SUPPLEMENTARY INFORMATION

Enhanced universal quantification of biomolecules using element MS and generic standards: application to intact protein and phosphoprotein determination

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ABSTRACT: Analysis of results on the correction of signal variations and signal enhancement for S, P, As, Se, Br, and I, under the addition of CH_4:Ar and CO_2:Ar. LC-MS/MS analysis of tryptic digestion of β-casein sample. capHPLC-ICP-MS/MS quantification of *Pseudechis papuanus* venom sample using CH_4:Ar and CO_2:Ar.

CONTENTS

STUDY OF PLASMA BEHAVIOR

Table S1. Average quantitative error (%) along the capHPLC-ICP-MS(QQQ) analysis (0 - 50% mobile phase B) for P, S, As, Br, Se, and I, at each of the CO_2:Ar flows assayed. Uncertainty corresponds to one standard deviation of the mean.

	P	S	As	Br	Se	I
0 mL/min	270 ± 263	54 ± 53	335 ± 332	90 ± 69	340 ± 272	189 ± 142
10 mL/min	18.9 ± 19.9	6.6 ± 8.4	8.6 ± 9.2	11.3 ± 11.3	9.0 ± 8.7	6.5 ± 5.5
20 mL/min	4.1 ± 3.8	5.3 ± 4.2	2.2 ± 2.8	2.3 ± 1.1	1.3 ± 1.3	4.5 ± 5.1
30 mL/min	1.1 ± 1.0	4.1 ± 3.6	1.9 ± 2.0	0.6 ± 0.5	1.8 ± 1.8	3.5 ± 2.9
40 mL/min	1.7 ± 1.5	4.8 ± 3.8	4.3 ± 5.1	0.8 ± 0.7	1.8 ± 1.5	3.7 ± 3.2
50 mL/min	1.3 ± 1.3	5.4 ±4.8	3.6 ± 5.0	0.9 ± 0.7	1.9 ± 1.6	4.4 ± 4.1
60 mL/min	4.4 ± 5.2	7.9 ± 5.2	13.9 ± 18.6	6.2 ± 2.3	2.9 ± 1.4	5.5 ± 5.4
70 mL/min	2.3 ± 2.6	4.6 ± 3.8	18.0 ± 28.1	1.8 ± 1.6	2.0 ± 2.1	4.4 ± 3.6
80 mL/min	4.0 ± 3.5	4.5 ± 3.7	19.6 ± 30.1	1.3 ± 1.1	2.4 ± 1.8	3.6 ± 2.8
90 mL/min	2.7 ± 2.5	4.6 ± 4.5	16.4 ± 23.4	1.4 ± 1.1	2.1 ± 2.1	4.0 ± 3.4
100 mL/min	3.1 ± 3.0	4.1 ± 4.3	16.6 ± 22.7	2.6 ± 2.5	1.5 ± 1.7	3.1 ± 2.9

Table S2. Average quantitative error (%) along the capHPLC-ICP-MS(QQQ) analysis (0 - 50% mobile phase B) for P, S, As, Br, Se, and I, at each of the CH_4:Ar flows assayed. Uncertainty corresponds to one standard deviation of the mean.

	P	S	As	Br	Se	I
0 mL/min	270 ± 263	54 ± 53	335 ± 332	90 ± 69	340 ± 272	189 ± 142
10 mL/min	28.5 ± 30.4	13.5 ± 15.8	42.0 ± 55.6	23.1 ± 23.4	15.9 ± 16.2	8.7 ± 8.7
20 mL/min	18.8 ± 21.0	7.4 ± 10.2	32.4 ± 45.6	16.1 ± 15.1	9.1 ± 7.9	2.2 ± 1.8
30 mL/min	14.7 ± 15.4	5.3 ± 5.4	27.9 ± 40.3	11.1 ± 9.6	7.0 ± 5.7	1.3 ± 1.2
40 mL/min	12.3 ± 13.5	4.0 ± 3.3	24.0 ± 34.3	7.2 ± 7.0	5.2 ± 4.7	1.6 ± 2.0
50 mL/min	11.4 ± 12.1	3.1 ± 2.8	24.7 ± 36.3	12.5 ± 7.8	6.2 ± 5.5	1.9 ± 1.8
60 mL/min	11.3 ± 13.8	3.6 ± 3.6	26.1 ± 38.6	3.4 ± 3.3	6.5 ± 5.4	1.1 ± 0.7
70 mL/min	13.1 ± 14.3	4.8 ± 4.4	25.5 ± 39.2	4.4 ± 5.4	6.9 ± 6.0	1.0 ± 0.8
80 mL/min	12.4 ± 14.0	4.4 ± 3.8	25.2 ± 38.0	4.5 ± 5.3	6.2 ± 5.0	1.3 ± 1.1
90 mL/min	10.5 ± 12.4	3.5 ± 3.2	22.3 ± 31.7	5.2 ± 5.4	4.6 ± 4.7	0.9 ± 0.8
100 mL/min	11.6 ± 13.1	4.4 ± 3.3	22.3 ± 32.3	5.4 ± 5.5	6.6 ± 6.5	0.8 ± 1.1

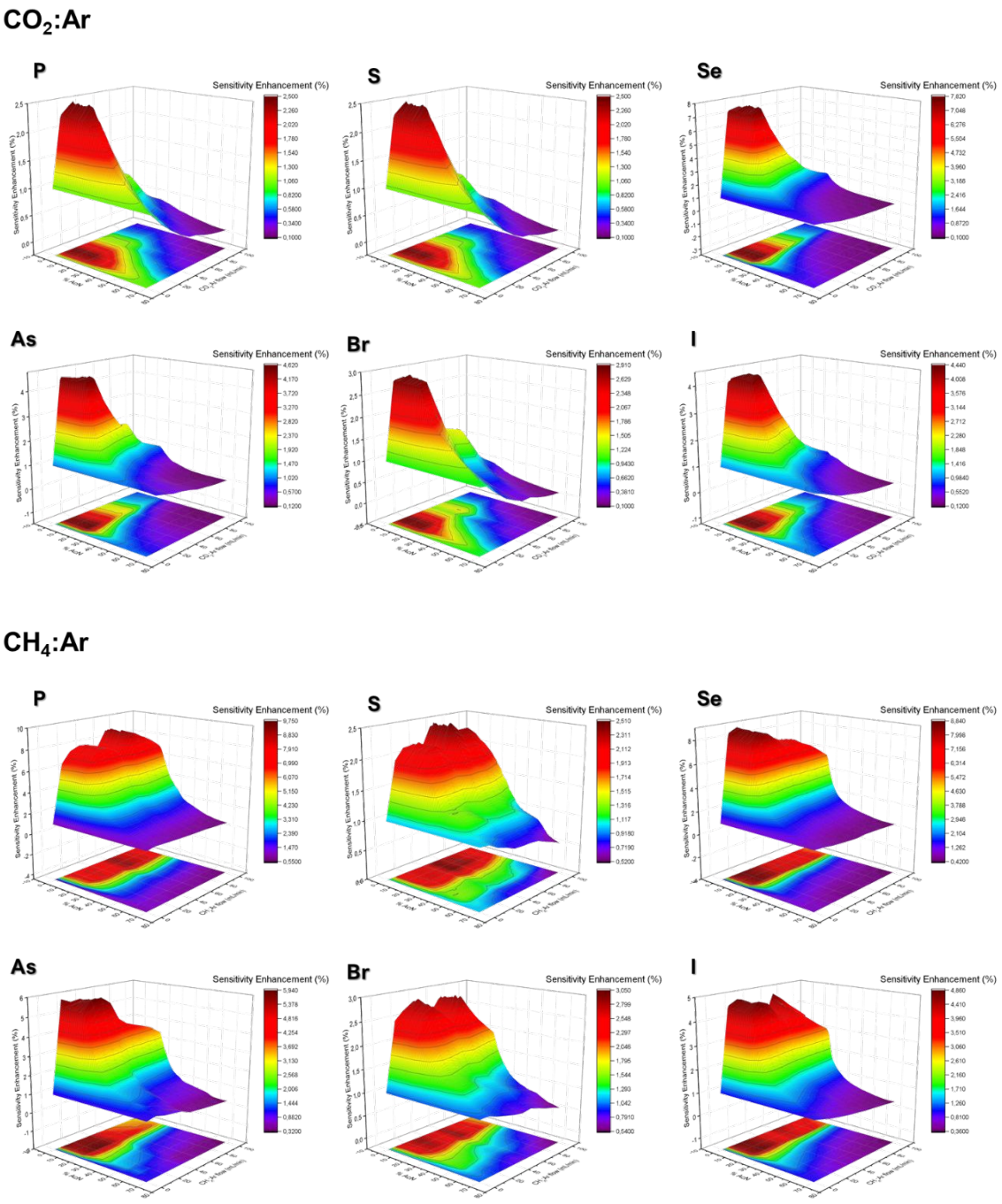

Figure S1. 3D-maps representing signal sensitivity enhancement along capHPLC-ICP-MS(QQQ) gradients (0 – 70 % ACN) for P, S, As, Se, Br, and I, under each of the CO_2:Ar (up) and CH_4:Ar (down) flows assayed.

COMPARISON OF SNAKE VENOM (*PSEUDECHIS PAPUANUS*) QUANTITATIVE ANALYSIS WITH CO_2 AND CH_4

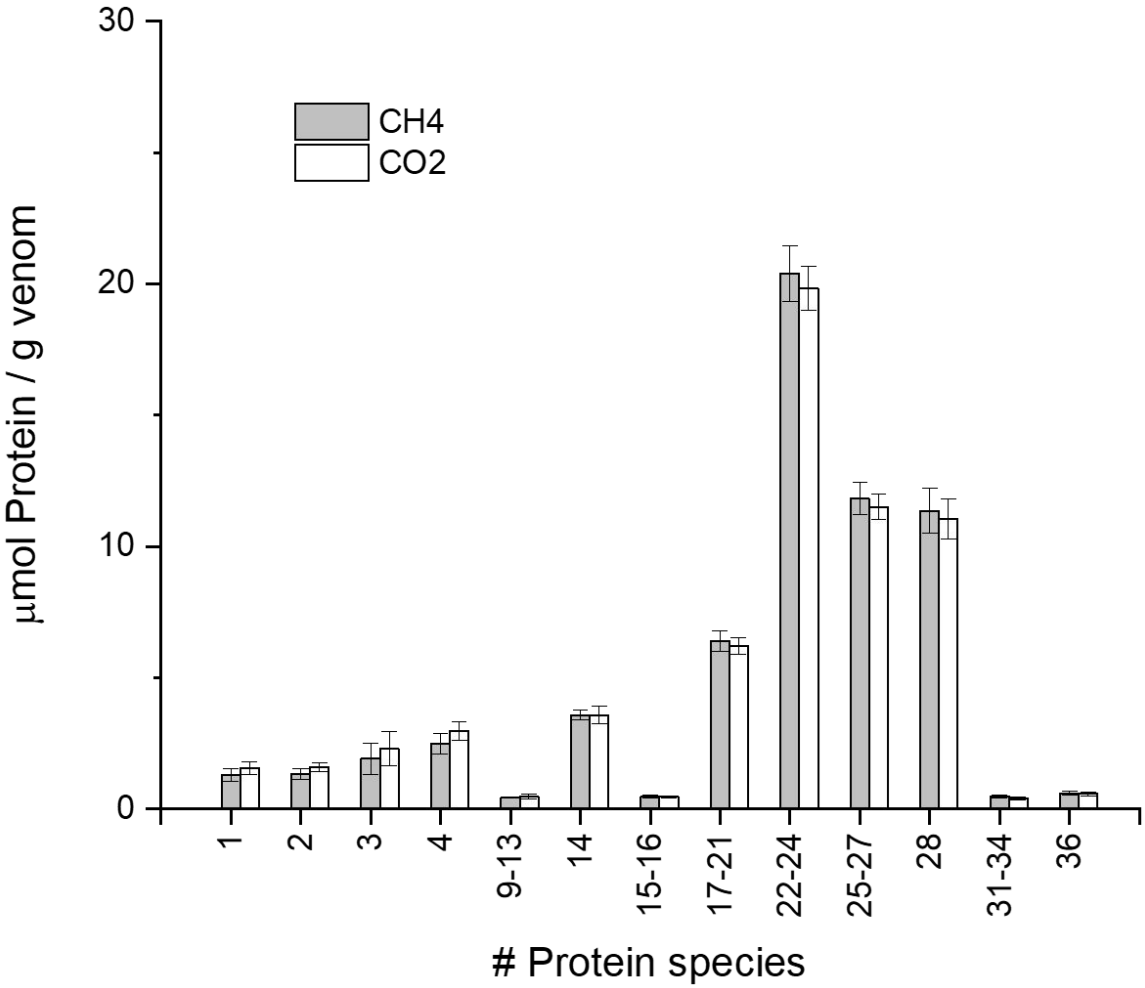

Figure S2. Calculated S-based absolute quantification (n=3) of *Pseudechis papuanus* protein species with capFIA-HPLC-ICP-MS/MS using CH_4:Ar and CO_2:Ar.

LC-MS/MS ANALYSIS OF β-CASEIN TRYPTIC DIGEST

Experimental Procedure

Protocol carried out for enzymatic tryptic digestion of β-casein was adapted from Rebecchi *et al*.[1] Briefly, protein sample was dissolved in 400 µL of 50 mM ammonium bicarbonate containing 6 M urea (pH 7.8). Reducing agent TCEP was added to the sample to a final concentration of 5 mM, and the mixture was shaken for 1 h at room temperature. Next, alkylant reagent iodoacetamide solution was added to a final concentration of 10 mM and the mixture was shaken in the dark for 1 h. Then, sample was diluted in ammonium bicarbonate to dilute urea to 1 M. Digestion was performed with trypsin (1:30 w/w, enzyme:protein ratio). Sample was incubated at 37ºC for 18 h. After digestion, 1 µL acetic acid per 100 µL solution was added to stop trypsin reaction. Finally, sample was stored at – 20ºC until use.

Enzymatic digest of the sample was analyzed with reversed-phase liquid chromatography with ESI-QToF detection in MS and MS/MS mode. Raw data from the analysis was uploaded to software-free online tool MASCOT MS/MS Ions Search, in order to identify protein species corresponding to detected tryptic peptides. MASCOT search conditions were: fixed modifications (Carbamidomethyl), variable modifications (phosphorylation ST, phosphorylation Y), peptide tolerance (1.2 Da), MS/MS tolerance (0.6 Da), peptide charge (2+, 3+, 4+), allowed miscleavages (1).

Results obtained are summarized in the next table:

Table S3. Results obtained in MASCOT MS/MS Ions Search on the LC-MS/MS analysis of bovine β-casein tryptic digest sample.

Protein hit	Score*
Beta-casein – Bos taurus (Bovine)	518
Kappa-casein – Bos taurus (Bovine)	208
Alpha-s1-casein – Bos taurus (Bovine)	201
Beta-lactoglobulin – Bos taurus (Bovine)	151
Alpha-s2-casein – Bos taurus (Bovine)	150

*MASCOT score >41 implies 95% confidence in unequivocal presence of the protein in the sample.

[1] Rebecchi, K. R.; Go, E. P.; Xu, L.; Woodin, C. L.; Mure, M.; Desaire, H. *Anal. Chem*. **2011**, 83, 8484−8491.