Evaluation of the spectral accuracy of mass spectrometers using compounds containing Cl or Br atoms.

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Abstract

Current procedures for the evaluation of spectral accuracy of mass spectrometers are limited by the lack of certified isotopic reference materials and the high uncertainty in the isotopic composition of natural abundance molecules. The calculated uncertainties in the ratio M+1/M for natural abundance molecules containing any number of C, H, N and/or O atoms are close to 5% relative due to the natural variability of the isotopic composition of carbon. So, we have developed two alternative measurement procedures with much lower theoretical uncertainties for a better evaluation of spectral accuracy in both single and triple quadrupole analysers. The first method is based on the measurement of the M+2/M, M+4/M+2, etc. ratios for halogenated organic compounds containing either Cl or Br. The theoretical uncertainties for these ratios because of natural variability are in the order of 0.3 to 1.0% making them suitable for the evaluation of spectral accuracy with the additional advantage that there is no need to take into account other limitations such as cluster purity or poor mass resolution. This procedure was applied to the evaluation of a single quadrupole GC-MS instruments using natural abundance PCB and PBDE standards with satisfactory results. The second method can be applied to tandem instruments and takes advantage of the loss of two halogen atoms when PCB and PBDE standards are fragmented by Collision Induced Dissociation. Theoretical SRM transition ratios can be calculated as a pure combinatorial probability with theoretical uncertainties lower than 0.1%. By combining PCBs and PBDEs with different number of halogen atoms, a mass range from 100 to 700 u and abundance ratios from 0.1 to 10 can be evaluated. The use of penta-chlorinated PCBs and/or penta-brominated PBDEs is finally recommended for the evaluation of spectral accuracy of mass spectrometers with the EI source.

Keywords: Spectral accuracy, Triple quadrupole, PCBs, PBDEs, Theoretical uncertainties.

Introduction

Spectral accuracy in molecular Mass Spectrometry can be defined as the capability of a mass spectrometer to provide mass spectra in agreement with the theoretical isotope distribution of a molecule calculated from the isotopic composition of its constituting atoms [1]. There are different fields in which accurate molecular isotope distribution measurements are required such as the determination of elemental compositions [1, 2], metabolic studies with enriched isotopes [3], the determination of the enrichment of labelled molecules [4] and in analytical measurements using Isotope Dilution Mass Spectrometry (IDMS) in combination with Isotope Pattern Deconvolution [5] to cite only a few examples.

In the last few years several works have reported on the evaluation of the spectral accuracy of different mass spectrometers including, Orbitrap [6-9] and TOF [9-10] analysers. The reason for this interest is the fact that, the combination of exact mass determinations and accurate isotope distribution measurements, helps in narrowing down the number of possible formulas which can be assigned to an unknown compound [1, 2]. In most cases, spectral accuracy was evaluated by comparison of the experimental M+1/M ratios in certain target molecules with theoretically derived ratios calculated from the isotope composition of their constituting atoms. In other cases [1,7], spectral accuracy is measured from the whole isotope distribution as the square sum of the differences between the expected and measured abundances. The problem with these procedures is twofold: first, there are intrinsic errors associated with the measurements because of limitations in the mass spectrometer (detector non-linearity, lack of adequate mass resolution, signal processing, etc.) or due to the lack of "chemical" purity of the measured cluster. Second, the theoretically derived abundances will be influenced by the actual isotope composition of carbon taken to calculate the isotope distribution. We have calculated that M+1/M ratios, used in many cases to evaluate spectral accuracy [9], may have uncertainties of the order of 5% relative because of the natural variability of the isotope composition of carbon and other elements. These problems were tackled many years ago in elemental mass spectrometry [11, 12] using certified isotopic reference materials. Intrinsic errors in the measurement of elemental isotope ratios such as mass bias, detector dead-time effects, spectral interferences, isotopic discrimination, etc. have been detected and corrected using certified reference materials with very low theoretical uncertainties [11, 12]. Unfortunately, no reference materials certified for molecular isotope distributions exist so this correction procedure cannot be implemented in molecular mass spectrometry.

Several intrinsic problems have been reported when evaluating the spectral accuracy of mass spectrometers both in high and low resolution analysers. For example, in high resolution, signal processing in Fourier transform may cause peak distortion for neighbouring peaks [13] and the non-linear response of Orbitrap mass spectrometer may affect IDMS calculations [14]. In their excellent review Wang and Gu [1] indicated that the low abundance peaks in the isotope distribution of $C_{62}H_{111}N_{11}O_{12}^+$ with a high resolution TOF instrument were underrepresented in comparison with the monoisotopic mass with a 9.4% overall spectral error. This behaviour is unexplained as it is the contrary to what we would have expected from detector saturation. Another source of intrinsic problems for spectral accuracy evaluation is the purity of the measured mass cluster which will cause spectral interferences. For example, when using Electron Ionization (EI) sources the purity of the mass cluster may affect the results obtained [4] because of the possible coexistence of $(M)^+$ and $(M \pm H)^+$ in the ion source for certain molecules forming unexpected spectral interferences. Additionally, low resolution quadrupole analyzers may suffer for tailing into adjacent peaks causing a distortion of the measured isotope distributions [4]. The afore-mentioned problems and the recent development of a GC/Quadrupole-Orbitrap mass spectrometer [15], prompted us to try and develop procedures which could be applied to better evaluate mass spectrometers for spectral accuracy.

All these intrinsic problems affect the spectral accuracy of molecular mass spectrometers. The correction of those errors has been approached from different points of view. Wang and Gu [1] use a comprehensive mass spectral calibration procedure using the whole isotope distribution while in our laboratory we have employed multiple linear regression for the correction of cluster purity and peak tailing [4]. However, in most cases, spectral accuracy is evaluated only from the measurement of M+1/M ratios [6, 8-10]. Whatever the correction procedure employed, the high uncertainty of the theoretical isotope distributions, make experimental and theoretical M+1/M ratios not a good choice for the evaluation of mass spectrometers in terms of spectral accuracy. An ideal method should be, at least, free of intrinsic problems related to the purity of the cluster in the ion source and the lack of spectral resolution in quadrupole analyzers. Additionally, theoretically derived ratios should have very low theoretical uncertainties to better pinpoint spectral accuracy problems such as those described for Orbitraps and TOF instruments. In this paper we propose two alternative procedures for the evaluation of spectral accuracy both for single and tandem mass spectrometers. These procedures are implemented here only in single and triple quadrupole analyzers coupled to Gas Chromatography using an EI source. However, the basic idea is general and they could be easily implemented for other single stage or tandem mass spectrometers including TOF and Orbitrap analysers and other ion sources. In our procedures Polychlorinated Biphenyls (PCBs) and Polybrominated diphenyl ethers (PBDEs) are proposed as test compounds and the ratios M+2/M, M+4/M+2, etc. selected for the evaluation of spectral accuracy. These ratios have theoretical uncertainties of the order of 0.3 to 1.0% and are not affected by intrinsic problems such as cluster purity in the EI source or the poor mass resolution of quadrupole analyzers.

Experimental

Reagents and materials

A 10 µg mL^{-1} mixture of six PCBs (congeners 18, 52, 101, 138, 180, 194) in heptane was purchased from Sigma-Aldrich (St. Louis, MO, USA). Similarly, a 50 µg mL^{-1} mixture of five PBDEs (congeners 28, 47, 100, 154, 183) was purchased from Accustandard Inc. (New Haven, USA). Additionally, individual 50 µg mL^{-1} certified standards of 7 PBDEs congeners (28, 47, 99, 100, 153, 154 and 183), obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) were employed. Working standard solutions were prepared by dilution of the stock solutions in isooctane (Sigma-Aldrich) or preconcentrated by evaporation of the solvent (PCBs) and were stored in the dark at 4°C until use.

Instrumentation

The GC-MS single quadrupole instrument consisted of a GC model 6890N (Agilent Technologies, Waldbrom, Germany) fitted with a split/splitless injector and equipped with a MSD model 5975B (Agilent Technologies, Tokyo, Japan). The chromatographic separation was carried out using a low polarity capillary column HP-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) purchased to J&W Scientific (Folsom, CA, USA).

The GC-MS/MS instrument consisted of a GC model Agilent 7890A (Agilent Technologies, Tokyo, Japan) fitted with a split/splitless injector coupled to an Agilent 7000 triple quadrupole Mass Spectrometer equipped with an EI source. The chromatographic separation was carried out using a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) purchased to Agilent technologies (Santa Clara, USA). Operating conditions have been included in the supplementary material Table S1.

Procedures

Measurement of isotope distributions in the single quadrupole instrument.

All isotope distributions were measured as relative peak area abundances in selective ion monitoring (SIM) mode by selecting 10-12 consecutive masses corresponding to the molecular ion of each compound starting from the monoisotopic mass. Isotopologue ratios were measured as peak area ratios for the corresponding masses. Five injections were performed and the final results correspond to the average and standard deviation of the five individual isotope distributions or isotope ratios.

Measurement of isotope distributions in the triple quadrupole instrument.

All isotope distributions were measured as relative peak area abundances obtained by selected reaction monitoring (SRM). For this purpose, one mass corresponding to the molecular ion cluster was selected in the first quadrupole for each compound, fragmented in the collision cell and the different product ions corresponding to the loss of two halogen atoms were measured in the second mass analyser using the same dwell time. All measurements were performed under the “unit” mass resolution mode on both quadrupole analysers which corresponds to an isolation window of about 0.6 to 0.7 mass units wide. Five injections were performed and the final results correspond to the average and standard deviation of the five individual isotope distributions or isotopologue ratios. When a different precursor ion was evaluated the whole procedure was repeated.

Calculation of theoretical isotope distributions and their uncertainties.

Theoretical isotope distributions and their uncertainties for the molecular ions were calculated using an updated and modified version of the Isotope Distribution Calculator excel spreadsheet which can be downloaded from the supplementary material of a previous work [4]. Details of the calculation procedure employed were given elsewhere [17, 18]. Theoretical isotope distributions for the product ions in tandem instruments were calculated using the software IsoPatrn developed by Ramaley and Cubero-Herrera [16]. The uncertainties of the isotope distributions provided by IsoPatrn were calculated using the Kragten spreadsheet procedure [19] assuming that the uncertainty in the isotope composition of carbon was the main source of uncertainty. In all cases, the atomic isotope abundances and their uncertainties employed to calculate the molecular isotope distributions corresponded to the last IUPAC values [20]. An updated version of the Isotope Distribution Calculator (IDC montecarlo.xlsm) using the new isotope composition of the elements [20] and employing Montecarlo simulations to calculate the uncertainties can be downloaded from the supplementary material of this publication.

Results and discussion

Theoretical uncertainties for the M+1/M and M+2/M ratios in organic compounds.

The updated representative isotopic composition (mole fraction) of the elements is given in reference [20] as “the isotopic composition of chemicals and/or natural materials that are likely to be encountered in the laboratory”. For carbon this corresponds to the ranges [0.9884, 0.9904] for carbon-12 and [0.0096, 0.0116] for carbon-13. These ranges “represent the observed interval of isotope-abundance variation in natural materials” [20]. Assuming that we have a rectangular distribution the standard uncertainty for the isotope composition of carbon can be calculated by dividing the range given in [20] by the square root of 12. When we combine several carbon atoms to form an organic molecule the isotope composition of this molecule can be calculated based on a binomial or polynomial distribution [17]. Additionally, and because of the uncertainties in the isotope composition of carbon, this molecular isotope distribution will also have uncertainties which can be calculated either by spreadsheet procedures [17] or Montecarlo simulations [21]. For example, for an hypothetical C_{60} molecule the relative isotope composition for M, M+1 and M+2 at nominal masses 720, 721 and 722 can be calculated as 0.5276(167), 0.3392(63) and 0.1072(72) respectively. The numbers in parentheses correspond to the calculated standard uncertainties using a Montecarlo simulation and assuming that the range of isotope compositions tabulated for carbon correspond to a rectangular distribution [17]. As can be observed, the isotope abundance for the monoisotopic ion M at mass 720 has a large relative uncertainty of 3.20% and those for the M+1 and M+2 ions are 1.9 and 6.7%, respectively.

When we calculate the theoretical abundance ratios M+1/M or M+2/M we obtain 0.6428 and 0.2032 respectively. Those theoretical ratios will have an associated uncertainty which can be calculated using Montecarlo simulations [21]. So, using Montecarlo simulations we can calculate the standard uncertainties for the M+1/M and M+2/M ratios obtaining 0.0318 and 0.0199 respectively (other similar values could be obtained when running a second Montecarlo simulation). These values correspond to a relative uncertainty of 5.0% for the M+1/M ratio and of 10.0% for the M+2/M ratio respectively. Similar values (between 4.5 and 5.5% depending on the simulation) were obtained for different number of carbon atoms in the molecule up to 512 carbon atoms. It seems that the relative uncertainty for the ratio M+1/M is independent from the number of carbon atoms in the molecule. In our opinion, these theoretical uncertainties in the molecular isotope distributions, due to the natural variation in the isotope composition of carbon, are too high for these ratios to be employed as reference values in the evaluation of the spectral accuracy of mass spectrometers. Unfortunately, these high relative uncertainties are constant and independent from the number of carbon atoms in the molecule. Moreover, the presence of other atoms in the molecule such as hydrogen, oxygen or nitrogen does not provide a significant decrease of the theoretical uncertainties. For example the M+1/M ratio for a peptide of formula $C_{50}H_{71}O_{12}N_{13}$ can be calculated to be 0.6001 with a relative uncertainty of 5.3%. Similar uncertainty values were obtained for other smaller sized peptides.

According to these results, there is a need to develop either reference materials certified for their molecular isotope distributions or reference procedures with much smaller theoretical uncertainties to better evaluate the spectral accuracy of modern mass spectrometers. The best alternative is to use reference materials certified in their carbon isotope composition but those materials do not exist nowadays for the purpose of calibrating organic mass spectrometers. So, we have explored the other alternative and searched for reference measurement procedures with smaller theoretical uncertainties.

We have considered certain organic compounds containing several chlorine or bromine atoms that provide smaller theoretical uncertainties. For example, we have calculated the theoretical uncertainties for the ratios M+1/M and M+2/M for the families of Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl ethers (PBDEs) as a function of the number of halogen atoms in the molecule. In these calculations we have taken into account the natural variability of carbon, oxygen, hydrogen and the corresponding halogen. The results are shown in Figure 1A for PCBs and in Figure 1B for PBDEs. As can be observed, the M+1/M ratios have theoretical uncertainties of the order of 5-6% while for the M+2/M ratios these uncertainties decrease drastically as the number of halogen atoms in the molecule increase to values between 0.9 to 1.0% for PCBs and between 0.3 to 0.4% for PBDE. For PBDEs we have represented also the M+4/M+2 ratios with theoretical uncertainties below 0.4% for 2 or more bromine atoms in the molecule. So, these compounds and these alternative ratios would be a better alternative as reference values for the evaluation of spectral accuracy of single quadrupole analysers.

Evaluation of spectral accuracy in single quadrupole analysers.

We measured the ratio M+1/M for several PCBs and PBDEs. For the PCBs the theoretical ratio M+1/M is approximately constant (between 0.1296 and 0.1289 depending on the number of halogen atoms in the molecule) while a range of 0.1300 to 0.1295 was calculated for PBDEs containing between 3 and 6 bromine atoms. Figure 2 shows the results obtained. As it can be observed, a large systematic error was obtained which, for PCBs, depended on the number of chlorine atoms in the molecule. This systematic error can be ascribed to the tailing of the mass peaks at the low mass range in our quadrupole analyser as described previously [18]. The M+2 peak in halogenated compounds is larger than the M+1 peak and its tailing will affect the abundance of the M+1 peak creating these large positive errors which, for PBDEs, approached 25% relative. These systematic errors observed for the M+1/M ratios are intrinsic for the quadrupole analyser and the electron ionisation source and could be corrected by different approaches [1, 4].

Figure 3 shows the experimental results obtained both for PCBs and PBDEs for the ratios M+2/M, M+4/M+2, M+6/M+4 and M+8/M+6 in comparison with the theoretical ratios. The error bars correspond to the standard deviation of 5 independent injections of the

PCB or PBDE mixture. Experimental relative standard deviations ranged between 0.2 and 2% depending on the compound and ratio measured. The slope of the weighed least squares line for PCBs was 0.9978 while a value of 0.9946 was obtained for the PBDEs. In the same figure we have included the relative error by plotting R_{exp}/R_{th} on the right axis. As can be observed, relative errors are lower than 2% for most of the measurements except for those performed at R_{exp} values close to 0. According to these results, the single quadrupole analyser used was able to measure accurately isotopologue ratios between 0.01 and 4 when using the halogenated compounds as test substances. The fact that the measurements employ isotopologues which are separated by two mass units avoids the need for the correction of systematic errors. So, the tailing of the peak at mass M-1 and the possible spectral interferences because of the presence of M-H ions in the ion source do not influence the measured ratios. Also, the fact that the theoretical ratios used have lower theoretical uncertainties than the M+1/M ratios helped us to provide more confidence in the spectral accuracy results.

Evaluation of spectral accuracy in triple quadrupole analysers.

The main fragmentation reaction both for PCBs and PBDEs by Collision Induced Dissociation (CID) corresponds to the loss of two halogen atoms. We have included two mass spectra in the supplementary information Figure S1 to show this point.

If we measure several product ions from the same precursor ion using the SRM mode in a triple quadrupole instrument, the relative abundance between the different transitions will depend only on pure mathematical permutations and will be independent from the natural isotope abundances. This is illustrated graphically in Figure 4 for two selected precursor ions of a hexa-brominated diphenyl ether. The precursor ion at mass 639.5 u corresponds mainly to the isotopologue $^{12}C_{12}{}^{1}H_{4}{}^{16}O_{1}{}^{79}Br_{5}{}^{81}Br_{1}$ being the only possible losses $^{79}Br_2$ and $^{79}Br^{81}Br$ with 10 and 5 possible permutations, respectively. On the other hand, the precursor ion at mass 645.5 u corresponds mainly to the isotopologue $^{12}C_{12}{}^{1}H_{4}{}^{16}O_{1}{}^{79}Br_{2}{}^{81}Br_{4}$ being the possible losses $^{79}Br_2$, $^{79}Br^{81}Br$ and $^{81}Br_2$ with 1, 8 and 6 possible permutations respectively.

One can directly calculate the ratios of the possible permutations to obtain the theoretical ratios that should be obtained when measuring the different SRM transitions. However, the presence of ^{18}O, $^{13}C_2$ or $^{2}H_2$ in the precursor ion will disturb slightly these predictions. Therefore, we have used the software IsoPtrn [16] to calculate the actual probabilities for the fragmentation of the precursor ions. Taking again the example of the hexa-brominated diphenyl ether, the probability for the fragmentation of the isotopologue at mass 639.5 is 0.6672 for the loss of $^{79}Br_2$ and 0.3328 for the loss of $^{79}Br^{81}Br$. In the same way, the actual probability calculated for the fragmentation of mass 645.5 is 0.0685 for the loss of $^{79}Br_2$, 0.5342 for the loss of $^{79}Br^{81}Br$ and 0.3973 for the loss of $^{81}Br_2$. If we consider now the ratio of possible permutations, taking the 1, 8 and 6 possible permutations for mass 645.5 the probabilities are 1/15, 8/15 and 6/15 which resulted in 0.0667, 0.5333 and 0.4000 respectively. These values are very close to those calculated by the software IsoPtrn [16].

We have calculated the theoretical abundances for the fragmentation of different PCBs and PBDEs in a triple quadrupole analyser and those data have been included in the supplementary material Tables S2 and S3. In order to evaluate spectral accuracy we need to measure the experimental ratios of the SRM transitions and compare them with the theoretical ratios. However, we also need to know the uncertainty of those theoretical ratios which will depend mainly on pure mathematical permutations so the effect of the natural variations of the isotopic composition of the elements is expected to be negligible. For this purpose, we have applied the Kragten procedure [19] in the following fashion: we calculated the fragment ion distribution with IsoPtrn for PCBs and PBDEs using the nominal IUPAC abundances for all the elements and then again using synthetic elements Xc (carbon), Xo (oxygen), Xcl (chlorine) and Xbr (bromine) with the abundances altered by their standard uncertainties due to natural variation. It was observed that only the natural variation of carbon affected slightly the distributions calculated. The theoretical ratios and their uncertainties for PCBs and PBDEs are included in the supplementary material Tables S4 and S5. We can summarize here that the ratios range from 0.066 to 7.55 for PCBs and from 0.100 to 9.70 for PBDEs. Also, and in most cases, the theoretical uncertainties of those ratios were lower than 0.1% relative making these compounds and these fragmentation reactions suitable for the evaluation of spectral accuracy in tandem instruments.

Experiments were performed at two different concentration levels (10 and 20 µg g^{-1}) for both the PCB and the PBDE mixtures to study concentration-derived effects. The concentrations were selected at the µg g^{-1} level to improve counting statistics. The results found for 5 independent injections and all the SRM transitions studied are given in Tables S6 to S9 in the supplementary material. The average and the standard deviations for all measurements are included in those tables. The first conclusion was that we did not observe concentration effects on the measured SRM transition ratios. Only the standard deviations improved at higher concentrations due to counting statistics particularly for the highly chlorinated and brominated compounds. In order to see any effect related to the mass of the precursor and fragment ion considered we divided the experimental ratios by the theoretical ratios and represented them as a function of the mass of the precursor ion considered. Figure 5 shows the results for the higher concentration studied (20 µg g^{-1}) where we can observe that there is no influence of the mass of the precursor ion on the experimental SRM ratios. Only the data obtained for the highly brominated compounds (hexa- and hepta-brominated) showed high uncertainties due to the low peak areas observed and the extremely high ratios measured. For PCBs the experimental uncertainties were always of the order of 1-2% relative with spectral accuracy errors lower than 2% on average. The penta-chlorinated PCB showed the best results in terms of precision and accuracy. For PBDEs the experimental uncertainties were between 1 and 3% for tri-, tetra- and penta-brominated compounds with much higher uncertainties for hexa and hepta-brominated. Spectral accuracy errors for PBDEs were, on average, lower than 3%. For 10 µg g^{-1} the results were similar. These results are given in Figure S2 of the supplementary material. Finally, and in order to study the effect of the measured SRM ratios on spectral accuracy we represented the results obtained versus the theoretical ratios for ratios up to 4 in Figure 6. The data for PCBs fit a straight line of slope 0.9949 which indicates a good agreement between the theoretical and experimental ratios. For PBDEs we also observe a good fit of the data but with somehow larger uncertainties. In the same figure we have plotted also in the right axis the ratio between the experimental and theoretical ratios both for PCBs and PBDEs. For this range of ratios spectral accuracy errors were in general lower than 2% for PCBs and PBDEs indicating the good performance of the triple quadrupole analyser for the measurement of isotope distributions.

Conclusions

The comparison of the experimental M+1/M ratios with theoretically derived ratios calculated from the isotope composition of their constituting atoms is the usual procedure to evaluate spectral accuracy in Mass Spectrometry. However, the high uncertainty of theoretically derived ratios due to the natural variability of the isotope composition of carbon may lead to significant errors when compounds of different natural or human-made origin are employed. Additional errors can arise from the lack of spectral purity when using EI sources or from the tailing into adjacent peaks that cause distortion of the measured isotope distributions when using low resolution quadrupole analysers [4]. For these reasons we consider that comparing experimental and theoretical M+1/M ratios is not a good choice for the evaluation of mass spectrometers in terms of spectral accuracy. Therefore we have developed here two different procedures for the evaluation of spectral accuracy in single and tandem mass spectrometers. First, we propose the measurement of the M+2/M, M+4/M+2, M+6/M+4 and M+8/M+6, etc. ratios in organic molecules containing several chlorine or bromine atoms for their comparison with the theoretical ratios as an alternative procedure to evaluate the mass accuracy of single quadrupole instruments. Using a mixture of several PCBs and PBDEs we have demonstrated that although M+1/M ratios have theoretical uncertainties of the order of 5% the uncertainty in M+2/M ratios decrease drastically with the number of halogen atoms in the molecule approaching values between 0.3 to 1.0% for both families of compounds. Experimental values obtained both for PBDEs and PCBs agreed well with the theoretical values and the obtained relative standard deviations ranged between 0.2 and 2% depending on the compound and ratio measured. Penta-chlorinated PCBs or penta-brominated PBDEs showed the best results and could be recommended as test substances to evaluate the spectral accuracy of single quadrupole analysers without the need of additional corrections [1, 4]. They have low theoretical uncertainties and convenient ratios between 0.2 and 2 for M+2/M, M+4/M+2, M+6/M+4 and M+8/M+6.

Secondly, for the evaluation of the spectral accuracy in tandem MS instruments we propose the measurement of SRM transitions ratios when chlorinated or brominated compounds fragment in a collision cell by losing two chlorine or bromine atoms. We have demonstrated that theoretical SRM transition ratios can be easily calculated as a pure combinatorial probability with theoretical uncertainties lower than 0.1%. Experiments performed at different concentration levels of several PCBs and PBDEs showed good agreement between the theoretical and experimental ratios regardless the mass of the precursor ion. Best results were obtained again with penta-chlorinated PCBs or penta-brominated PBDEs so these compounds could be recommended as test substances in order to evaluate the spectral accuracy of tandem mass spectrometers. Fortunately for us the results obtained both in the single and triple quadrupole analysers were in good agreement with the theoretical ratios. So, we are confident on the spectral accuracy characteristics of our single and triple quadrupole analysers. Of interest now could be the evaluation of other mass spectrometers, particularly TOF and Orbitrap instruments, using the same procedure and to convince the manufacturers of organic mass spectrometers to include a test of spectral accuracy in the evaluation of their instruments.

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Legends for Figures.

Figure 1. Theoretical relative uncertainties (RSD%) for the M+1/M and M+2/M ratios as a function of the number of halogen atoms in the molecule for (A) PCBs and (B) PBDEs. Note that for PBDEs the ratios M+4/M+2 have also been included.

Figure 2. Comparison of the experimental M+1/M ratios measured in a single quadrupole GC-MS instrument with the theoretical ratios for PCBs (grey circles) and PBDEs (white circles). The expected agreement is shown with the two horizontal lines.

Figure 3. Comparison of the experimental M+2/M, M+4/M+2, M+6/M+4 and M+8/M+6 ratios with the theoretical ratios obtained for different PCBs and PBDEs by single quadrupole GC-MS. The error bars correspond to the standard deviation of 5 independent injections of the PCB or PBDE mixture.

Figure 4. Possible permutations for the loss of two bromine atoms in the hexa-brominated PBDE at masses 639.5 (top) and 645.5 (bottom)

Figure 5. Spectral accuracy of the triple quadrupole instrument, expressed as the ratio between the experimental and theoretical ratios, for PCBs and PBDEs injected at 20 ppm as a function of the mass of the precursor ion selected in the first quadrupole.

Figure 6. Comparison of the experimental ratios measured in the triple quadrupole instrument with the theoretical ratios for PCBs and PBDEs.