuales, en la actualidad no existen detectores con la sensibilidad adecuada. Cambiando la configuración del sistema, al usar un flujo de $^{18}$O en lugar de uno de $^{16}$º, como en los anteriores capítulos, se consiguió distinguir el oxígeno proveniente del fondo del oxígeno del analito. Esta metodología además consigue detectarlos al tener una respuesta equimolar, pudiendo usar una cuantificación independiente de la especie. El sistema se validó mediante el análisis de un material de referencia y de una muestra real de diésel adicionada con compuestos de oxígeno.

Se puede concluir, que le objetivo general de la Tesis Doctoral, de desarrollar metodologías elementales y selectivas mediante el uso de la GC y la MS fue conseguido exitosamente, siendo validadas todas las metodologías mediante su aplicación a muestras reales y materiales certificados de referencia.

# Conclusions

The present Doctoral Thesis aimed at a critical evaluation of methodologies for the selective analysis of different elements in energetic samples, making use of methodologies based on gas chromatography coupled to different mass spectrometry detectors, such as GC-ICP-MS/MS and the new combustion-MS detector.

The GC-ICP-MS/MS technique is possibly one of the most sensitive in terms of heteroatom speciation analysis, however, it is very limited to a significant number of elements since its ionization source, i.e. the plasma, is at atmospheric pressure. This prevents any element naturally occurring in the surrounding air from being measured without obtaining a huge background signal, which, on one side, would prevent detection of the element at low concentration levels and, on the other hand, would quickly saturate the detector. Thus, with GC-ICP-MS, elements such as C, H, N or O are limited or directly prohibited.

During the present doctoral thesis, a series of objectives already listed have been addressed, mainly undertaken with two detectors coupled to the GC. The ICP-MS/MS, allowed in Chapter 1 the characterization of Cl compounds in samples of great complexity, such as pyrolysis oils obtained through plastics. In this chapter it was possible to develop a selective methodology for the detection of Cl obtaining an equimolar response, which allowed the use of generic standards to carry out the quantification of the species. A methodology was developed that allowed the analysis by speciation, as well as the analysis of the total Cl content without chromatographic separation. The methodology was validated with the use of a certified reference material.

During the following chapters, the technique used was GC-combustion-MS. This methodology was developed during the doctoral thesis in collaboration with the instrument company, Shimadzu. In chapter 2, the first prototype designed was extended towards the detection of compounds impossible to detect by ICP-MS/MS such as C, H, N in addition to S. By instrumental modification of a commercial GC-MS equipment, the installation of a combustion furnace between the column and the MS, allowed the quantitative combustion of the compounds eluting from the column to the same species, the corresponding oxide of each element (C-CO2, H-H2O, N-NO, S-SO2). This subsequently allows a species-independent ionization in the MS, ultimately allowing the use of generic standards to carry out the quantification of unknown species. The technique was optimized for different temperature and flow parameters. In addition, the technique was validated by analysing several types of diesel whose S and N content was known.

In this context, during Chapter 3, the prototype was evaluated as a selective detector of N. In this chapter, the new equipment developed by Shimadzu also came into play. Thus, the characteristics of both devices were compared in terms of sensitivity and reproducibility of the results. The equipment was also critically compared with a detector dedicated exclusively to the selective detection of N, the NCD. In analytical terms of linearity and equimolarity, similar results were obtained for both, however, a considerable improvement in the detection limit of up to two orders of magnitude was observed with respect to the NCD and of one order with respect to the Agilent prototype. Several samples were analysed achieving similar results in terms of chromatographic profile with both detectors, in addition to obtaining statistically indistinguishable quantitative results.

During the present Doctoral Thesis, a short stay was carried out with the objective of characterizing the samples that were analysed during chapters 1, 2 and 3 by means of two-dimensional gas chromatography. It was possible to correlate the selective profiles obtained in the ICP-MS and combustion-MS detectors with the two-dimensional chromatograms obtained during the stay for most of the samples. It is true, however, that the chlorine compounds obtained during Chapter 1 were difficult to identify and only one of them could be confirmed with adequate reliability.

Finally, during chapter 4, the development of the GC-combustion-MS detector reached one of the high points, allowing the development of a methodology for the selective detection of O compounds, for which there are currently no detectors with adequate sensitivity. By changing the configuration of the system, using a flow of 18O instead of one of 16º, as in the previous chapters, it was possible to distinguish the oxygen coming from the background from the oxygen of the analyte. This methodology also manages to detect them by having an equimolar response, being able to use a species-independent quantification. The system was validated by analysing a reference material and a real diesel sample added with oxygen compounds.

It can be concluded that the general objective of the Doctoral Thesis, to develop elemental and selective methodologies through the use of GC and MS was successfully achieved, being validated all the methodologies through their application to real samples and certified reference materials.

# SUGERENCIAS PARA TRABAJOS FUTUROS

A lo largo de la presente Tesis Doctoral se han desarrollado diferentes metodologías todas ellas basadas en la cromatografía de gases acoplada a distintos detectores selectivos, de cara a obtener cuantificaciones independientes de la especie. Si bien, varios de los capítulos llegan a un fin, otros abren puertas a posibles aplicaciones nuevas tanto en analitos como en muestras.

- Por ejemplo, en el caso del ICP-MS/MS, uno de los trabajos que podría ser interesante es la determinación de especies de Si. Este elemento es alto complejo, dada su gran abundancia en todas las partes del sistema de GC. Por ello se requiere el uso de condiciones específicas de cara a obtener respuestas no interferidas por el sistema. Aunque se hicieron algunas pruebas durante la presente tesis doctoral, el uso de condiciones genéricas no permitió detectar las especies con suficiente sensibilidad, quedando, por tanto, una futura línea a explorar.
- El detector desarrollado conjuntamente con la empresa Shimadzu de GC-combustión-MS, ha sido, evaluado no solo como detector multielemental para C, H, S y N sino que también se extendió su aplicabilidad hacia la detección selectiva de nitrógeno. Este mismo proceso puede llevarse a cabo con un elemento de también gran interés como es el S. Además, la disponibilidad de otro detector en el grupo de investigación que permite la detección selectiva de S, el ICP-MS/MS, permitiría una comparación directa en cuanto a sensibilidad y capacidad análisis de matrices complejas.
- De nuevo, del artículo número III, se deriva algún estudio más en el que sería interesante profundizar. El hecho de detectar también selectivamente el H de las especies, permitiría llevar a cabo estudios en los que evaluar el detector para obtener información del grado de insaturación, relacionado con el ratio H/C en muestras complejas, lo que podría llevar a estudios relacionados con la destilación simulada.

- En cuanto a la detección selectiva de oxígeno abordada en el capítulo 4, existe un gran número de experimentos realizados durante la presente tesis doctoral, y que aportan información respecto al proceso fundamental que sufren las especies en el horno de combustión. Todo este experimental será evaluado para su publicación en el futuro.
- Por supuesto, existen un número grande de elementos que son interesantes de cara a analizar en cromatografía de gases, como el F, Cl, Br, P, Si… siguiendo la teoría planteada en los capítulos 2 y 3, la combustión de especies que contengan estos elementos deberían generar especies volátiles que pueden ser detectadas en el MS. Si se consiguiera una detección equimolar para todos estos elementos, se daría un salto de calidad en el detector, al poderse considerar ya un verdadero detector multielemental, abarcando un gran número de elementos que no se pueden medir por ICP-MS/MS (como son el C, H, N, F…).

# INFORME DE PUBLICACIONES

El trabajo desarrollado en la presente Tesis Doctoral ha sido publicado en revistas científicas de primer nivel, en revistas específicas de Química Analítica y en revistas multidisciplinares, todas ellas indexadas en el JCR (Journal Citation Report), en el primer cuartil de cada categoría. Los dos trabajos enviados para su publicación también se esperan que sean publicados en revistas del primer quartil de Química Analítica y multidisciplinar. Acontinuación se enumeran los artículos presentados en la Tesis Doctoral.

- **Recent advances in GC-ICP-MS: Focus on the current and future impact of MS/MS technology**. García Bellido, J., Freije-Carrelo, L., Moldovan, M. and Ruiz Encinar, J. Trends in Analytical Chemistry (2020), 130, 115963. DOI: 10.1016/j.trac.2020.115963
- **Quantitative multiplexed elemental (C, H, N and S) detection in complex mixtures using gas chromatography**. Freije-Carrelo, L., García Bellido, J., Alonso Sobrado, L., Moldovan, M. Bouyssiere, B., Giusti, P. and Ruiz Encinar, J. Chemical Communications (2020), 56, 2905-2908. DOI: 10.1039/C9CC09842A
- **Potential of GC-Combustion-MS as a Powerful and Versatile Nitrogen-Selective Detector in Gas Chromatography**. Javier García-Bellido, Laura Freije-Carrelo, Montserrat Redondo-Velasco, Marco Piparo, Mariosimone Zoccali, Luigi Mondello, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti, and Jorge Ruiz Encinar. Analytical Chemistry (2023), 95, 11761–11768 DOI: 10.1021/acs.analchem.3c01943
- **Assessing GC-ICP-MS/MS determination of chlorine-containing compounds in plastic-based pyrolysis oil samples.** Javier García Bellido, Montserrat Redondo-Velasco, Mariomone Zoccali, Luigi Mondello, Pierre Giusti, Mariella Moldovan, and Jorge Ruiz Encinar. *A la espera de ser enviado a la revista Talanta*.
- **Sensitive and Selective Determination of Oxygenated Compounds in complex samples using GC-combustion-MS with isotopic oxygen as oxidizing gas.** Javier García Bellido,

Montserrat Redondo-Velasco, Laura Freije-Carrelo, Mariella Moldovan, Brice Bouyssiere, Pierre Giusti and Jorge Ruiz Encinar. *A la espera de ser enviado.*

En la siguiente tabla se presenta un informe con los factores de impacto de las publicaciones presentadas.

| **Revista** | **Factor de Impacto** | **Categoría** | **Puesto** | **Año JCR** |
|---|---|---|---|---|
| Trends in Analytical Chemistry | 12.3 | Química Analítica | 1/87 (Q1) | 2020 |
| Chemical Communications | 6.22 | Química, multidisciplinar | 44/178 (Q1) | 2020 |
| Analytical Chemistry | 7.4 | Química Analítica | 7/86 (Q1) | 2022 |
| Talanta (En espera de ser enviado) | 6.1 | Química Analítica | 9/86 (Q1) | 2022 |
| Journal of the American Chemical Society (En espera de ser enviado) | 15.0 | Química, multidisciplinar | 17/178 (Q1) | 2022 |

# PUBLICACIONES Y TRABAJOS DERIVADOS

Artículo científico VI.

**Título**: *GC-ICP-MS/MS Instrumental Setup for Total and Speciation Sulfur Analysis in Gasolines using Generic Standards*

**Autores**: Laura Freije-Carrelo, Javier García-Bellido, Francisco Calderón-Celis, Mariella Moldovan, Jorge Ruiz Encinar

**Referencia**: *Analytical Chemistry, **2019**, 91, 7019-7024*

**DOI**: 10.1021/acs.analchem.9b01199

analytical chemistry

Technical Note

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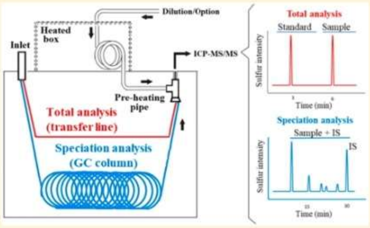
# GC-ICP-MS/MS Instrumental Setup for Total and Speciation Sulfur Analysis in Gasolines using Generic Standards

Laura Freije-Carrelo, Javier García-Bellido, Francisco Calderón-Celis, Mariella Moldovan,* and Jorge Ruiz Encinar*

Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain

*Supporting Information*



**ABSTRACT:** Quantitative characterization of sulfur-containing petroleum derivatives is mainly limited by the large number of potential targets present and the matrix effects suffered due to the high-carbon-containing matrices. Herein we describe the instrumental modifications required in a commercial GC-ICP-MS/MS instrument, and their corresponding optimization, for turning it into a compound-independent quantitative technique for both total and speciation sulfur analysis in gasolines. Additionally, carbon-derived matrix effects were made negligible for direct and fast total S analysis, making the use of relatively complex isotope-dilution strategies not necessary anymore. An absolute detection limit of 0.3 pg of S was achieved, which is, to the best of our knowledge, more than 1 order of magnitude below the ones reported for other sulfur GC selective detectors. The precision was below 3% RSD. Total analysis was performed by flow-injection analysis through a transfer line and external calibration, whereas speciation analysis was carried out by chromatographic separation and internal standardization. In both cases, simple generic standards were used, which enabled us to get rid of specific S-containing standards, which were sometimes not available or unstable. The proposed method was successfully applied to total and speciation sulfur analysis of a commercial gasoline sample and validated with a certified-reference-material (ERM-EF213) gasoline. The approach has proved to be simple, fast, robust, and convenient for implementation in routine laboratories, as demonstrated by the successive analyses of 50 gasoline samples in 3 h without any instrumental drift.

Petroleum derivatives are highly complex samples consisting of a mixture of hydrocarbons and heteroatom-containing compounds, the most abundant one being sulfur (up to 8% in crude oil), which can be found in different functional groups, mainly thiols, sulfides, and thiophenes. The relevance of these compounds lies in their corrosive nature, which causes catalyst poisoning in refining processes, and in their pollution impact on the environment.[1,2] Maximum permissible sulfur contents are already regulated. For instance, a legal limit of 10 ppm (w/w) sulfur in diesel fuels and gas oils has been established in Europe (Directive 2009/30/EC).[3] In addition to total sulfur analysis, quantitative speciation of the individual S-containing compounds is also advisable in petroleum samples for the optimization of the desulfuration processes and for environmental studies.[1,4]

Different techniques for direct total sulfur analysis in petroleum derivatives have been reported, such as wavelength-dispersive X-ray fluorescence[5] or ultraviolet fluorescence.[6] These techniques, however, suffer from poor limits of detection (LODs, around 1 ppm) and poor precision and accuracy, and in most cases, matrix effects have to be accounted for. On the other hand, speciation analysis of the S-containing compounds in light and middle distillates is commonly performed by gas chromatography (GC) coupled to sulfur-selective detectors. Importantly, quantification of these sorts of samples might be challenging given the high number of S-containing compounds present. Therefore, compound-independent calibration (CIC) would be very convenient because it would avoid the use individual standards, which are sometimes not available or are unstable, for each target compound. Flame photometric detectors (FPDs) and pulsed flame photometric detectors (PFPDs) have been used. However, responses in FPDs are usually nonlinear, are relatively nonuniform, and can be quenched by coelution of hydrocarbons.[7,8] Atomic-emission detectors (AEDs) can also be applied for S-specific detection, but there is considerable controversy with regard to the possibility of performing CIC.[9,10] Sulfur chemiluminescence detectors (SCDs) have proved to be superior to other detectors, are widely used for sulfur speciation in petroleum derivatives, and have LODs in the low-parts-per-billion range. However, the presence of heavy

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organic matrix can lead to significant signal drift, resulting in stringent operation and maintenance procedures.[9]

Alternatively, inductively coupled plasma mass spectrometry (ICP-MS) could be a really sensitive and convenient technique for both total and speciation sulfur analysis as it is able to provide species-independent responses. Nevertheless, direct liquid total analysis of petroleum samples by ICP-MS requires conscientious selection of the introduction technique to prevent large quantities of carbon reaching the plasma, which can be accomplished by using low-flow nebulizers, cooled spray chambers, or alternative procedures.[11−13] Direct injection by thermal vaporization and ICP-MS has also been employed. Unfortunately, matrix effects and signal drifts caused by coelution of hydrocarbons demand isotope dilution using $^{34}$S-labeled volatile standards. Even so, only 0.1 $\mu$L of sample could be injected, and nitrogen-gas bracketing injections were needed.[14] On the other hand, ICP-MS can also be used as GC selective detector for sulfur speciation in petroleum-related samples.[15] Once more, the main difficulty lies in the carbon-matrix effects caused by coelution of hydrocarbons with the S-containing compounds; therefore, accurate sulfur speciation could only be performed by species-specific[16] or online[17] isotope dilution. Although this last approach avoids the use of individual standards, it still requires the synthesis, characterization, and online addition of an $^{34}$S-labeled gas standard that is more complicated than in the case of the use of $^{34}$S-labeled standards in HPLC. Recently, the development of ICP-MS/MS technology opened the door to interference-free sulfur detection, as already demonstrated in numerous applications.[18−20] Although sulfur profiles have already been reported using GC-ICP-MS/MS,[21] the application of ICP-MS/MS as a quantitative, compound-independent sulfur GC detector has not been carefully optimized and demonstrated so far. This work presents a new instrumental GC-ICP-MS/MS setup able to provide both total and speciation sulfur quantitative analysis at the very-low-parts-per-billion level in petroleum derivatives without the need for complex isotope-dilution procedures.

## EXPERIMENTAL SECTION

**Standards and Samples.** Dibutylsulfide (DBS, purity 100%), ethyl phenyl sulfide (EPS, 97%), benzothiophene (BT, 97%), methylbenzothiophene (MBT, 96%), dibenzothiophene (DBT, 98%), bromocyclohexane (CXB, 99%), and hexane were purchased from Sigma-Aldrich. Dimethyl sulfide (DMS) in argon-gas standard (2.9 ± 0.3 ppm S, v/v) was obtained from Linde AG. $CO_2$/Ar (10:90) and $O_2$/Ar (20:80) were supplied by Air Liquide. Commercial gasoline was obtained from a regular gas station in Asturias, Spain. The certified reference material, ERM-EF213, consisting of real "sulfur-free" gasoline (9.1 ± 0.8 ppm S, w/w), was obtained from BAM.

**Instrumentation.** A GC 7890B (Agilent Technologies) was coupled to a triple quad ICP-MS, ICP-MS/MS (Agilent 8800) by means of a heated GC-ICP-MS interface (Agilent Technologies). A gas-sampling valve (GSV) located outside the oven and heated at 200 °C, was connected to the GC inlet in order to allow the injection of 1 mL of the DMS gas standard when necessary. An Ar pipe was taken out of the oven and attached to the heated GSV box. GC and ICP-MS/MS conditions are detailed in Table S-1 of the Supporting Information, and a diagram of the setup is shown in Figure 1. Total and speciation sulfur analyses were performed using an inert transfer line (fused silica deactivated, 5 m × 0.32 mm i.d., Agilent Technologies) and a J&W HP-5 column (30 m × 0.32 mm i.d. × 0.25 $\mu$m), respectively. Manual injection was used for liquid samples. $CO_2$/Ar (10:90) was introduced online using a T-connection, and a Mass Flow Controller (MFC) PR4000B (MKS Instruments).

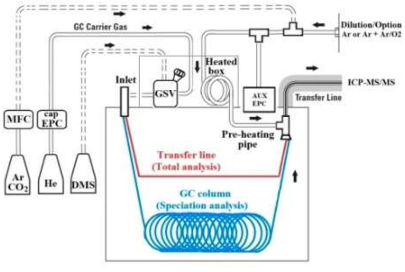

Figure 1. Diagram of the GC-ICP-MS/MS setup for total analysis (transfer line, red line) or speciation analysis (GC column, blue line). Dimethyl sulfide gas standard (DMS) and Ar/$CO_2$ (dotted lines) were only used for the assessment of species-independent responses and matrix effects.

**Procedures.** *Assessment of the Species-Independent Sulfur Response.* DMS (1 mL) was injected by the GSV and the transfer line at different temperatures (80 to 320 °C). Sulfur areas were computed with the preheating Ar pipe inside and outside the oven. Then, 1 $\mu$L of a mixture (0.3 ppm S, w/w) of DBS, EPS, BT, MBT, and DBT was injected through a GC column, using both splitless and split modes (1:6 and 1:10 ratios). Response factors and recoveries were computed using DBS as an internal standard (IS).

*Assessment of Carbon-Matrix Effects for Sulfur Detection.* DMS (1 mL) was injected through the GSV and the transfer line at increasing flows of the $CO_2$/Ar mixture (from 2 to 5 mL/min in steps of 0.5 mL/min). To do so, $CO_2$/Ar was mixed online with the option-flow $O_2$/Ar gas (8%) and dilution-flow−option-flow Ar gas through a T-connection 5/32″ (John Guest), as shown in Figure 1. $O_2$/Ar gas was added to prevent carbon buildup on the cones during the working time.

*Direct Total Volatile-Sulfur Analysis by GC-ICP-MS/MS.* Total sulfur analysis was carried out by injecting the sample into the transfer line. Detailed conditions are shown in Table S-1. An external-calibration curve was built by injecting 1 $\mu$L of a mixture of BT in hexane (3−12 ppm S, w/w) and CXB, which was added as the IS (ca. 13 ppm Br). Then, 1 g of gasoline was spiked along with with 13 ppm Br. For standard additions, 0.2 g of a mixture containing increasing concentrations of BT standard and constant concentrations of CXB was added to 10 g of the commercial gasoline.

*Sulfur Speciation Analysis by GC-ICP-MS/MS.* Detailed conditions are shown in Table S-1. Commercial and CRM gasoline samples were spiked with DBT as the IS (0.5 and 2 ppm S, respectively).

## RESULTS AND DISCUSSION

**Instrumental Modifications To Obtain Species-Independent Sulfur Response.** When performing CIC in GC-ICP-MS, the quantitative transmission of all the compounds along the GC and the GC-ICP-MS interface is required, as well as species-independent ionization in the ICP-MS plasma. In the new commercial Agilent GC-ICP-MS interface, the flow from the column is mixed online with the Ar carrier (a mix of the "dilution" and "option" flows) previously heated by means of a preheating pipe (ca. 4.5 m) located inside the GC oven. Therefore, the temperature of the Ar carrier changes dramatically along a regular GC gradient. To evaluate whether the S response could be influenced by this effect, triplicate injections of DMS gas were made using the GSV and the fused silica transfer line at different oven temperatures (80−320 °C). In this way, inlet and column issues were avoided and only the oven temperature played a role. Surprisingly, as illustrated in Figure 2, DMS S areas increased significantly during the experiment (up to a 55%); therefore, sulfur CIC was hampered. Such results pointed to the likelihood that the Ar-carrier flow, which greatly influences the sensitivity, could change significantly as the temperature of the oven increases. In order to demonstrate this assumption, an auxiliary electronic pressure control (Aux EPC) was installed on the GC and directly connected to the Ar-flow line using a "T" (Figure 1) to monitor the back-pressure along the gradient. As can be seen in Figure S-1, the back-pressure increased significantly (56%) with the temperature of the oven. We decided then to move the pipe outside the oven and place it in the GSV box in order to still heat the Ar carrier but at a constant temperature of 200 °C. Pressure values measured under such instrumental conditions remained unchanged with temperature (Figure S-1, <7% variation). Moreover, triplicate injections of DMS gas were then repeated, and the S areas obtained were stable with temperature (3% RSD), as clearly shown in Figure 2.

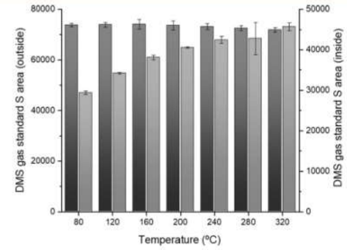

**Figure 2.** Peak areas for the DMS gas standard from 80 to 320 °C with the Ar preheating pipe inside (light gray) and outside the oven (dark gray). Uncertainty bars correspond to 2 × SD ($n = 3$).

Once the stability of the S response factor along the GC was achieved, a solution containing five S compounds (DBS, EPS, BT, MBT, and DBT), which represent the families typically present in petroleum derivatives, was injected onto a GC column (Figure S-2) using different split ratios (splitless, 1:6, and 1:10). The first-eluting compound (DBS) was used as the IS for the quantification of the other four, so that possible variations in the S response factor due to different transmission efficiencies at the inlet−column could be assessed. As shown in Table S-2, recoveries ranging from 97 to 104%, were obtained, demonstrating that CIC of S can be safely carried out with the customized GC-ICP-MS/MS setup.

The LOD ranged from 380 to 420 ppt S depending on the species (absolute detection limit of ca. 0.3 pg of S). To the best of our knowledge, this LOD is more than 1 order of magnitude lower than the ones reported for other S-selective detectors, such as SCD or ICP-MS (both in ppb levels)[9] and similar to those obtained with the GC-ICP-MS/MS instrument.[21] Interestingly, the LODs reported in this last case were more than 1 order of magnitude different depending on the S-containing species analyzed, which seems to support the need for the instrumental modifications proposed herein to obtain sulfur CIC when using the commercial GC-ICP-MS/MS instrument. The linear range obtained covers more than 2 orders of magnitude (1−360 ppb S, Figure S-3).

**Assessment of Carbon-Matrix Effects for Sulfur Detection in Petroleum Derivatives.** It is well-known that coelution with carbon-containing compounds may produce sulfur-matrix effects, and strategies such as isotope dilution were developed to overcome them.[15,17] Therefore, once the species-independent S signal was demonstrated, we wanted to assess the occurrence of carbon-matrix effects in the plasma. In order to keep full control on the amount of carbon reaching the plasma, injections of DMS were made through the GSV connected to the transfer line, whereas flows of the $CO_2$/Ar mixture (10:90) were added online, as shown in Figure 1. The carbon signal was monitored as $^{29}CO^+$ to obtain a rough profile of the added carbon. A minimum flow of 2 mL/min (limited by the MFC used) and a maximum flow of 5 mL/min were tested, corresponding to 107 and 268 μg/min C, respectively. Note that 268 μg/min C is equivalent to a flow-injection analysis (FIA) of 1 μL of pure gasoline eluting in 2.5 min. The FIAgram and DMS sulfur areas obtained are shown in Figure 3A,B, respectively. Remarkably, both the RSD of the S areas for individual triplicates at each carbon content and the RSD for all injections were around 1% ($n = 24$), proving that the occurrence of matrix effects was negligible within the tested range.

These results nonetheless are in disagreement with previous studies in which analysis of S-containing species in petroleum products showed signal variations attributable to carbon-matrix effects.[17] In order to clarify this issue, a study under conditions that bore a close resemblance to such works (RF power of 700 W; no cell gas; no optional Ar/$O_2$ gas; and single quad, SQ) was carried out.[17] The FIAgram and computed areas from GC-ICP-MS are shown in Figures S-4 and 3B, respectively. In contrast to our results, this second set showed a significant correlation between S signal and carbon added to the plasma. Interestingly, this decrease is consistent with the negative peaks observed in the S background and is caused by hydrocarbon coelution when using GC-ICP-MS (SQ).[17] Finally, we wanted to corroborate that matrix effects were also negligible when analyzing a C-containing matrix in the liquid phase. For this purpose, direct injection in splitless mode of 1 μL of pure hexane was made, and a continuous sulfur signal was monitored from the DMS gas mixed online with the Ar flow. As can be clearly seen in Figure S-5, elution of 1 μL of hexane (547 μg of C) over 2.5 min did not produce any distortion in the continuous S signal. Therefore, the use of the GC-ICP-MS/MS setup proposed herein seems to allow S measurement without significant matrix effects, which opens the door to sulfur CIC in high-carbon-content samples without the need for isotope dilution.

**Direct Total Volatile-Sulfur Analysis of Gasoline by GC-ICP-MS/MS.** After assuring the compound-independent S

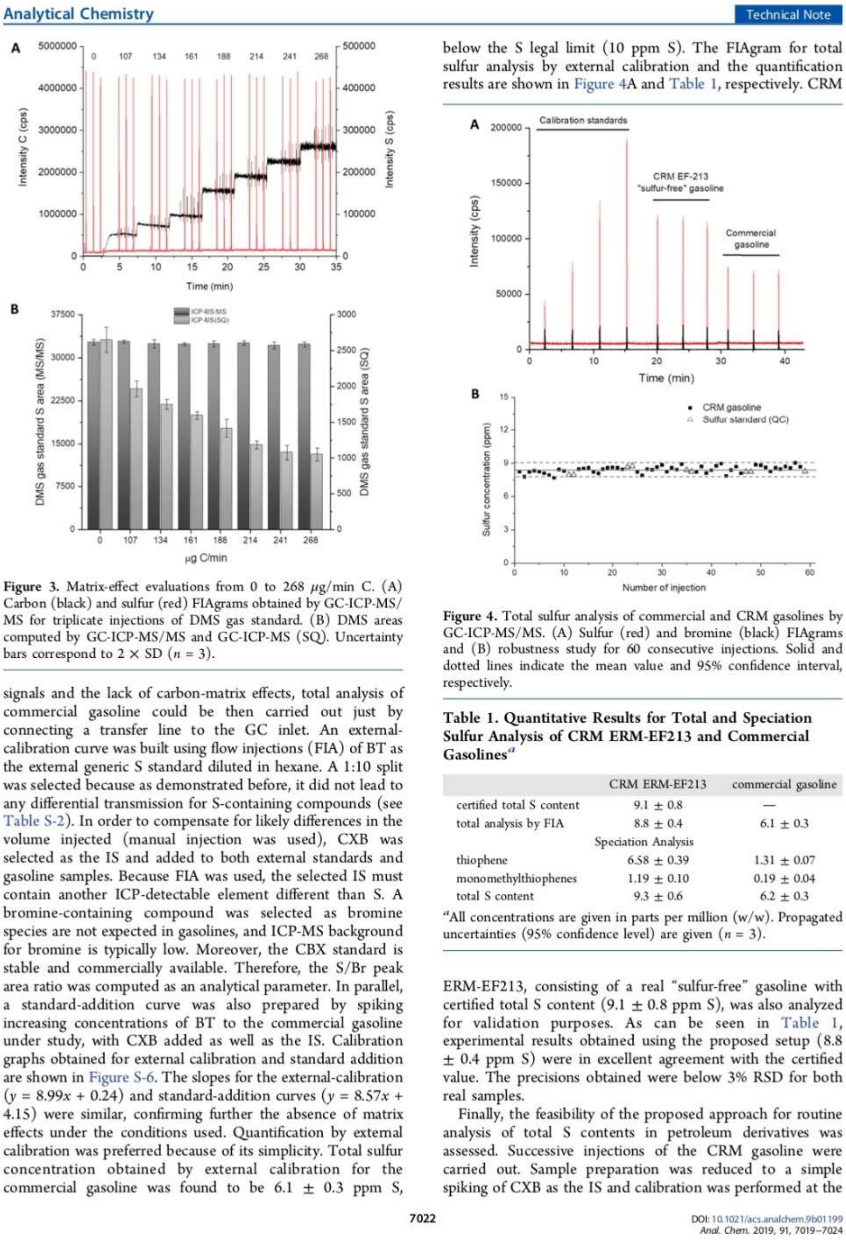

**Figure 3.** Matrix-effect evaluations from 0 to 268 μg/min C. (A) Carbon (black) and sulfur (red) FIAgrams obtained by GC-ICP-MS/MS for triplicate injections of DMS gas standard. (B) DMS areas computed by GC-ICP-MS/MS and GC-ICP-MS (SQ). Uncertainty bars correspond to 2 × SD ($n = 3$).

signals and the lack of carbon-matrix effects, total analysis of commercial gasoline could be then carried out just by connecting a transfer line to the GC inlet. An external-calibration curve was built using flow injections (FIA) of BT as the external generic S standard diluted in hexane. A 1:10 split was selected because as demonstrated before, it did not lead to any differential transmission for S-containing compounds (see Table S-2). In order to compensate for likely differences in the volume injected (manual injection was used), CXB was selected as the IS and added to both external standards and gasoline samples. Because FIA was used, the selected IS must contain another ICP-detectable element different than S. A bromine-containing compound was selected as bromine species are not expected in gasolines, and ICP-MS background for bromine is typically low. Moreover, the CBX standard is stable and commercially available. Therefore, the S/Br peak area ratio was computed as an analytical parameter. In parallel, a standard-addition curve was also prepared by spiking increasing concentrations of BT to the commercial gasoline under study, with CXB added as well as the IS. Calibration graphs obtained for external calibration and standard addition are shown in Figure S-6. The slopes for the external-calibration ($y = 8.99x + 0.24$) and standard-addition curves ($y = 8.57x + 4.15$) were similar, confirming further the absence of matrix effects under the conditions used. Quantification by external calibration was preferred because of its simplicity. Total sulfur concentration obtained by external calibration for the commercial gasoline was found to be 6.1 ± 0.3 ppm S, below the S legal limit (10 ppm S). The FIAgram for total sulfur analysis by external calibration and the quantification results are shown in Figure 4A and Table 1, respectively. CRM ERM-EF213, consisting of a real "sulfur-free" gasoline with certified total S content (9.1 ± 0.8 ppm S), was also analyzed for validation purposes. As can be seen in Table 1, experimental results obtained using the proposed setup (8.8 ± 0.4 ppm S) were in excellent agreement with the certified value. The precisions obtained were below 3% RSD for both real samples.

**Figure 4.** Total sulfur analysis of commercial and CRM gasolines by GC-ICP-MS/MS. (A) Sulfur (red) and bromine (black) FIAgrams and (B) robustness study for 60 consecutive injections. Solid and dotted lines indicate the mean value and 95% confidence interval, respectively.

**Table 1. Quantitative Results for Total and Speciation Sulfur Analysis of CRM ERM-EF213 and Commercial Gasolines**[a]

| | CRM ERM-EF213 | commercial gasoline |
|---|---|---|
| certified total S content | 9.1 ± 0.8 | — |
| total analysis by FIA | 8.8 ± 0.4 | 6.1 ± 0.3 |
| | Speciation Analysis | |
| thiophene | 6.58 ± 0.39 | 1.31 ± 0.07 |
| monomethylthiophenes | 1.19 ± 0.10 | 0.19 ± 0.04 |
| total S content | 9.3 ± 0.6 | 6.2 ± 0.3 |

[a] All concentrations are given in parts per million (w/w). Propagated uncertainties (95% confidence level) are given ($n = 3$).

Finally, the feasibility of the proposed approach for routine analysis of total S contents in petroleum derivatives was assessed. Successive injections of the CRM gasoline were carried out. Sample preparation was reduced to a simple spiking of CXB as the IS and calibration was performed at the

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beginning of the experiment. A solution of BT in hexane (spiked with CXB) was injected twice every 10 gasoline samples as the QC standard. The results obtained are shown in Figure 4B. As can be seen, no signal drift was apparent along the 60 injections performed (3 h of total analysis time). Notably, RSD of the 50 gasoline samples was as low as 3.6%. Similar precision (3.0%, $n = 10$) was obtained for the QC standards. Interestingly, the long sequence of real complex samples did not produce any detrimental effects on the performance of the setup. Moreover, absolute peak areas obtained for S and Br in the QC injections did not show any specific trends (7% RSD), which seems to indicate that the sequence could have been extended without any problem. In fact, the liner, the transfer line, and the ICP cones did not need to be cleaned after the analysis. Sample throughput was as high as 3 min per sample.

**Speciation Sulfur Analysis of Gasoline by GC-ICP-MS/MS.** Speciation sulfur analysis was carried out for both the commercial and CRM gasolines by simply exchanging the transfer line with a GC column. DBT was added as the IS, as it is not naturally present in gasolines. Its computed S response factor was used for quantification of the S species present. The obtained chromatograms are shown in Figures 5 and S-7 for the CRM and commercial gasolines. Quantitative results for the major species (thiophene and monomethylthiophenes isomers) as well as the total S concentration, calculated using the area sum of all the S peaks obtained in the chromatogram, are given in Table 1. Total sulfur contents determined by speciation analysis were found to be statistically indistinguishable from those determined in the direct total flow-injection analyses, for both the commercial gasoline (6.2 ± 0.3 ppm S) and CRM (9.3 ± 0.6 ppm S), which internally validates our results and demonstrates again the negligible influence of the matrix in the direct analysis of total S contents. Moreover, the thiophene and monomethylthiophene concentrations found in the CRM gasoline (6.58 ± 0.39 and 1.31 ± 0.07) fitted very well with the previously reported contents obtained using GC-ICP-MS and online isotope-dilution analysis (6.62 ± 0.12 and 1.33 ± 0.09, respectively),[17] which can be considered the reference method.

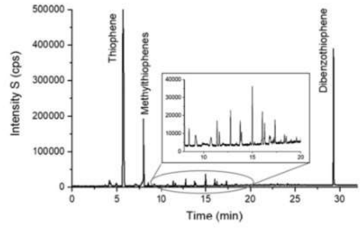

**Figure 5.** GC-ICP-MS/MS chromatogram of CRM EF-213 gasoline spiked with dibenzothiophene as the internal standard.

## ■ CONCLUSIONS

We present herein the instrumental modifications and careful optimization required in a commercial GC-ICP-MS/MS instrument to make it possible to obtain independent quantification of S species. A number of independent experiments were also conducted in order to demonstrate the absence of C-based matrix effects when directly analyzing gasoline samples. Under such instrumental conditions, isotope-dilution strategies are not necessary anymore. These unique characteristics make the developed setup a valuable alternative for total and speciation S analysis in petroleum-related samples using simple and generic S and Br standards with sulfur LODs as low as 400 ppt (w/w). Notably, it is also simple, fast, and robust and thus very convenient for its implementation in petroleum laboratories.

Total or speciation sulfur quantifications can be performed without any pretreatment of the sample using one single instrumental setup just by connecting the GC inlet either to a transfer line (total analysis) or to a column (speciation analysis). It is worth mentioning that the use of a GC equipped with two inlets, one connected to a transfer line, the other to the column, and both to the GC-ICP-MS transfer line by means of a two-hole ferrule, as previously described elsewhere for gas-sample analysis,[22] would further simplify the approach. Such integrated setup could be useful for high-throughput total sulfur quantification (screening). Notably, robustness of the approach would allow long sequence analyses of such real samples, whereas speciation could be later performed exclusively on those whose total S contents would be significant.

Finally, most of the exceptional features obtained for S analysis could be extended to species containing other ICP-detectable elements and amenable to analysis by GC, such as P-, Si-, Br-, or Cl-containing compounds.[23,24] This would extend the scope of the application from petroleum derivatives to biogas, natural gas, or environmental samples.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b01199.

> Instrumental parameters, recoveries, and chromatograms for a model mixture of sulfur compounds; figures for CIC and matrix assessment; calibration curves and chromatogram of the gasoline sample (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

*E-mail: moldovanmariella@uniovi.es (M.M.).
*E-mail: ruizjorge@uniovi.es (J.R.E.).

### ORCID

Francisco Calderón-Celis: 0000-0003-0331-1595
Mariella Moldovan: 0000-0001-6697-4252
Jorge Ruiz Encinar: 0000-0001-6245-5770

### Notes

The authors declare no competing financial interest.

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# SUPPORTING INFORMATION FOR

# GC-ICP-MS/MS Instrumental Set-up for Total and Speciation Sulfur Analysis in Gasolines using Generic Standards

**Laura Freije-Carrelo, Javier García-Bellido, Francisco Calderón-Celis, Mariella Moldovan*, Jorge Ruiz Encinar***

Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain

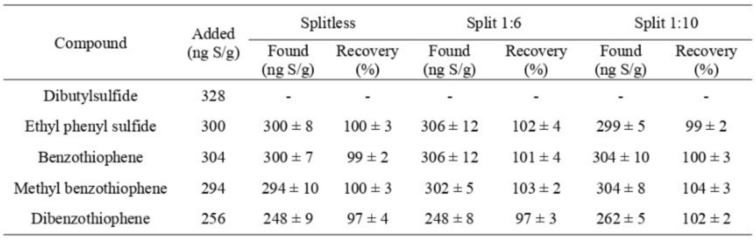
*Authors for correspondence: Dr. Mariella Moldovan (moldovanmariella@uniovi.es), Dr. Jorge Ruiz Encinar (ruizjorge@uniovi.es)

**Instrumental parameters, recoveries and chromatograms for a model mixture of sulfur compounds, figures regarding CIC and matrix assessment, calibration curves and chromatogram of the gasoline sample (PDF).**

**Table S-1.** GC and ICP-MS/MS conditions.

| | |
|---|---|
| **ICP-MS parameters** | |
| RF power | 1550 W |
| Sampling depth | 8 mm |
| Dilution gas | 0.55 L/min |
| Option flow (Ar:$O_2$) | 8% |
| Cell flow ($O_2$) | 25% |
| Cones type | Pt cones |
| ICP torch | GC-ICP-MS interface Agilent Torch |
| m/z measured | $^{32}S \rightarrow {}^{48}SO$ |
| | $^{79}Br \rightarrow {}^{79}Br$ |
| | $^{13}C \rightarrow {}^{29}CO$ |
| **GC parameters, total analysis** | |
| Transfer line flow | 1 mL/min |
| Oven temperature | 250 ºC |
| Injection mode | Split |
| Split ratio | 1:10 |
| Inlet temperature | 250 ºC |
| Injection volume | 1 µL |
| **GC parameters, speciation analysis** | |
| Column | HP-5 |
| Colum flow | 1 mL/min |
| Oven gradient | 40 ºC (2 min), 6ºC/min to 260 ºC |
| Injection mode | Split |
| Split ratio | 1:6 |
| Inlet temperature | 250 ºC |
| Injection volume | 1 µL |
| **Gas sampling valves (GSV)** | |
| Injection volume | 1 mL |
| GSV box temperature | 200 ºC |
| **GC interface** | |
| Interface temperature | 250 ºC |

**Table S-2.** Found concentration and quantitative recoveries of S-containing compounds model mixture obtained by GC-ICP-MS/MS calculated using dibutylsulfide as internal standard. The uncertainties indicate standard deviations for n = 3 injections.



| Compound | Added (ng S/g) | Splitless | | Split 1:6 | | Split 1:10 | |
|---|---|---|---|---|---|---|---|
| | | Found (ng S/g) | Recovery (%) | Found (ng S/g) | Recovery (%) | Found (ng S/g) | Recovery (%) |
| Dibutylsulfide | 328 | - | - | - | - | - | - |
| Ethyl phenyl sulfide | 300 | 300 ± 8 | 100 ± 3 | 306 ± 12 | 102 ± 4 | 299 ± 5 | 99 ± 2 |
| Benzothiophene | 304 | 300 ± 7 | 99 ± 2 | 306 ± 12 | 101 ± 4 | 304 ± 10 | 100 ± 3 |
| Methyl benzothiophene | 294 | 294 ± 10 | 100 ± 3 | 302 ± 5 | 103 ± 2 | 304 ± 8 | 104 ± 3 |
| Dibenzothiophene | 256 | 248 ± 9 | 97 ± 4 | 248 ± 8 | 97 ± 3 | 262 ± 5 | 102 ± 2 |

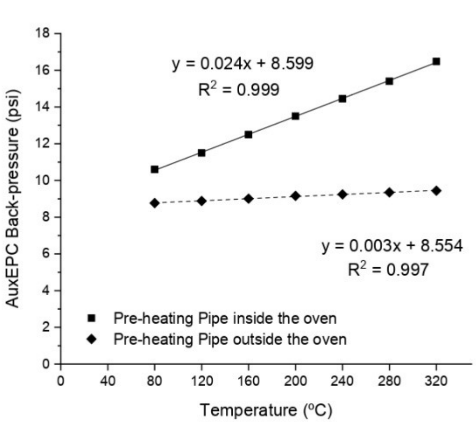


**Figure S-1.** Ar back-pressure, monitored by the AuxEPC installed, when the Ar pre-heating pipe was located inside and outside the oven (attached to the GSV box at 200 ºC).

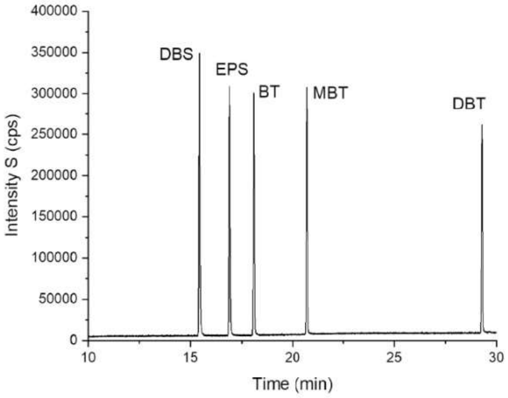


**Figure S-2.** GC-ICP-MS/MS chromatogram of S-containing compounds model mixture (300 ppb S, w/w), consisting of dibutylsulfide, ethyl phenyl sulfide, benzothiophene, methylbenzothiophene, dibenzothiophene (listed by elution order).

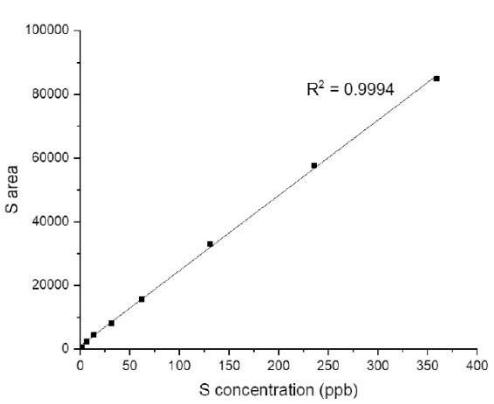


**Figure S-3.** Calibration curve from 1 to 360 ppb S of benzothiophene standard in hexane (w/w). 1 μL injected (splitless conditions) in the GC column.

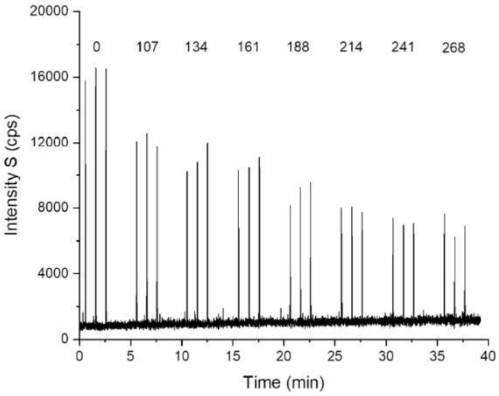


**Figure S-4.** Sulfur FIAgram obtained by GC-ICP-QMS for triplicate injections of DMS gas standard while adding increasing amounts of Ar:$CO_2$ to the plasma (from 0 to 268 μg C/min). Instrumental conditions were selected according to reference 17 (RF power 700 W, no cell gas, no optional Ar:O2 gas, single quad) for comparison purposes.

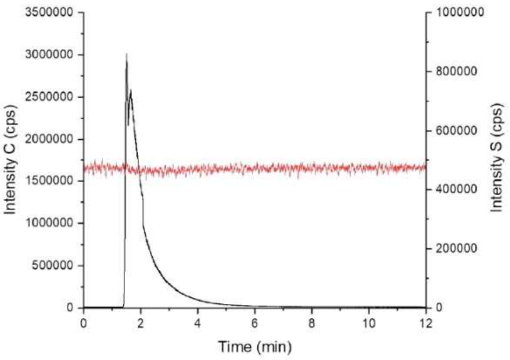


**Figure S-5.** Sulfur (red) and carbon (black) FIAgrams obtained for injection of pure hexane in total analysis conditions (splitless mode). A constant flow (2.5 mL/ min) of the DMS standard (2.9 ppm v/v in Ar) was added to the plasma.

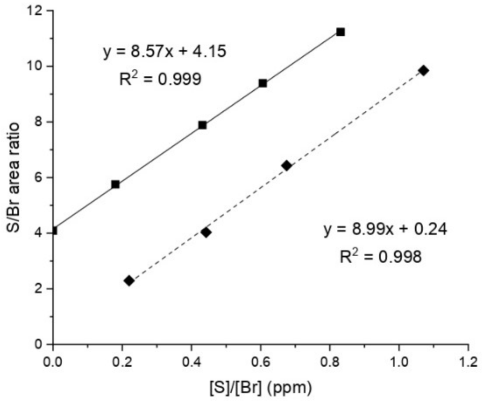


**Figure S-6.** External calibration curve (hexane, dotted line) and standard additions (continuous line) for sulfur total analysis in a commercial gasoline using benzothiophene standard spiked with bromocyclohexane.

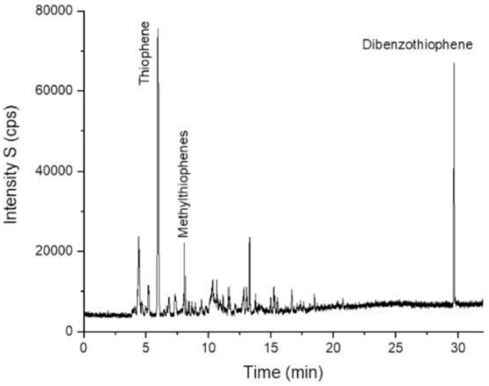


**Figure S-7.** GC-ICP-MS/MS sulfur chromatogram of a commercial gasoline spiked with dibenzothiophene as internal standard.