SUPPLEMENTARY MATERIAL

Determination of methionine and selenomethionine in food matrices by gas chromatography mass spectrometry after aqueous derivatization with triethyloxonium salts

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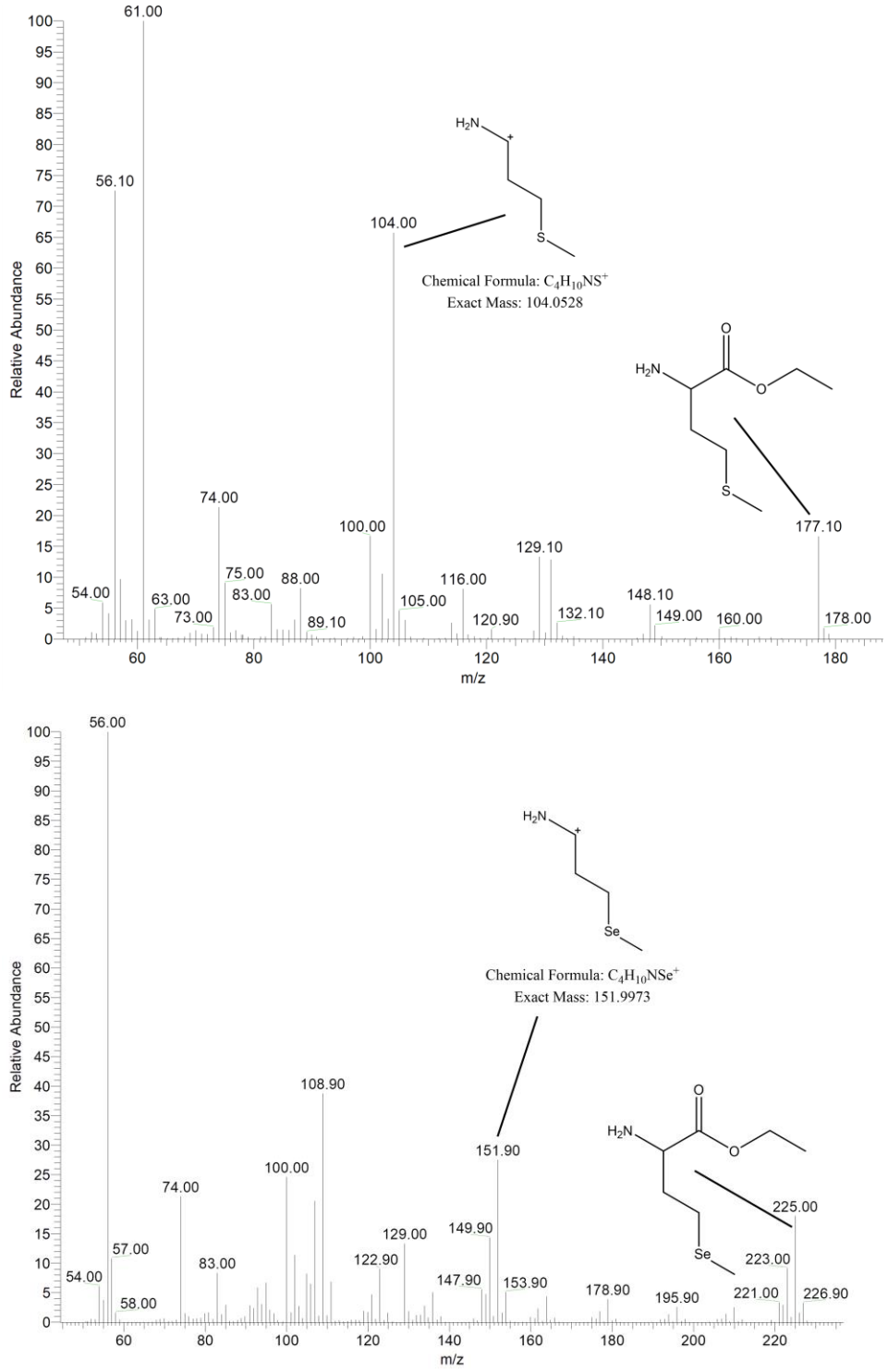

Figure S1. Mass spectra of Met (top) and SeMet (bottom) monoethylated forms. Only the ^{80}Se (49.61% natural abundance) isotopologues and their fragments, here labeled by the corresponding structures, were considered in this study.

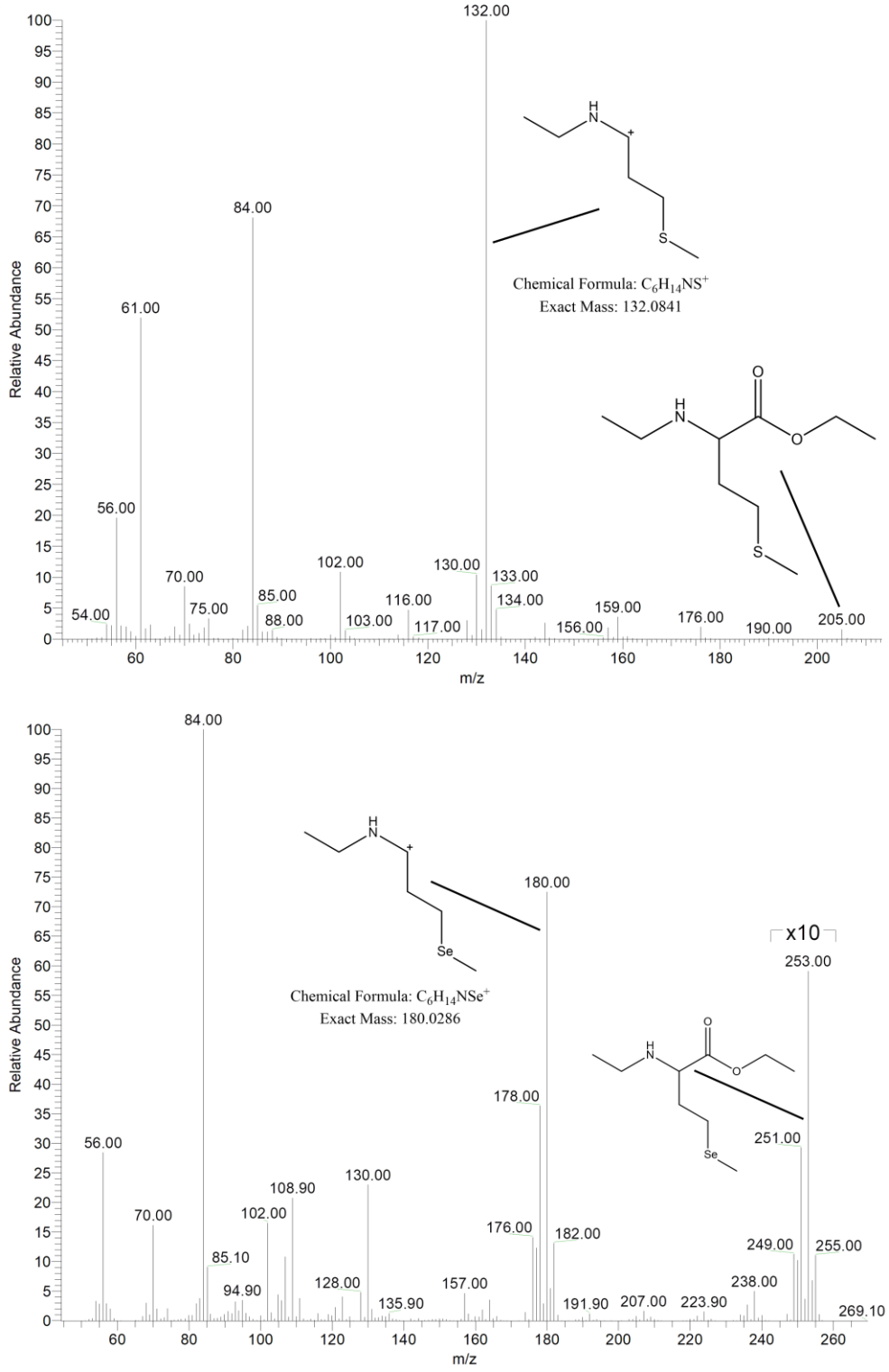

Figure S2. Mass spectra of Met (top) and SeMet (bottom) diethylated forms. Only the ^{80}Se (49.61% natural abundance) isotopologues and their fragments, here labeled by the corresponding structures, were considered in this study. The portion of the spectrum containing the molecular ion of Et_2SeMet was magnified to better appreciate the characteristic isotopic pattern due to the presence of a selenium atom.

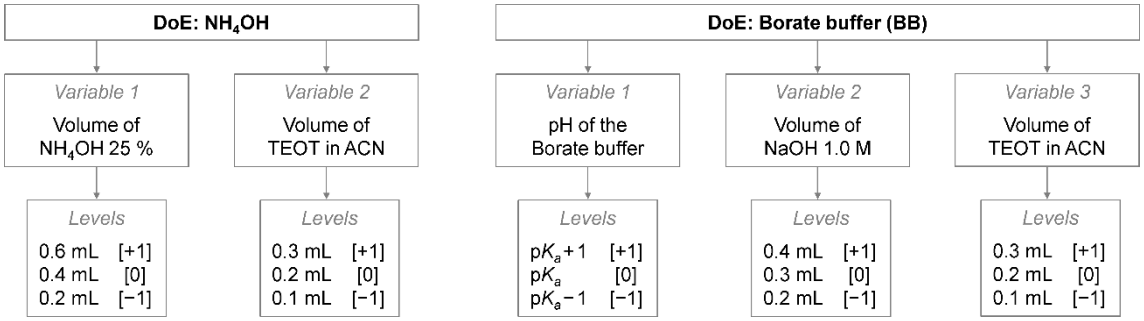

Figure S3. Optimizing the pH of the TEOT derivatization. On the left: a two-variables DoE for the optimization of the ammonium buffer. On the right: a three-level variables DoE for the optimization of the borate buffer. For each variable, the three levels were selected and their codified value is reported in square brackets.

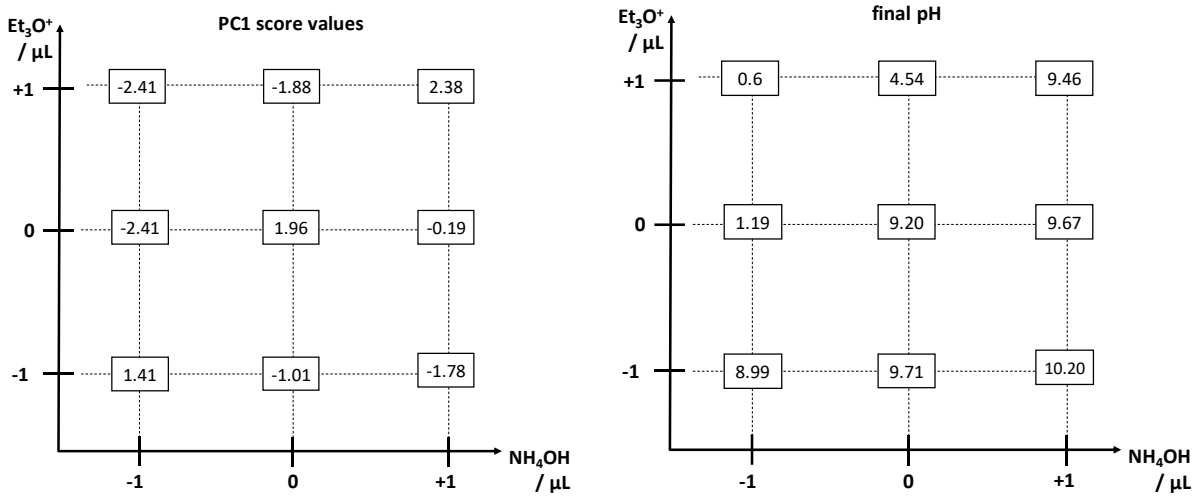

Figure S4. PC1 values (on the left) and final pH (on the right) in the space of process variables for NH_4OH DoE. Each number is the response of each experiment. Final pH values were measured with a pH-meter in the residual aqueous phases after each extraction.

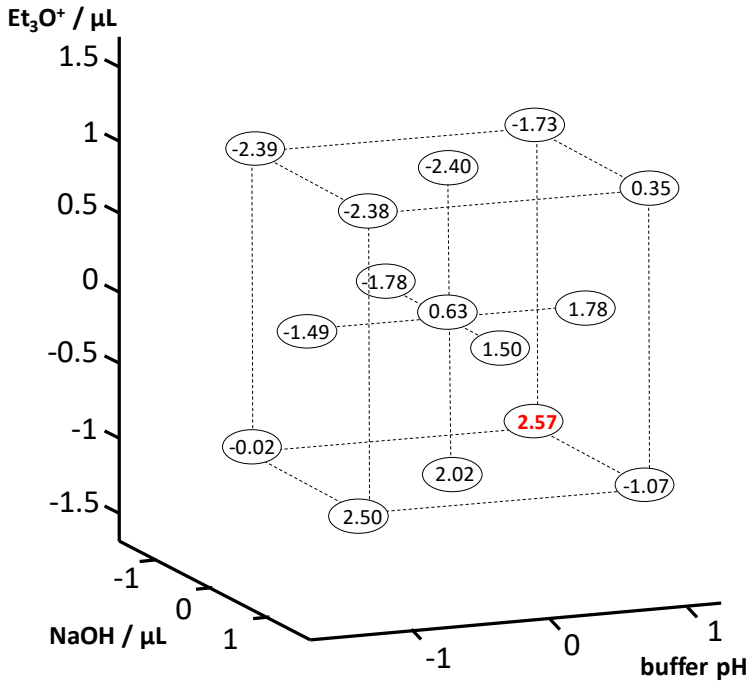

Figure S5. PC1 score values for each experiment performed in the space of process variables for BB DOE. Bold red number corresponds to the highest PC1 value obtained.

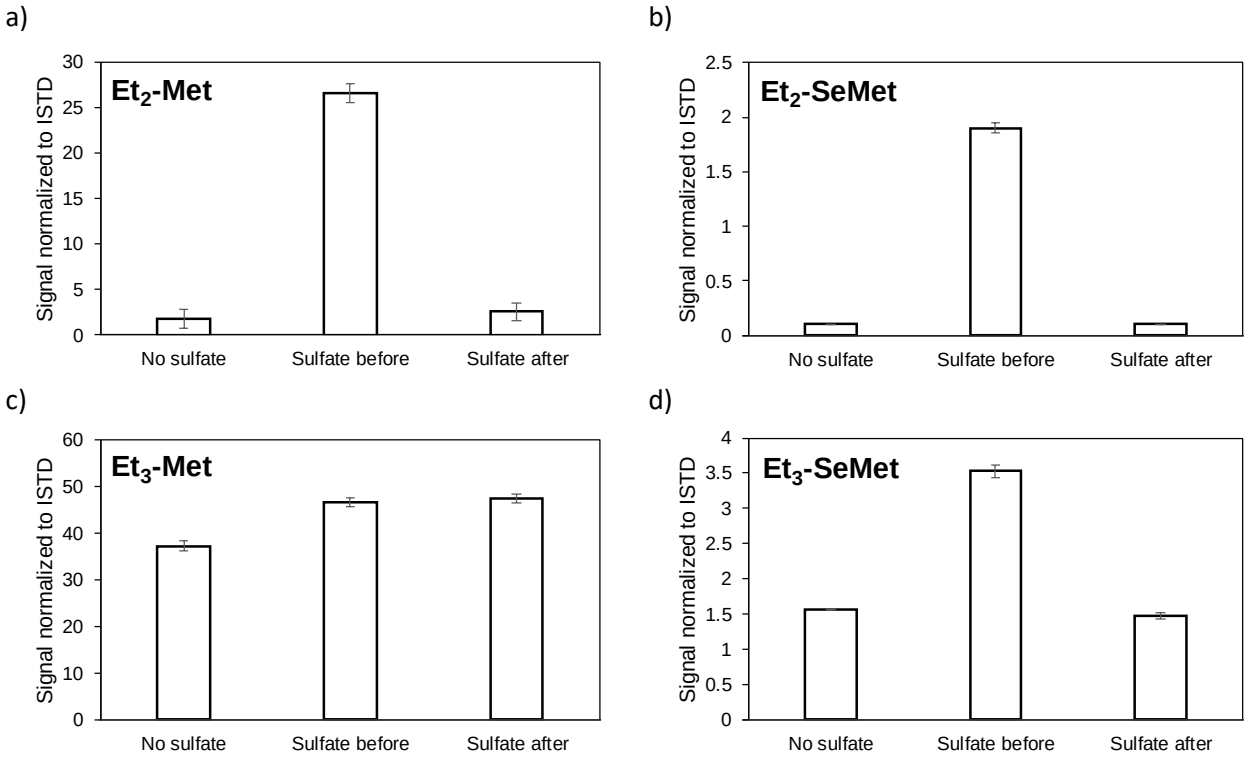

Figure S6. Different responses obtained with no sulfate, with sulfate added before derivatizing and after derivatizing for (a) Et_2-Met; (b) Et_2-SeMet; (c) Et_3-Met; (d) Et_3-SeMet.

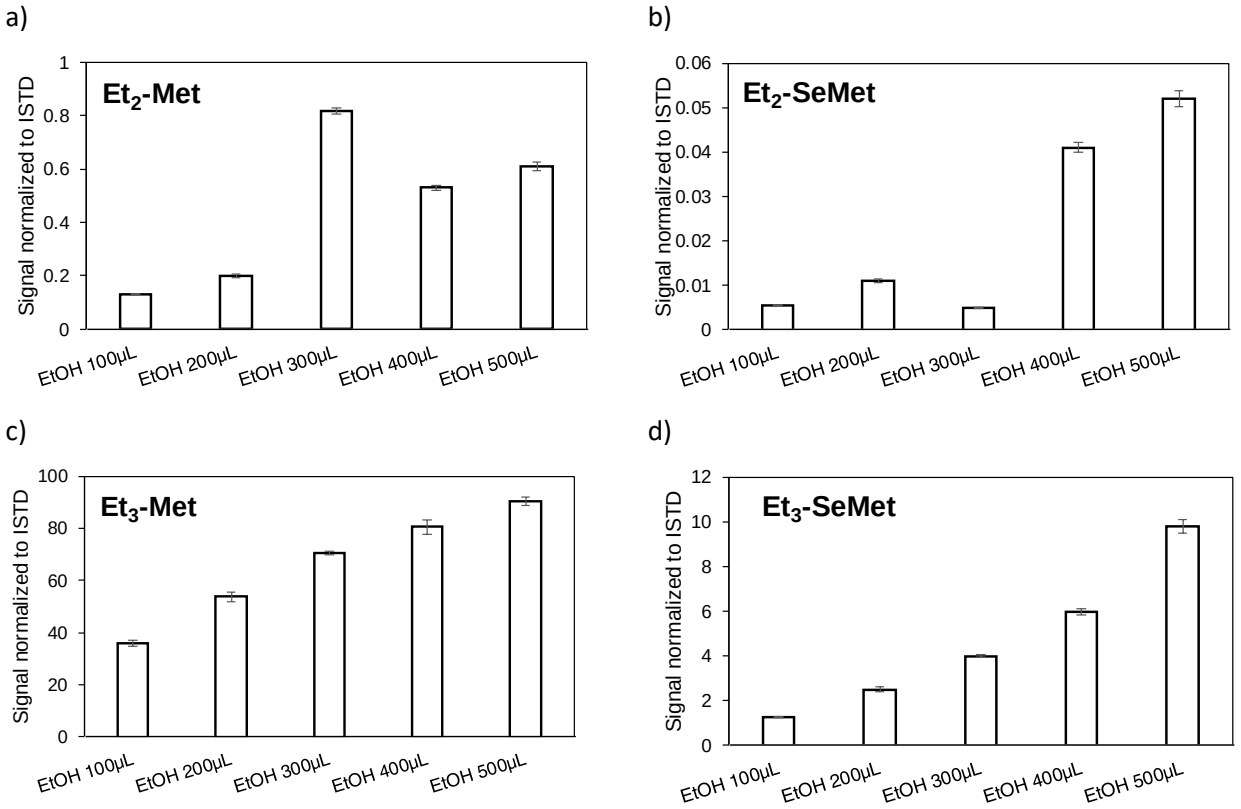

Figure S7. Increment of the responses proportional to the amount of EtOH added to the aqueous solution for (a) Et_2-Met; (b) Et_2-SeMet; (c) Et_3-Met; (d) Et_3-SeMet.

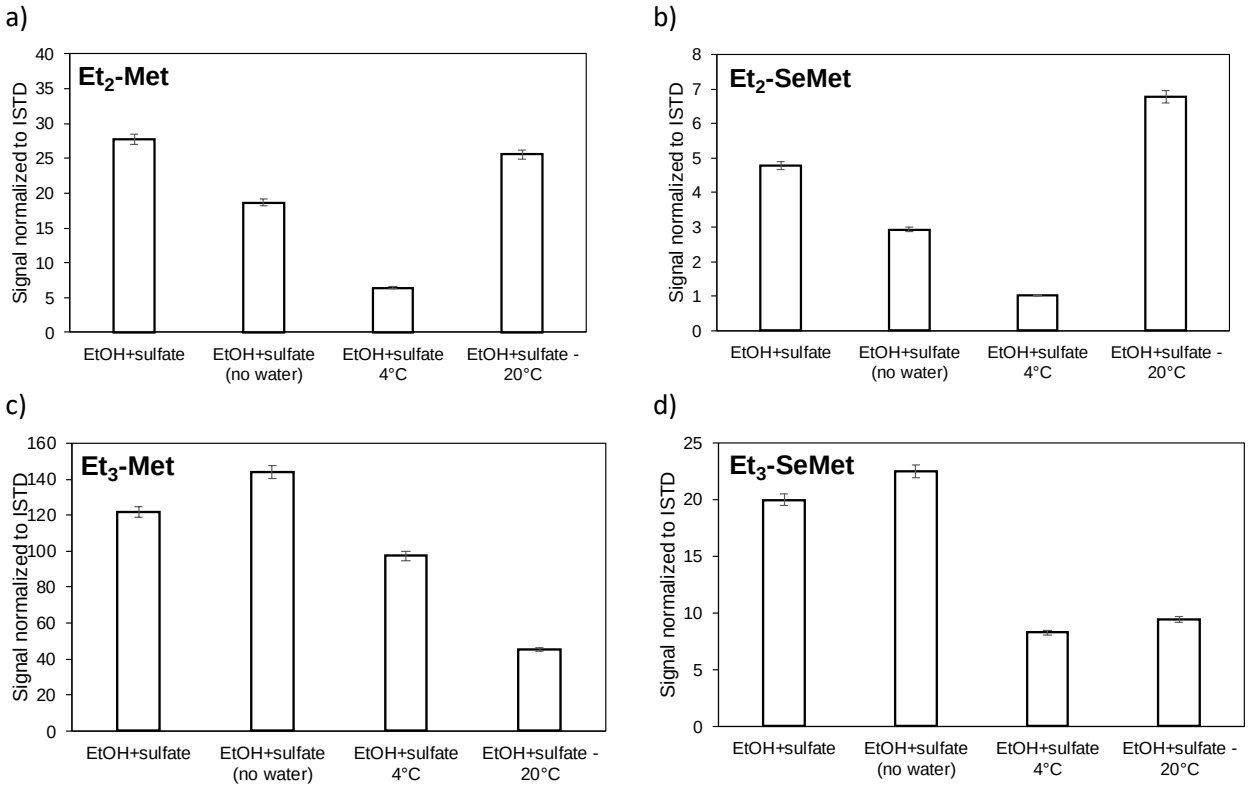

Figure S8. Responses of the experiments performed using 1 g of sodium sulfate and 500 µL of EtOH to improve method sensitivity for (a) Et_2-Met; (b) Et_2-SeMet; (c) Et_3-Met; (d) Et_3-SeMet.

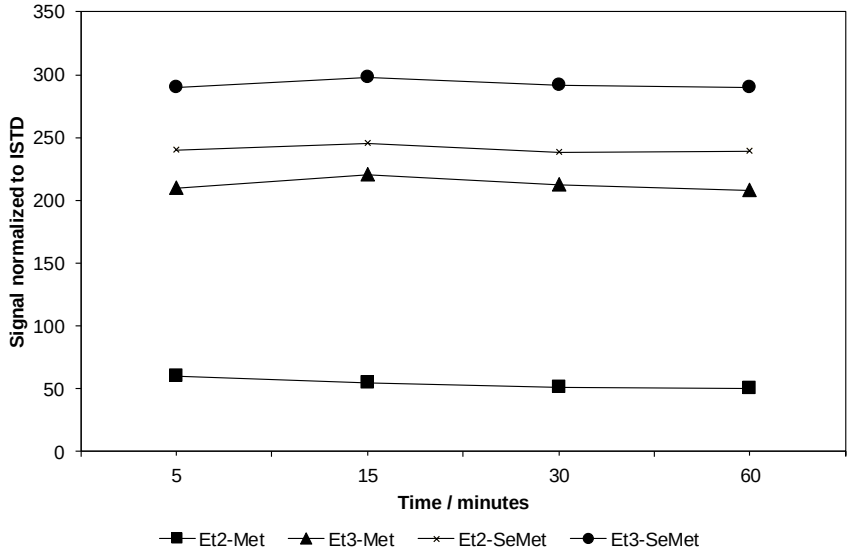

Figure S9. 4-point kinetic study employing simultaneous derivatization and extraction for Et_2-Met, Et_2-SeMet, Et_3-Met and Et_3-SeMet.

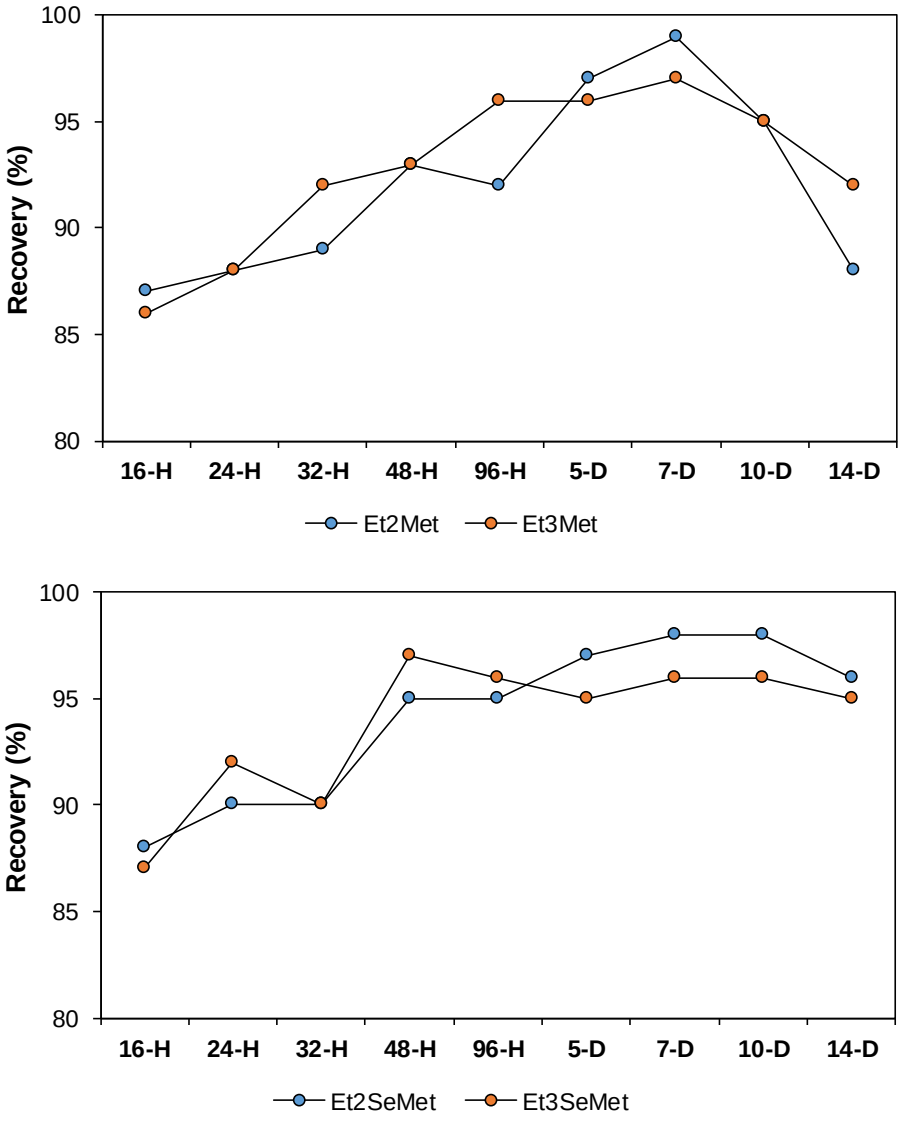

Figure S10. Evolution of Met and SeMet recovery from SELM-1 CRM, calculated respect to the certified values, with the extraction time (conventional oven, 110°C). All the extracts were analyzed after a 1:10 dilution and a neutralization with NaOH 10 M.

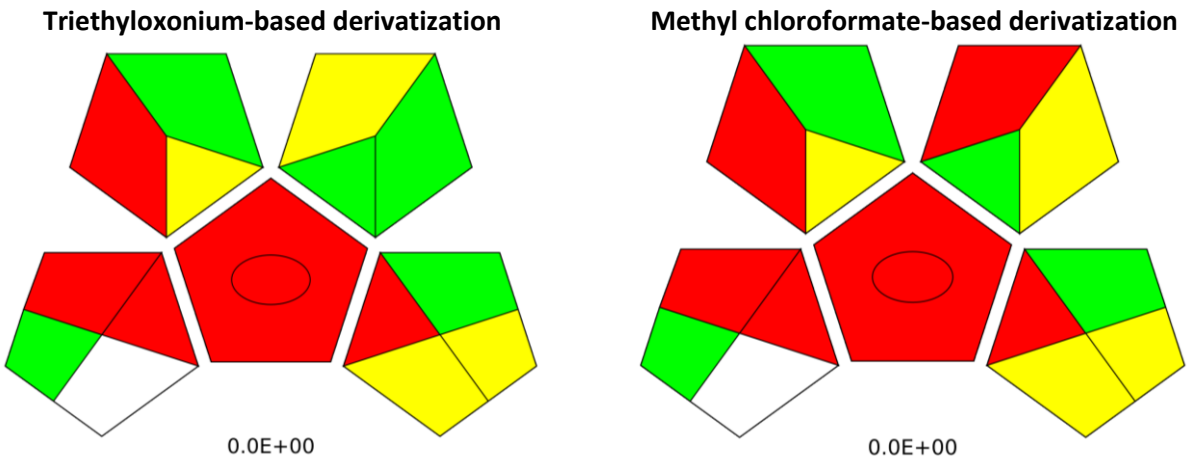

Figure S11. Comparison of triethyloxonium (present work) and methyl chloroformate [1] derivatization using the ComplexGAPI tool [2].

Table S1. Experimental matrix and experimental plan for the NH_4OH DoE.

exp	5% NH_4OH (µL)	1g Et_3O^+ /1 ml ACN (µL)	X_1	X_2
1	600	300	1	1
2	600	100	1	-1
3	200	300	-1	1
4	200	100	-1	-1
5	400	200	0	0
6	400	200	0	0
7	400	200	0	0
8	600	200	1	0
9	400	300	0	1
10	200	200	-1	0
11	400	100	0	-1

Table S2. Experimental matrix and experimental plan for the BB DoE.

exp	Buffer pH	1g Et_3O^+ /1 ml ACN (µL)	30% NaOH (µL)	X_1	X_2	X_3
1	10.2	300	400	1	1	1
2	10.2	300	200	1	1	-1
3	10.2	100	400	1	-1	1
4	10.2	100	200	1	-1	-1
5	8.2	300	400	-1	1	1
6	8.2	300	200	-1	1	-1
7	8.2	100	400	-1	-1	1
8	8.2	100	200	-1	-1	-1
9	10.2	200	300	1	0	0
10	9.2	200	400	0	0	1
11	9.2	300	300	0	1	0
12	8.2	200	300	-1	0	0
13	9.2	100	300	0	-1	0
14	9.2	200	200	0	0	-1
15	9.2	200	300	0	0	0
16	9.2	200	300	0	0	0
17	9.2	200	300	0	0	0
18	9.2	200	300	0	0	0

References

[1] Zhang, Xu, Lu Yang, and Zoltan Mester. "Determination of amino acids in selenium-enriched yeast by gas chromatography–mass spectrometry after microwave assisted hydrolysis." *Analytica chimica acta* 744 (2012): 54-59.

[2] Płotka-Wasylka, Justyna, and Wojciech Wojnowski. "Complementary green analytical procedure index (ComplexGAPI) and software." *Green Chemistry* 23.21 (2021): 8657-8665.