EVALUATION OF DIFFERENT INTERNAL STANDARDIZATION APPROACHES FOR THE QUANTIFICATION OF MELATONIN IN CELL CULTURE SAMPLES BY 1D- AND 2D-HPLC-ESI-MSMS

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Number of pages: 13

Number of Tables: 1

Number of Figures: 12

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Figure S1. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 233.2) as precursor ion (B) mass spectra for melatonin (MEL).

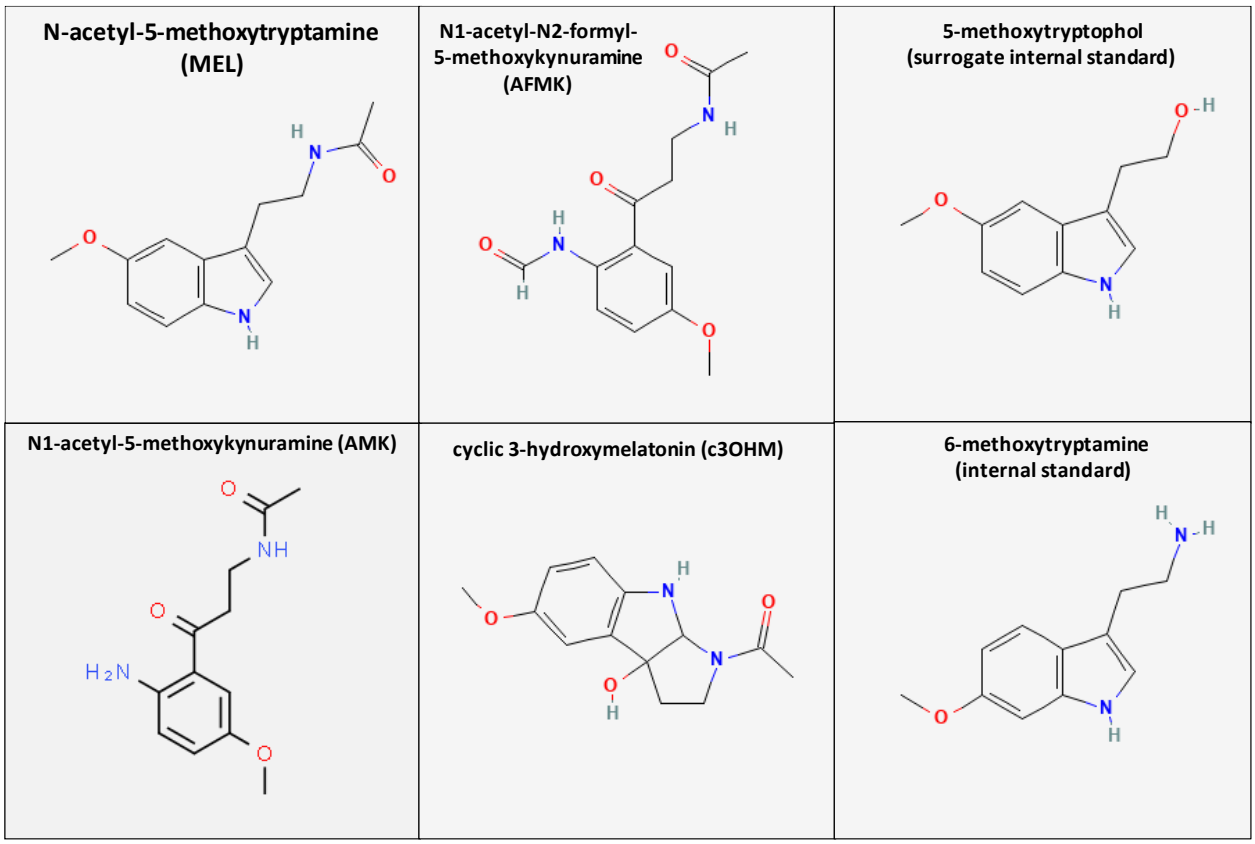


Figure S2. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 233.2) as precursor ion (B) mass spectra for melatonin (MEL)

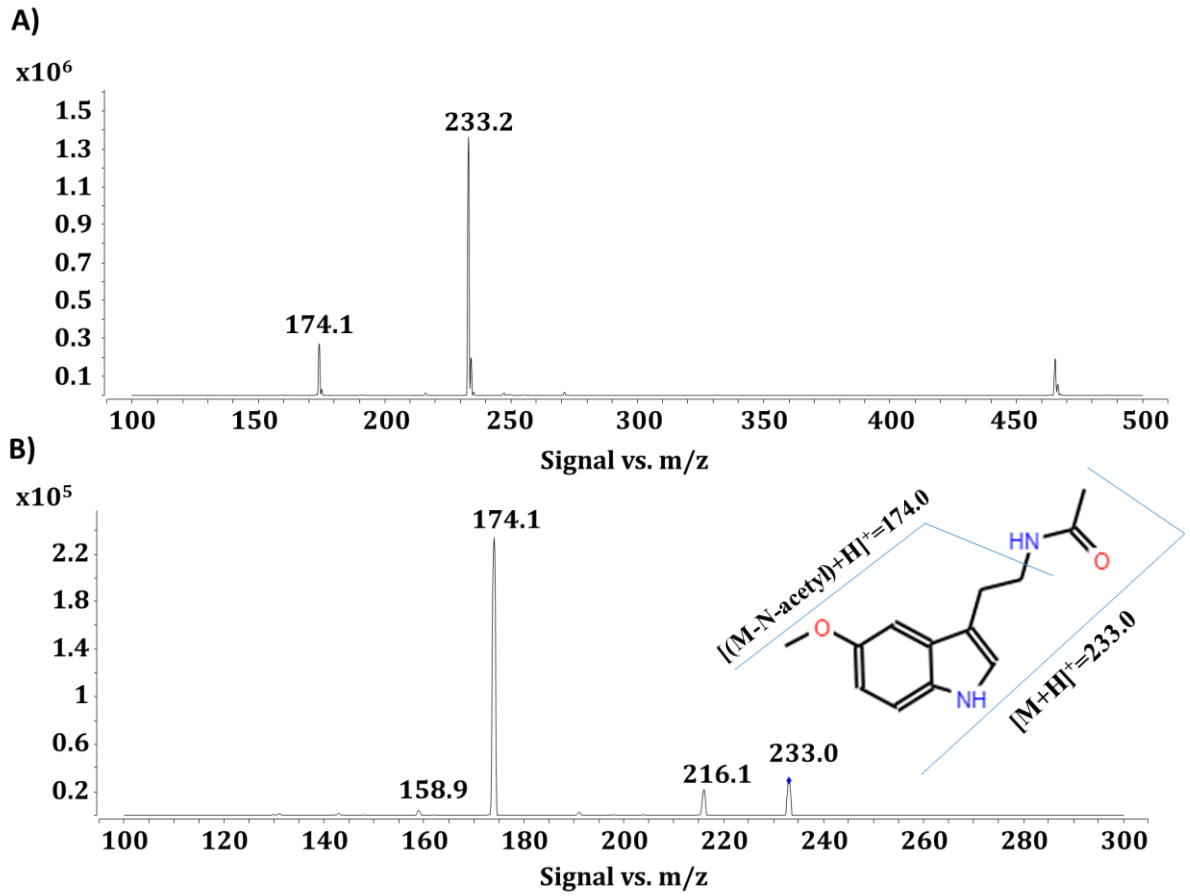


Figure S3. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 249.2) as precursor ion (B) mass spectra for cyclic 3-hydroxymelatonin (c3OHM)

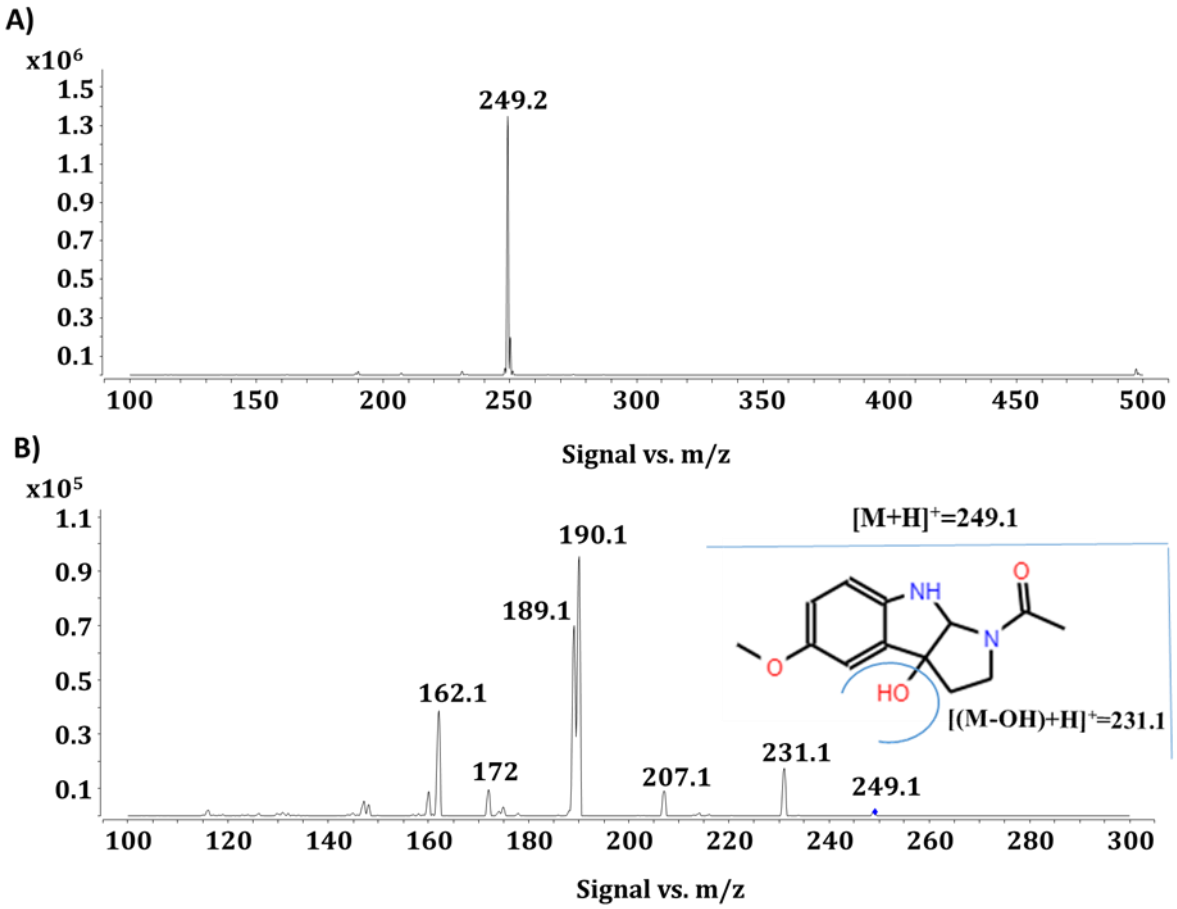


Figure S4. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 237.1) as precursor ion (B) mass spectra for N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK)

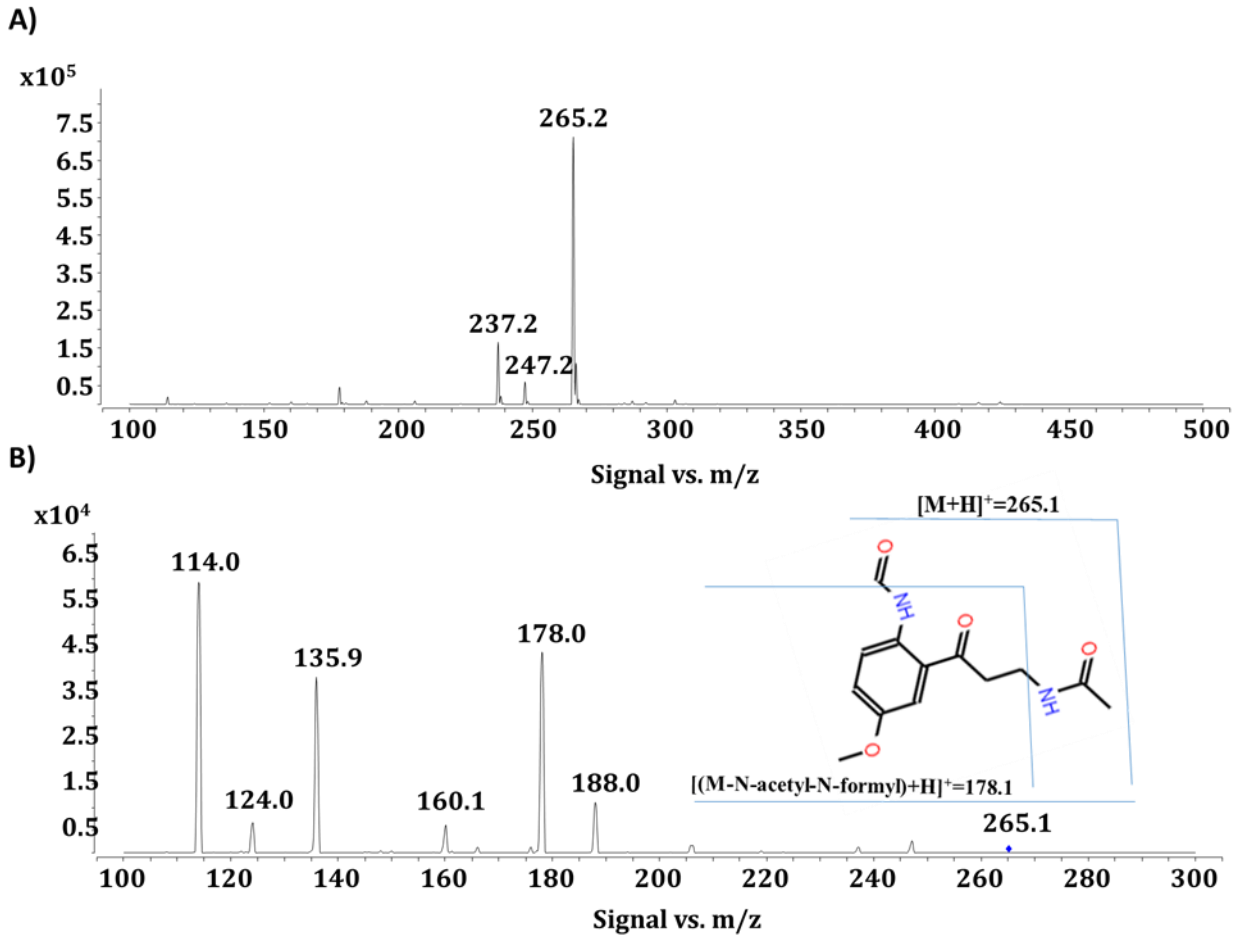


Figure S5. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 265.1) as precursor ion (B) mass spectra for N^1-acetyl-5-methoxykynuramine (AMK)

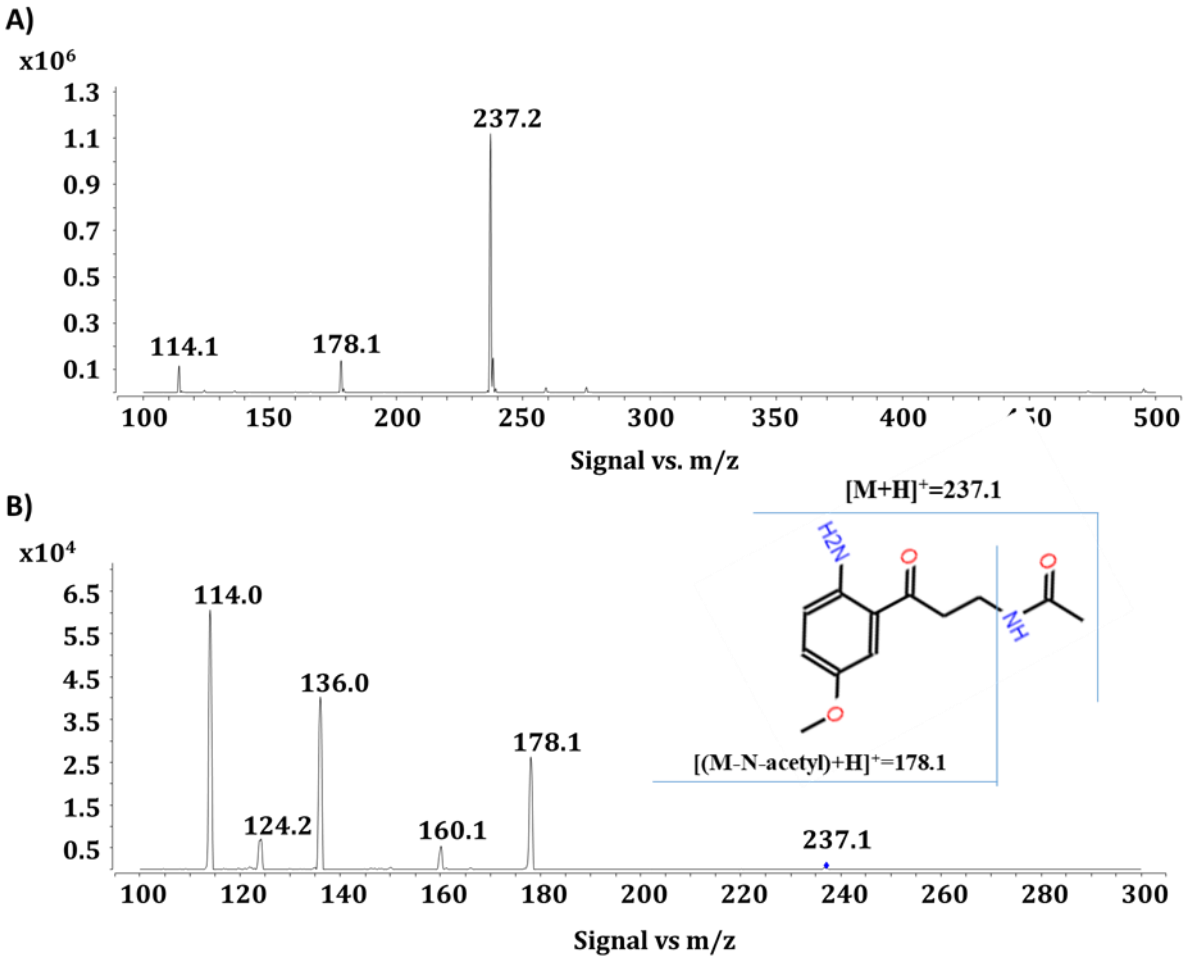
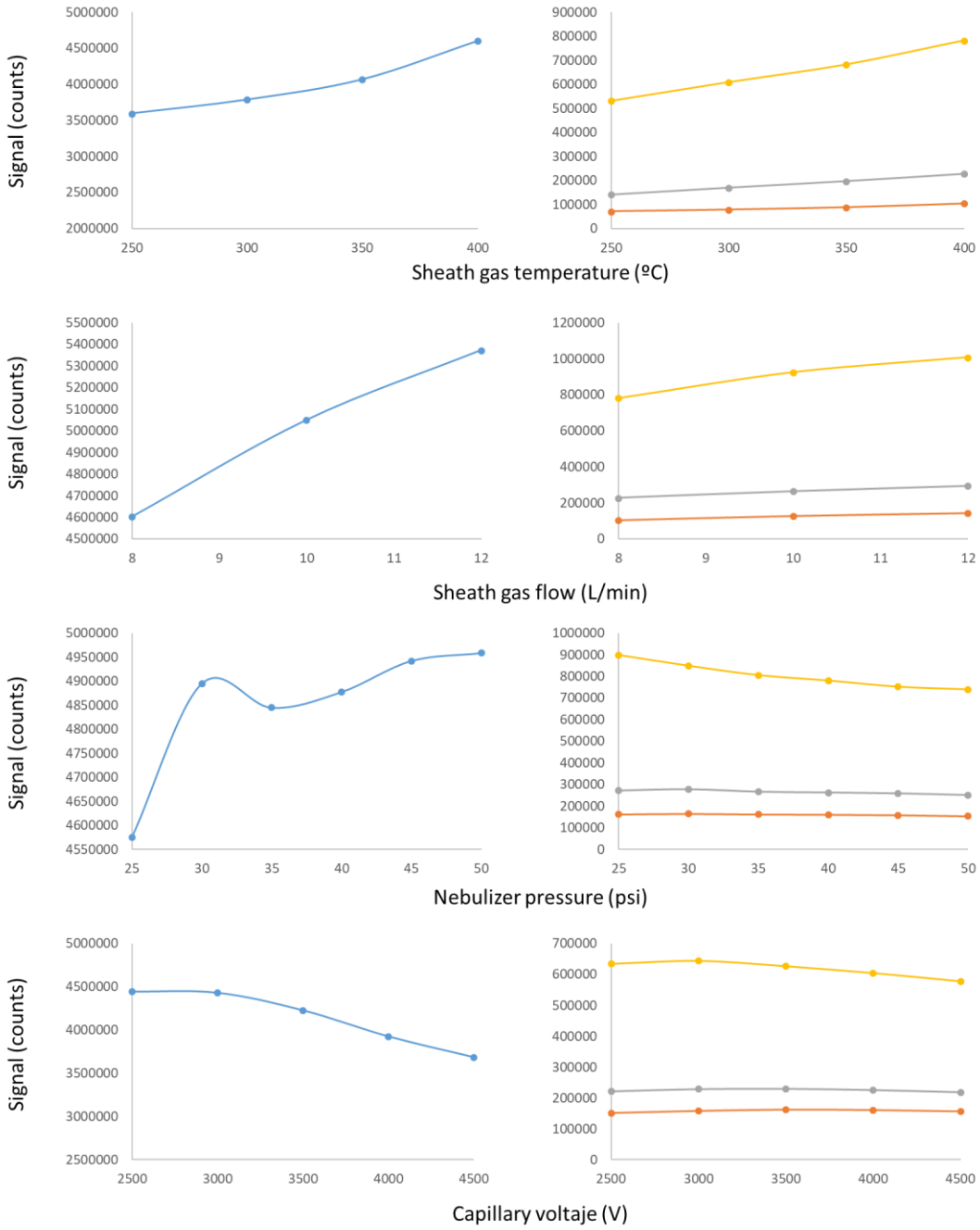
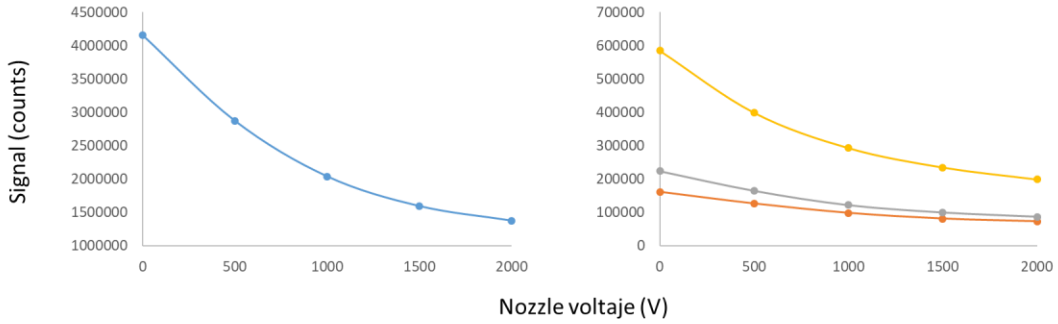


Figure S6. Optimization of the ion source parameters for MEL (•), c3OHM (•), AFMK (•) and AMK (•)

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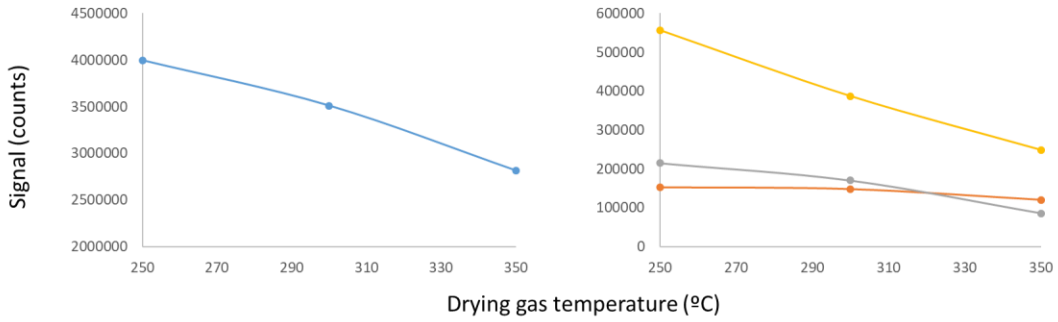
200000
100000
0
Nozzle voltaje (V)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200000
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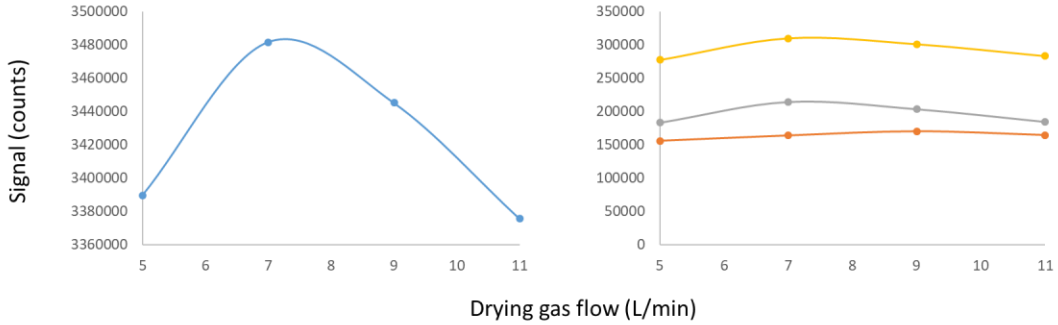
0
Drying gas temperature (ºC)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300000
250000
200000
150000
100000
50000
0
Drying gas flow (L/min)

Figure S7. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 192.2) as precursor ion (B) mass spectra for 5-methoxytryptophol (surrogate IS)

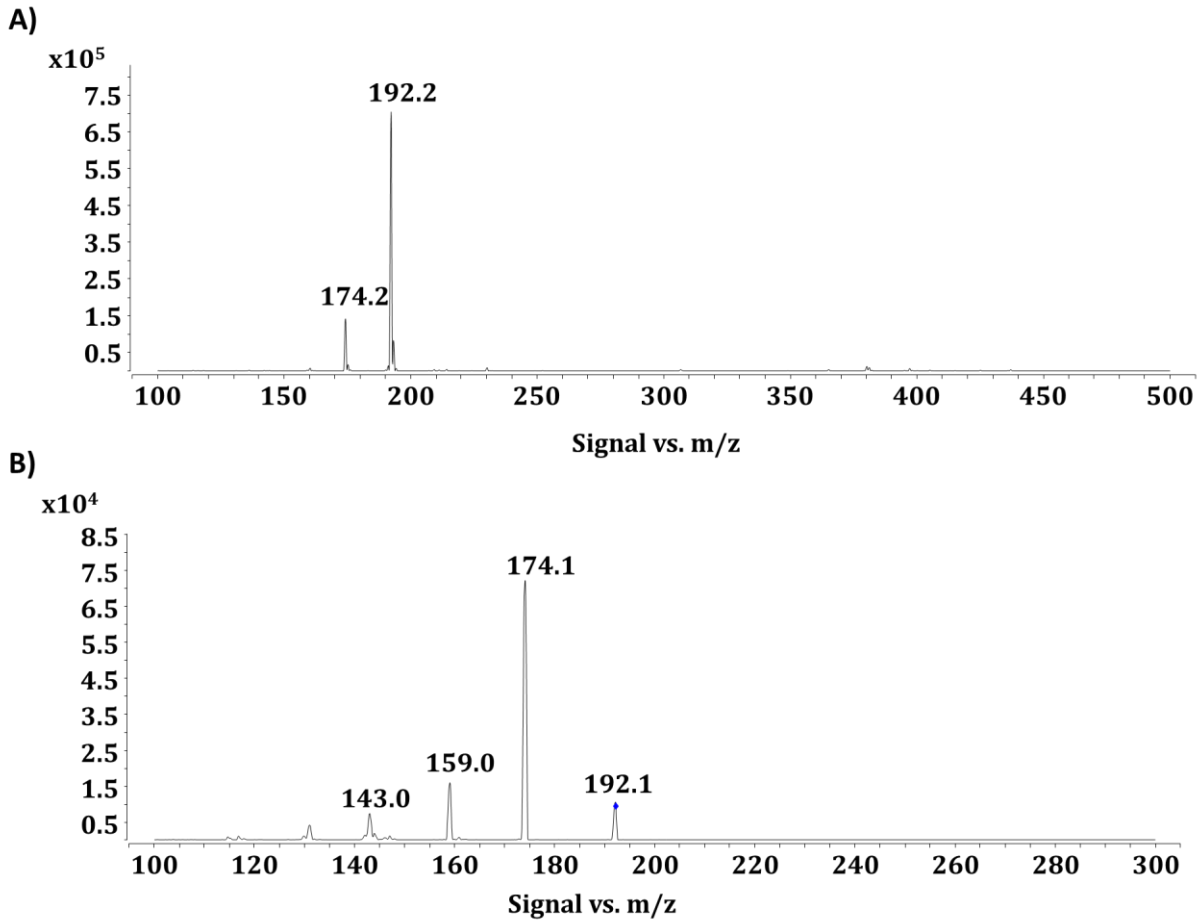


Figure S8. Scan (A) and product ion scan selecting $[M+H]^+$ (m/z = 190.9) as precursor ion (B) mass spectra for 6-methoxytryptamine (IS)

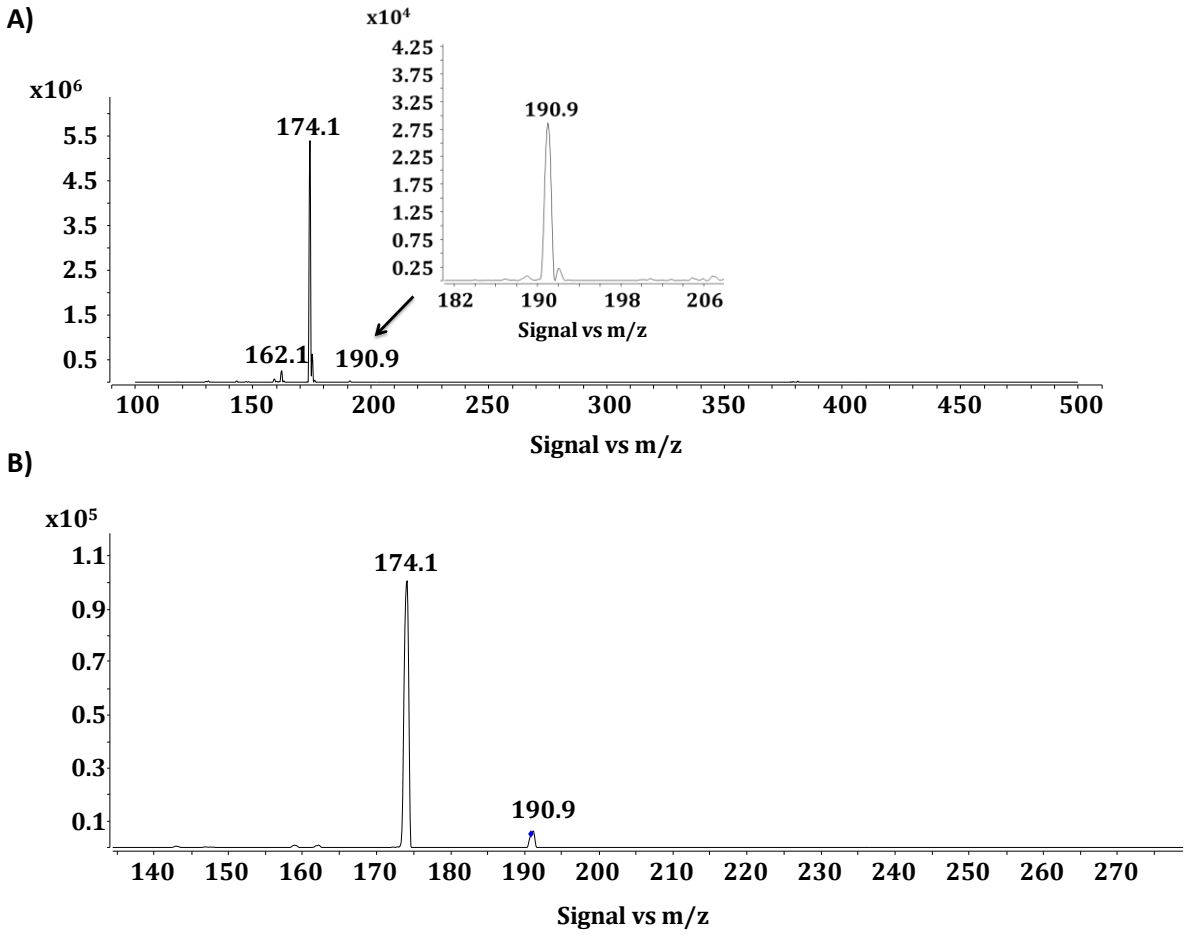


Figure S9. Plot of measured versus added MEL concentration of a homogenized PC3 cell culture sample fortified with 0, 0.5 and 1.8 µg g^{-1}. The samples were measured by 1D-LC-MSMS and the experimental concentrations calculated using four different strategies: 1) using the surrogate IS and the IS, 2) using only the surrogate IS, 3) using only the IS and 4) without using the surrogate IS or IS. n=3 independent replicates were performed for each concentration level and each replicate was injected in triplicate in the LC-MSMS. The points of the graphics correspond to individual injections in the LC-MSMS system.

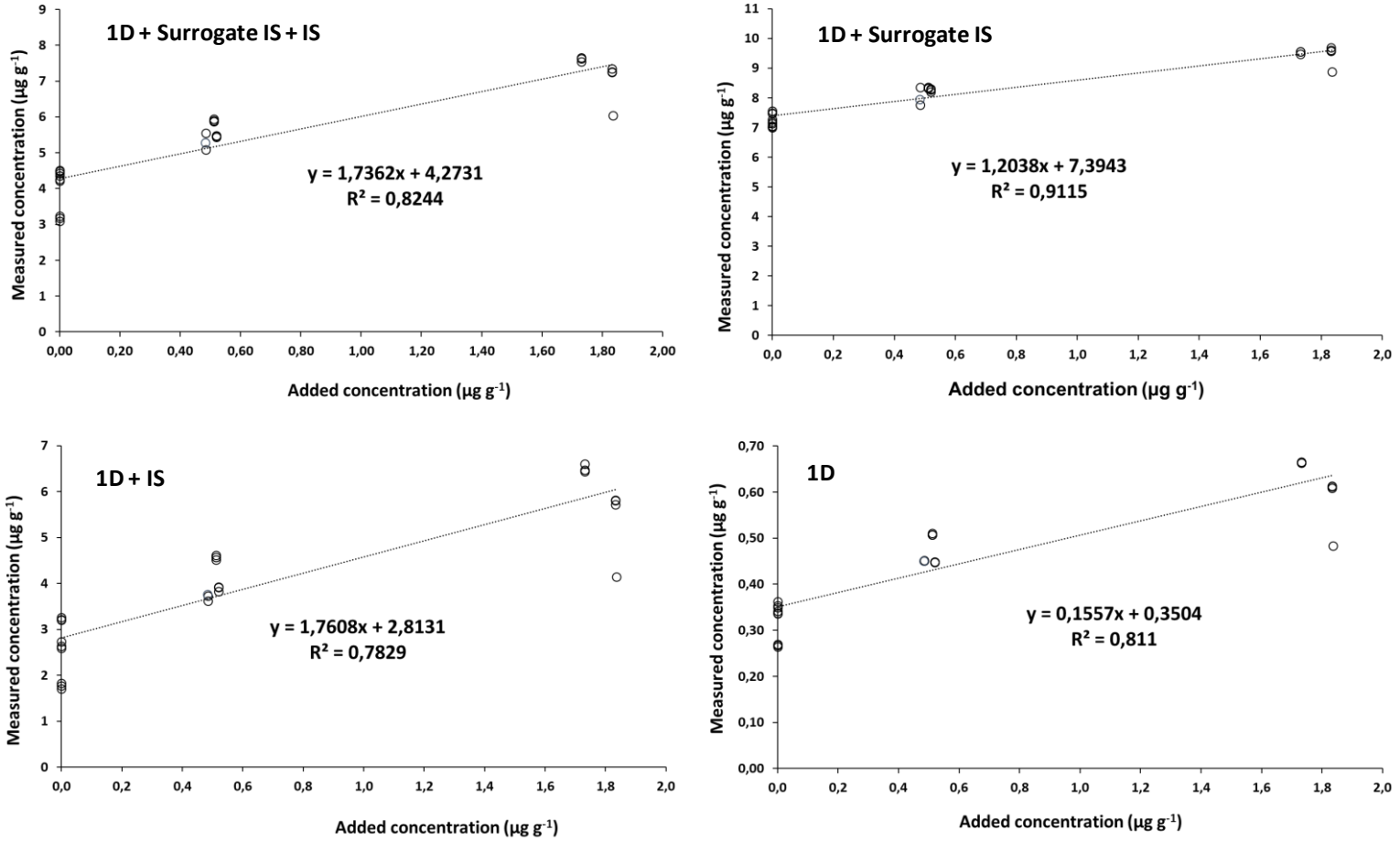


Figure S10. Plot of measured versus added MEL concentration of a homogenized PC3 cell culture sample fortified with 0, 0.5 and 1.8 µg g^{-1}. The samples were measured by 2D-LC-MSMS and the experimental concentrations calculated using four different strategies: 1) using the surrogate IS and the IS, 2) using only the surrogate IS, 3) using only the IS and 4) without using the surrogate IS or IS. n=3 independent replicates were performed for each concentration level and each replicate was injected in triplicate in the LC-MSMS. The points of the graphics correspond to individual injections in the LC-MSMS system.

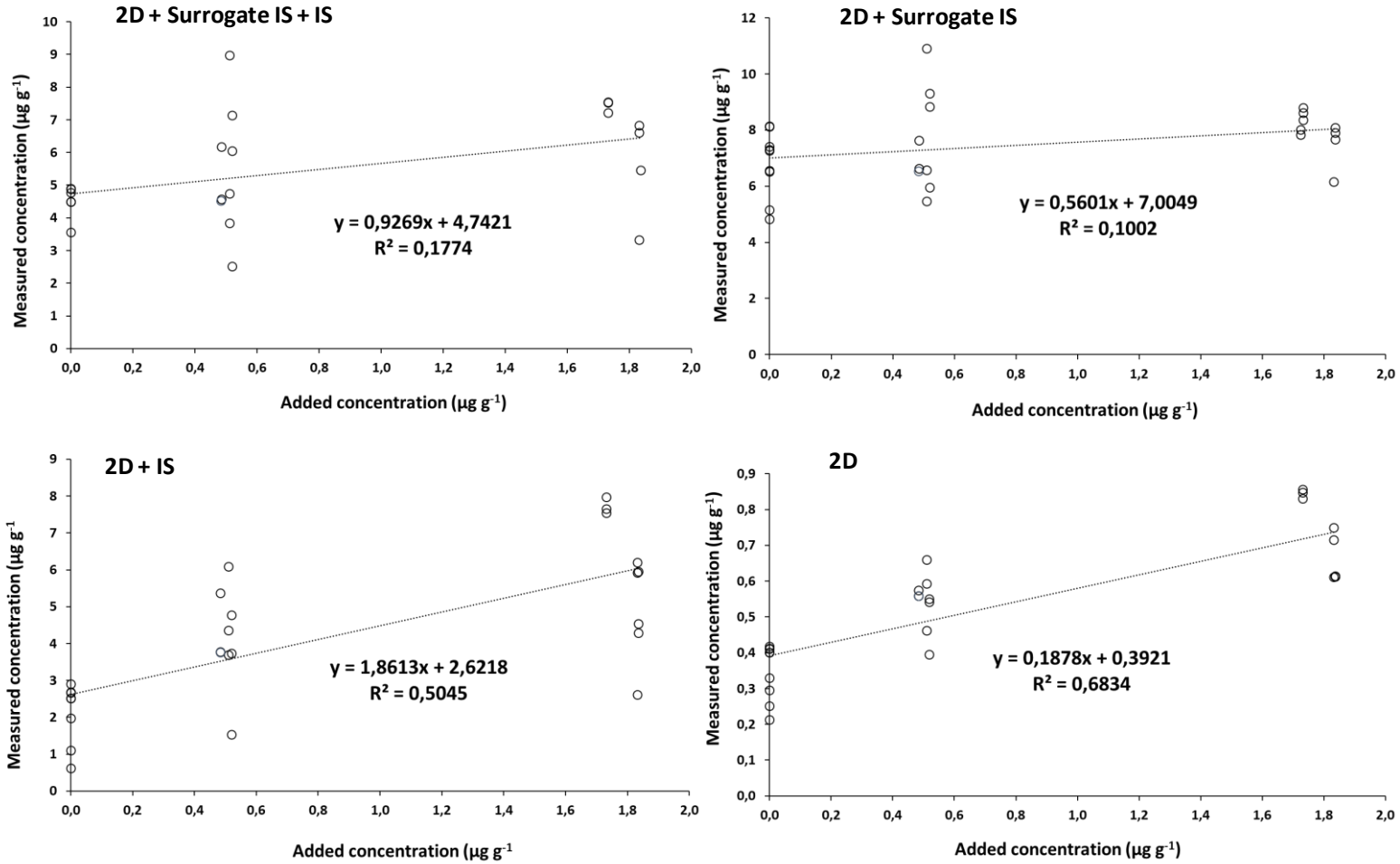
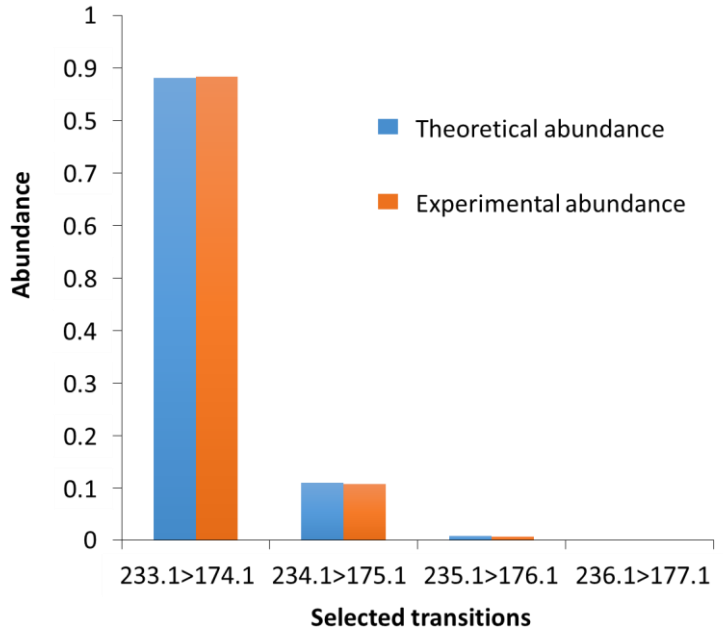


Figure S11. Comparison of theoretical and experimental isotope composition. Experimental values were obtained by 1D-LC-ESI-MS/MS measurement for selected SRM transitions.

A)



B)

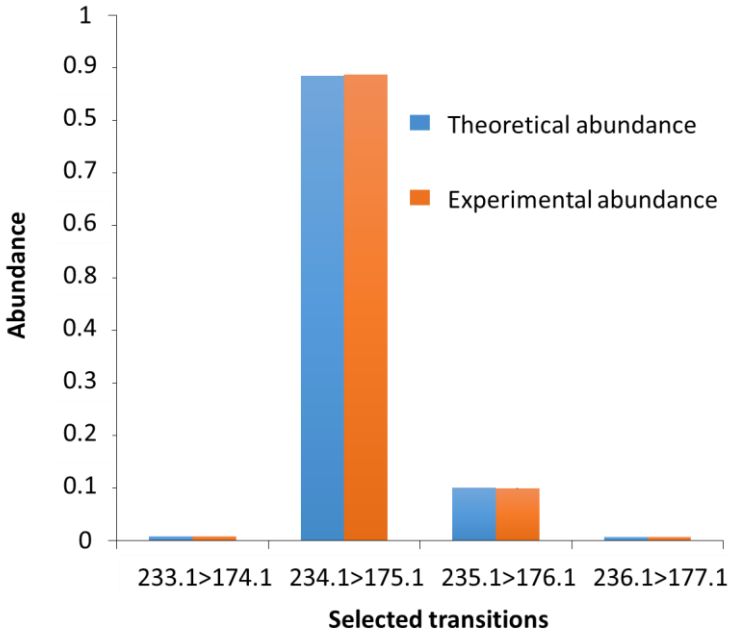


Table S1. Concentration values obtained for a $^{13}C_1$-MEL solution. The average concentration was obtained by reverse IDMS experiments performed in three independent blends.

Blend	Concentration (µg/g)	RSD (%)
1	1.062 ± 0.008	0.8
2	1.067 ± 0.002	0.2
3	1.070 ± 0.005	0.5
Average	**1.067 ± 0.006**	**0.6**

Figure S12. A) 1d-LC-MSMS and B) 2D-LC-MSMS chromatograms of blended solutions containing labelled and a certified standard of natural abundance MEL.