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RESEARCH ARTICLE

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Removal of isobaric interference using pseudo-multiple reaction monitoring and energy-resolved mass spectrometry for the isotope dilution quantification of a tryptic peptide

Alicia Maroto¹  | Dany Jeanne dit Fouque¹  | Rémy Lartia²  |
Antony Memboeuf¹ ¹Univ Brest, CNRS, UMR 6521 CEMCA, Brest, France²DCM, Univ Grenoble-Alpes, Grenoble, France

Correspondence

Antony Memboeuf, Univ Brest, CNRS, UMR 6521 CEMCA, F-29200 Brest, France.

Email: antony.memboeuf@univ-brest.fr

Abstract

Energy-resolved mass spectrometry (ERMS) and an isotopically labelled internal standard were successfully combined to accurately quantify a tryptic peptide despite the presence of an isobaric interference. For this purpose, electrospray ionisation tandem mass spectrometry (ESI-MS/MS) experiments were conducted into an ion trap instrument using an unconventional 8 m/z broadband isolation window, which encompassed both the tryptic peptide and its internal standard. Interference removal was assessed by determining an excitation voltage that was high enough to maintain a constant value for the analyte/internal standard peaks intensity ratio, thus ensuring accurate quantification even in the presence of isobaric contamination. Pseudo-multiple reaction monitoring (MRM) was employed above this excitation voltage to quantify the tryptic peptide. The internal standard calibration model showed no lack of fit and exhibited a linear dynamic range from 0.5 μM up to 2.5 μM . The detection limit was 0.08 μM . The accuracy of the method was evaluated by quantifying the tryptic peptide of three reference samples intentionally contaminated with the isobaric interference. All the reference samples were accurately quantified with $\sim 1\%$ deviation despite the isobaric contamination. Furthermore, we have demonstrated that this methodology can also be applied to quantify the isobaric peptide by standard additions down to 0.2 μM . Finally, liquid chromatography ERMS (LC ERMS) experiments yielded similar results, suggesting the potential of the proposed methodology for analysing complex samples.

KEYWORDS

energy-resolved mass spectrometry, gas phase collisional purification, isobaric interference, peptide quantification, pseudo-MRM, survival yield

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1 | INTRODUCTION

Multiple reaction monitoring (MRM) is a quantification technique, usually performed in triple-quadrupole instruments (QqQ). The general process of MRM scanning mode is to use the first quadrupole to select the precursor ion, the second quadrupole (an hexapole is nowadays more often used) as a collision cell to fragment the precursor ion by collision-induced dissociation (CID) and the third quadrupole to select at least two product ions, which are specific to the analyte.¹ However, MRM requires a good fragmentation yield in CID for the compounds to be analysed. In the context of iso-baric/meric interferences, pseudo-MRM serves as an alternative for analysing compounds that are difficult to fragment. It is based on monitoring, at high collision energy, only the precursor ions instead of the fragment ions. Pseudo-MRM corresponds then to a 'no mass loss' transition between MS¹ and MS².² It has shown good performances for the analysis of complex samples, due to the reduction of isobaric co-elution inside the collision cell.²⁻⁶ However, in pseudo-MRM, there is no clear indication of the optimal collision voltage to be applied in order to confirm the complete removal of isobaric interferences by CID.

Although other techniques have been developed to analyse iso-baric/meric mixtures, such as the kinetic method⁷⁻¹⁰ and, more recently, ion mobility mass spectrometry (MS),^{9,11-14} energy-resolved mass spectrometry (ERMS) can also provide reliable results for quantitative analysis in the presence of iso-meric/baric interferences.^{9,15-17} Crotti et al. have shown that accurate quantification of co-eluted isomers can be achieved by multilinear regression of ERMS.^{9,18-21} Complementary with the latter approach, Memboeuf et al. have shown that ERMS and, more specifically, the survival yield (SY) plots can be used as a tool for detecting iso-baric/meric contamination.¹⁵ Typically, SY curves of pure compounds are sigmoidal. However, if there is iso-baric/meric contamination, the SY curves may be distorted^{16,22} and could exhibit a plateau, if the fragmentation parameters of the two compounds differ sufficiently.^{15,17,22,23} This plateau provides two types of information. Firstly, the vertical position is correlated with the relative concentration of the iso-meric/baric compounds.^{15,17} Secondly, the horizontal position provides the minimum collision voltage necessary to perform a novel strategy, named 'gas-phase collisional purification' (GPCP).^{6,22} Essentially, GPCP occurs when the precursor ions of the target analyte are purified by fragmenting its iso-baric/meric interference entirely. This is also supposed to be done in pseudo-MRM when a high excitation voltage is applied in CID. However, in the GPCP strategy, the monitoring of SY at several excitation voltages provides a mean to ensure the complete purification of the analyte, which is not the case in pseudo-MRM. It should be noted that GPCP and pseudo-MRM can only be applied when the iso-baric/meric interference fragments at lower energies than the analyte.

Among the potential applications of the method, GPCP was combined with MS³ measurements for the quantitative analysis of the purity of synthetic samples.^{6,16} The feasibility of performing structural and quantitative analysis of isobaric mixtures by combining GPCP with in-source fragmentation was also demonstrated.^{23,24} Recently,

we have shown that GPCP can be done in the presence of an internal standard for the absolute quantification of a tryptic peptide.^{24,25} This proof of concept was illustrated by using sodium cationised poly(ethylene)-glycol as a dummy internal standard.²⁵ MS/MS measurements were conducted by direct infusion into an ion trap, using an unconventional broadband isolation window of 15 *m/z* to simultaneously isolate and excite the tryptic peptide and the dummy internal standard. Monitoring the analyte/internal standard peaks intensity ratio at several collision voltages clearly showed the complete fragmentation of the interference with the appearance of a plateau.²⁵ This is a clear and very robust indication of the complete purification of the analyte. This approach facilitates the selection of an optimal excitation voltage in pseudo-MRM, enabling full fragmentation of the iso-baric/meric interference while keeping the analyte of interest.

Going one-step further, the aim of this paper is to apply the GPCP strategy by replacing the dummy internal standard with an isotopically labelled internal standard and evaluate by means of statistics the reliability of this approach. We have applied this strategy to quantify a tryptic peptide (780.402 Da), in the presence of an isobaric interference (780.370 Da). A triply deuterated analogue of the tryptic peptide was used as internal standard (783.420 Da). It was used not only to quantify the tryptic peptide with an internal standard calibration model but also as a criterium to determine the optimal excitation voltage to perform pseudo-MRM. The optimal excitation voltage obtained from the internal standard was also compared with the information from the SY curves. The accurate quantification of the tryptic peptide was verified with intentionally contaminated reference samples. Furthermore, the use of the isotopically labelled internal standard allowed the quantification not only of the tryptic peptide but also of its isobaric interference. Finally, we have tested the feasibility of applying this strategy to liquid chromatography tandem mass spectrometry (LC-MS/MS) for the analysis of complex samples.

2 | EXPERIMENTAL SECTION

2.1 | Chemicals

Acetonitrile (HPLC-MS grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA), water (HPLC PLUS grade) was purchased from Carlo Erba Reagent (Val de Reuil, France), and formic acid was obtained from Amresco (Solon, Ohio, USA) with purity grade higher than 94.5%.

2.2 | Synthetic peptides

Synthetic peptides were synthesised at the Nanobio Platform (Université Grenoble-Alpes, Grenoble, France). The tryptic peptide contains an arginine amino acid residue (**ArgPept**, CH₃CO-ISTYTR-NH₂, monoisotopic mass 780.402 Da), and its internal standard corresponds to the methyl end-group deuterated peptide (**IS**, CD₃CO-ISTYTR-NH₂, monoisotopic mass 783.420 Da). The

isobaric interference is a peptide without any basic residue (*IntPept*, CH₃CO-YYPGQI-NH₂, monoisotopic mass 780.370 Da). All peptides were assembled on a Syro II peptides synthesiser using the Fmoc strategy described in our previous study.¹⁶

2.3 | Sample preparation

All the peptide samples were prepared in acetonitrile/water (7:3) with 0.1% formic acid. Several mixtures of the three peptides were prepared with the following molar ratios *IS/ArgPept/IntPept*: 1:1:0, 1:1:0.5, 1:1:1, 1:1:1.5, 1:1:2 and 1:0:2. Concentration of *IS* and *ArgPept* were fixed at 0.425 and 0.40 μM, respectively. The concentration of *IntPept* ranged from 0 to 0.8 μM. The five standards of the internal standard calibration curve have the same concentration for *IS* (1 μM) and the following concentrations for *ArgPept*: 0.5, 1.0, 1.5, 2.0 and 2.5 μM.

2.4 | MS

MS experiments were performed in positive ion mode using an ion trap mass spectrometer (HCTplus, Bruker Daltonics) equipped with an electrospray ionisation source (ESI, Agilent Technologies). The solutions were introduced by direct injection and electrosprayed via a syringe pump at a 2 μL·min⁻¹ flow rate. The nebulising gas (N₂) pressure was set at 10 psi, and the N₂ drying gas flow rate at 5 L·min⁻¹ heated at 300°C. Helium was used as trapping and collision gas at two different pressures: 2.02·10⁻⁵ and 3.00·10⁻⁵ mbar (uncorrected gauge reading). All data were acquired in the low resolution ultra-scan mode using a 26 000 (*m/z*)·s⁻¹ scan speed. The settings of the instrument were the following: capillary voltage 3.8 kV, end plate -0.5 kV, skimmer 40 V, cap exit 146.4 V and a trap drive at 68.7. The ion charge control option was used, except otherwise noted, to maintain ~100 000 ions inside the trap.

Pseudo-MRM experiments were performed using a 8 *m/z* isolation window centred at *m/z* 783.00, which corresponds to an intermediate mass between the protonated *ArgPept* and *IS*. Isolation was followed by an excitation delay of 200 ms and a CID stage with 100-ms excitation time and 8 *m/z* excitation width (with the same target mass as for the isolation stage). The ion charge control option was unchecked, and the accumulation time was fixed between 2 and 15 ms (depending on the concentration of the sample, see Tables S1 and S2) to maintain ~100 000 ions inside the trap and stabilise the signal. CID MS/MS spectra were obtained on 780–788 *m/z* range, after averaging 5 acquisitions and 2 rolling averages, and combined over 2-min acquisition at each excitation voltage. SY curves were plotted from energy-resolved tandem mass spectrometry (ERMS) spectra performed by isolating the precursor ions with an isolation window of 1.2 *m/z* followed by a fragmentation delay of 200 ms, fragmentation time of 100 ms and a fragmentation width of 10 *m/z*. The MS/MS spectra were acquired for 1 min at each excitation voltage. The 27% default mass cut-off was applied.

The LC-MS/MS measurements were performed by coupling a reversed-phase high-performance liquid chromatography (RP-HPLC) system (Dionex LC Packings Ultimate) to the ion trap spectrometer. Chromatographic analysis was performed with a Xterra C₁₈ column (3.5-μm particles, 1.0 i.d. × 100 mm length, Waters). The volume injected was 1 μL, and the flow rate was 0.5 mL·min⁻¹. The mobile phase was prepared in acetonitrile/water (7:3) with 1% formic acid in isocratic mode. As in direct infusion, a wide isolation window (8 *m/z* centred at *m/z* 783) was also applied to acquire by pseudo-MRM the extracted ion chromatograms (EICs) of *ArgPept* (*m/z* 781.4) and *IS* (*m/z* 784.4) at different excitation voltages. In those experiments, the trapping gas pressure was set at 3.00·10⁻⁵ mbar (uncorrected gauge reading).

DataAnalysis 3.3 (Bruker Daltonics, Bremen, Germany) was used for data acquisition and processing of mass spectra using default software parameters for data processing (background reduction, smoothing and peak centering). SY curves were calculated using the freely available LibreOffice software package.²⁶ The SciDAVis software (freely available) was used for data visualisation.²⁷ The SY and R curves were fitted using the SciDAVis built-in Boltzmann model.

3 | RESULTS AND DISCUSSION

3.1 | SY curves of the protonated peptides

ERMS experiments were conducted by measuring the MS/MS spectra of the three pure protonated peptides (*IntPept*, *ArgPept* and *IS*) at several excitation voltages ranging from 0.5 up to 1.8 V. Additionally, the MS/MS spectra of a mixture with a 1:1-M ratio of *ArgPept* and *IntPept* were measured at the same excitation voltages (MS/MS spectra can be found in Figure S1 at two excitation voltages: 0.88 V and 1.60 V).

The SY was calculated at each excitation voltage as the ratio of the precursor ions peak intensity and the total ion current (TIC).^{6,15,16,22,23,25}

$$SY = \frac{I_{\text{precursor}}}{I_{\text{precursor}} + \sum I_{\text{fragment}}}$$

where $I_{\text{precursor}}$ is the intensity of the precursor ions peak and I_{fragment} is the intensity of each fragment ions peak obtained from the MS/MS experiment. SY curves were then obtained by plotting SY against the excitation voltage (see Figure 1).

Figure 1 shows the SY curves of the three pure protonated peptides, which are sigmoidal. As previously noted,^{23,25} the SY curve of *ArgPept* exhibits a significant shift to higher excitation voltages compared with that of *IntPept*. This is due to the presence of an arginine residue in the tryptic peptide that enhances adduct ion stability by proton sequestration.^{28,29} On the contrary, the SY curves of *ArgPept* and *IS* almost perfectly overlap. This means that the isotopically labelled internal standard behaves equivalently to non-labelled peptide. This is consistent with the MS/MS spectra of *ArgPept* and *IS*,

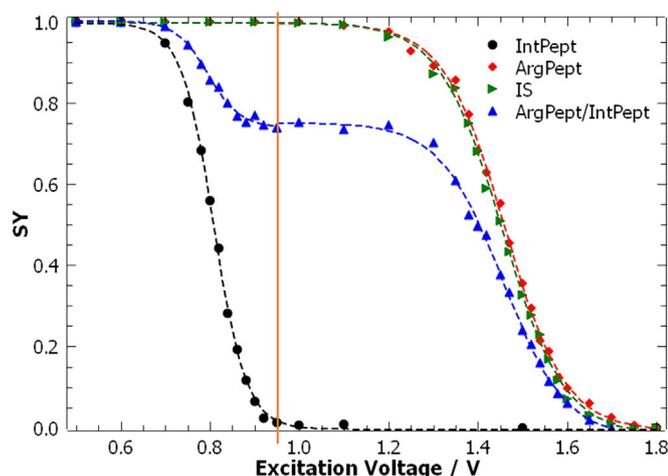


FIGURE 1 Survival yield (SY) curves for the pure protonated *IntPept* (black circles), *ArgPept* (red diamonds) and *IS* (green triangles). The SY curve of a mixture with a molar ratio 1:1 of *ArgPept* and *IntPept* (blue vertical triangles) is also shown. The ion trap pressure was $2.02 \cdot 10^{-5}$ mbar.

which exhibit identical behaviour (see Figure S1). However, the existence of three deuterium results in a 3 m/z shift for the fragment ions of *IS*.

Concerning the 1:1 mixture of *ArgPept* and *IntPept*, the SY curve is a linear combination of the SY curves of both protonated peptides. The SY curve of the 1:1 mixture displays a plateau because the precursor ions of *IntPept* are fully fragmented at lower excitation voltage than those of *ArgPept*. This plateau can be seen as an indicator of isobaric/meric contamination.^{15,22,23,25} Ideally, this plateau should be positioned precisely between the SY curves of the pure peptides, as the concentrations of both peptides are identical. However, this is not typically observed owing to different ionisation efficiencies or competition in ionisation. Figure 1 illustrates that, in this case, ion suppression is observed for *IntPept*, as the plateau is closer to the SY curve for *ArgPept*. The vertical position of the plateau can be correlated with the relative concentrations of both isobaric peptides through a calibration model, accounting then for the ionisation suppression/enhancement of the isobaric mixtures.¹⁵

The horizontal position of the plateau indicates the excitation voltage required to fully fragment the isobaric interference and perform ‘Gas-Phase Collisional Purification’ (GPCP).^{22,25} Figure 1 shows that *IntPept* is fully fragmented at 0.95 V (orange vertical line), and GPCP must be performed at a minimum excitation voltage of 0.95 V.

3.2 | Tryptic peptide peak intensity normalisation using internal standard as a criterium for gas phase collisional purification

As previously demonstrated, the plateau of the SY curve indicates the required excitation voltage to fragment all precursor ions of the isobaric interference. This voltage must be at least 0.95 V to allow

for the accurate quantification by pseudo-MRM of the analyte *ArgPept* in a sample that also contains the isobaric interference *IntPept*. The goal is to show that, rather than using TIC normalisation, this information can also be obtained by normalising the intensities of the precursor ions of *ArgPept* and *IS*.

For this purpose, we used an unconventional broadband isolation window of 8 m/z , which was centered at m/z 783 in order to isolate and excite simultaneously not only *ArgPept* but also *IS* (which can be seen as a parallel pseudo-MRM).^{30,31} The MS/MS spectra were acquired at excitation voltages ranging from 0.70 up to 1.25 V. Figure 2 displays the MS/MS spectra acquired in the range m/z 779 to m/z 787. This allowed for the monitoring of peaks related to the precursor ions of *ArgPept* and *IS* while excluding any of their fragment ions.

To normalise the intensity of the *ArgPept* protonated precursor ions (m/z 781.4) with the intensity of the *IS* protonated precursor ions (m/z 784.4), the following ratio, R , was calculated:

$$R = \frac{I_{\text{ArgPept}+ \text{IntPept}}^{m/z 781.4}}{I_{\text{IS}}^{m/z 784.4}}$$

If the sample also contains *IntPept*, it should be noted that the peak intensity at m/z 781.4 will include the contribution of *ArgPept* as well as of its isobaric interference, *IntPept*.

The MS/MS spectra in Figure 2 were acquired at two excitation voltages: 0.7 V (prior to *IntPept* fragmentation, left column) and 1.0 V (after complete fragmentation of *IntPept*, right column). Three distinct mixtures of peptides were MS/MS analysed to be presented in this figure: (A and B) a mixture of *ArgPept* and *IS* (top MS/MS spectra); (C and D) a mixture of *IntPept* and *IS* (MS/MS spectra at the centre); (E and F) a mixture of *ArgPept*, *IntPept* and *IS* (bottom MS/MS spectra). The concentration of *IS* (0.425 μM) was maintained constant for all three mixtures. For the mixtures containing *ArgPept*, the concentrations were 0.40 μM , and it was 0.80 μM for *IntPept*.

The MS/MS spectra obtained for the mixture *IS/ArgPept* are presented in Figure 2A,B. As expected, the peak intensities of *ArgPept* (m/z 781.7) and *IS* (m/z 784.7) are comparable at both excitation voltages. This finding is in agreement with the SY curves depicted in Figure 1, which indicate that neither *ArgPept* nor *IS* fragment at those excitation voltages. The slight differences in intensity are compensated by calculating the ratio, R . Comparable values of R were obtained at both excitation voltages: $R = 0.81$ for 0.70 V and $R = 0.83$ for 1.00 V.

Figure 2C,D shows the MS/MS spectra for the mixture *IS/Int-pept*. The MS/MS spectrum obtained at 0.7 V (Figure 2C) displays a peak at m/z 781.5, indicating the presence of *IntPept*. However, this peak disappears at 1.0 V (Figure 2D) because of the complete fragmentation of *IntPept* precursor ions at this excitation voltage. This clearly shows that GPCP has been successfully performed and that, as previously shown in the SY curves, this interference can be fully eliminated by employing pseudo-MRM at an excitation voltage of 1.0 V. The intensity of the peak at m/z 784.6 (which corresponds to *IS*) is

FIGURE 2 Tandem mass spectrometry (MS/MS) spectra obtained for three mixtures of peptides at two excitation voltages: 0.7 V (left-hand side) and 1.0 V (right-hand side); 8 m/z isolation window was applied at m/z 783 (blue diamond) before the collision-induced dissociation (CID) excitation stage. The MS/MS spectra correspond to a mixture *IS/ArgPept* at the top (A and B), a mixture *IS/IntPept* at the centre (C and D) and a mixture *IS/ArgPept/IntPept* at the bottom (E and F). For each MS/MS spectrum, R is calculated as the ratio of intensities obtained at m/z 781.6 and m/z 784.6. The intensity at m/z 781.6 corresponds to the analyte *ArgPept* and its isobaric interference *IntPept*, whereas the intensity at m/z 784.6 corresponds to the isotopically labelled *IS*.

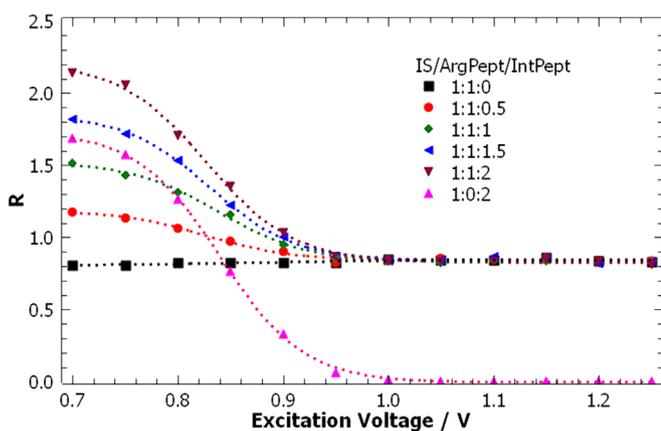
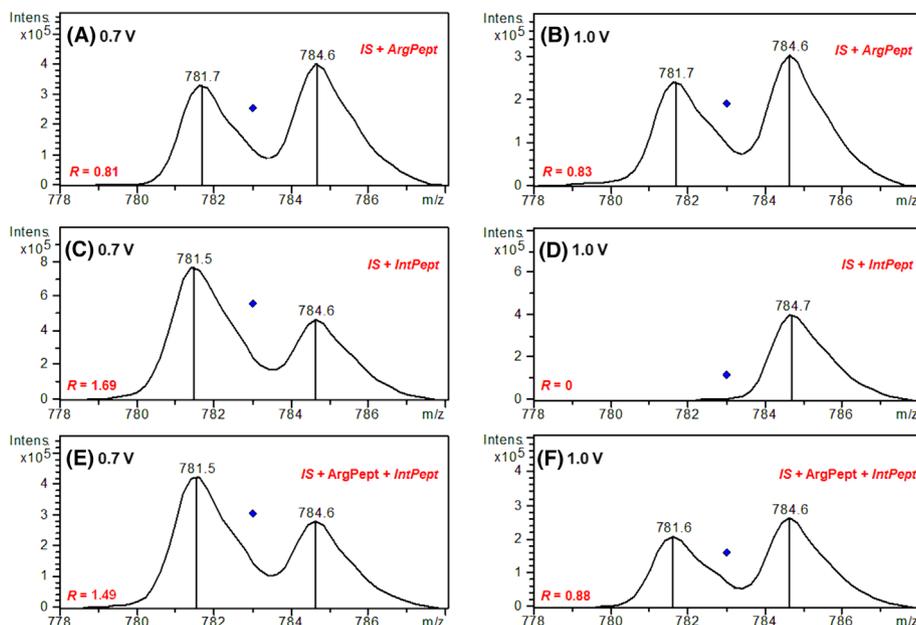


FIGURE 3 Ratio, R , of MS peak intensities for m/z 781.4 and m/z 784.4. R is plotted as a function of the collision-induced dissociation (CID) excitation voltage for six mixtures of *IS*, *ArgPept* and *IntPept*. All the tandem mass spectrometry (MS/MS) spectra were acquired with a broadband isolation window of 8 m/z centred at m/z 743. The trapping gas pressure was set to $2.02 \cdot 10^{-5}$ mbar.

very similar at both excitation voltages because *IS* does not fragment at such an excitation voltage. The ratio, R , was also calculated. At 0.7 V (prior to GPCP) $R = 1.69$ and drops down to 0 after applying GPCP at 1.0 V. This implies that changes in R due to excitation voltage are correlated to the existence of *IntPept* and indicate an isobaric interference.

The MS/MS spectra of Figure 2E,F correspond to a mixture of the three peptides *IS/ArgPept/IntPept*. It is clearly seen that at 0.7 V, the peak intensity at m/z 781.6 is much higher than at 1.0 V, whereas the peak intensity from *IS* at m/z 784.6 is almost identical at both excitation voltages. The decrease in intensity at m/z 781.4 results from the fragmentation of the *IntPept* precursor ions at 1.0 V (as for the mixture *IS/IntPept*). In addition, the decrease in R clearly indicates

isobaric contamination: R decreases from 2.12 at 0.7 V (Figure 2E) to 0.88 at 1.0 V (Figure 2F).

To monitor GPCP, the ratio, R , of MS peak intensities '*analyte/IS*' was plotted as a function of CID excitation voltage. Figure 3 shows the values of R obtained for six mixtures with the following molar ratios *IS/ArgPept/IntPept*: 1:1:0, 1:1:0.5, 1:1:1, 1:1:1.5, 1:1:2 and 1:0:2.

GPCP is clearly observed for the mixture containing only *IntPept* and *IS* (i.e., 1:0:2, pink triangles). For this mixture, the ratio R decreases monotonously as the excitation voltage is increased to reach a value of 0 above 0.95 V, meaning that all *IntPept* precursor ions are fragmented. This shows that an excitation of 0.95 V is sufficient to completely fragment *IntPept* by CID and perform GPCP to remove the interfering *IntPept*. This corresponds to the excitation voltage at which (1) the SY of *IntPept* becomes 0 and (2) a plateau is observed for the *ArgPept/IntPept* mixture (orange vertical line, see Figure 1). This means that, similarly to the SY curves, the internal standard provides, through the ratio R , a reliable indicator for GPCP. The plot of R for the 1:0:2 mixture is sigmoidal and is very similar to the SY curve for *IntPept* (Figure 1).

On the contrary, for a mixture of *ArgPept* and *IS* (i.e., 1:1:0, black squares), R remains almost constant at all excitation voltages ($R \approx 0.84$). As there is no *IntPept* in the mixture, R depends only on the relative concentrations of *ArgPept* and *IS*. Even if there were a slight variability in ionisation efficiency or some degree of fragmentation, this variability would be equally compensated by *IS*.

Ratios R obtained for mixtures of the three peptides as a function of the excitation voltages are actually linear combinations of the previous mixtures (i.e., Figure 3, pink triangles for 1:0:2 and black squares for 1:1:0). Similarly to the sample containing only *IntPept* and *IS*, R decreases gradually with increasing the excitation voltage. However, it does not reach $R = 0$ above 0.95 V. Instead, a plateau is observed from 0.95 up to 1.25 V. Regardless of the concentration *IntPept*, the

TABLE 1 One-way ANOVA table calculated from the ratios, R , of peak intensities measured at the plateau for five mixtures with different concentrations of *IntPept*. The concentrations of *IS* and *ArgPept* were the same for all samples. The between-groups variability corresponds to the amount of *IntPept* and the within-groups variability to the experimental fluctuations of R with the excitation voltage.

Source of variation	Sum of squares (SS)	Degrees of freedom	Mean squares (MS)	F	p value
<i>IntPept</i> concentration	$7.22 \cdot 10^{-4}$	4	$1.80 \cdot 10^{-4}$	1.274	0.306
Excitation voltage	$3.54 \cdot 10^{-3}$	25	$1.42 \cdot 10^{-4}$		
Total	$4.26 \cdot 10^{-3}$	29			

Abbreviation: ANOVA, analysis of variance.

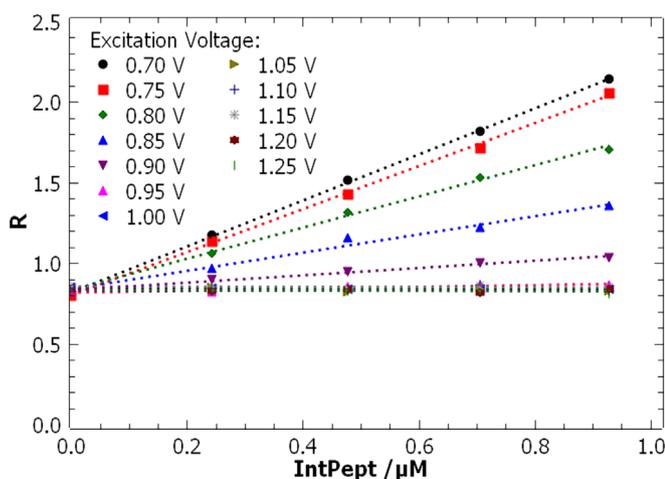


FIGURE 4 Linear dependence of R against the concentration of *IntPept* at 12 excitation voltages (from 0.70 to 1.25 V).

plateau is observed at $R = 0.84$ (with small variations due to experimental variability). This value exactly corresponds to the ratio obtained for the sample with only *ArgPept* and *IS* (i.e., 1:1:0, black squares). This shows that, independently of the concentration of *IntPept*, the samples can be purified by CID fragmentation. Therefore, even in the presence of *IntPept*, it is possible to quantify accurately the tryptic peptide, providing the excitation voltage exceeds 0.95 V.

One-way analysis of variance (ANOVA) was employed to compare statistically the means of ratios R , obtained at the plateau for the five peptide mixtures (R values showed in Table S3). The between-groups variability is associated with the amount of *IntPept*, whereas the within-groups variability corresponds to the experimental fluctuations due to excitation voltage. The homogeneity of variances within-groups was evaluated using the Levene test (p value = 0.31). The BoxPlot (Figure S2) displays comparable variabilities and R values in all the samples. The one-way ANOVA table reveals that the variability of R due to the amount of *IntPept* is not significant compared to experimental variability (p value > 0.05) (see Table 1). Consequently, the one-way ANOVA confirms that *IntPept* has been completely fragmented through the CID process, and the minor variations in R among samples are solely attributable to experimental variability and are independent of the amount of *IntPept*.

For excitation voltages below 0.95 V, it can be observed that the ratio is proportional to the amount of *IntPept* in the sample. This is

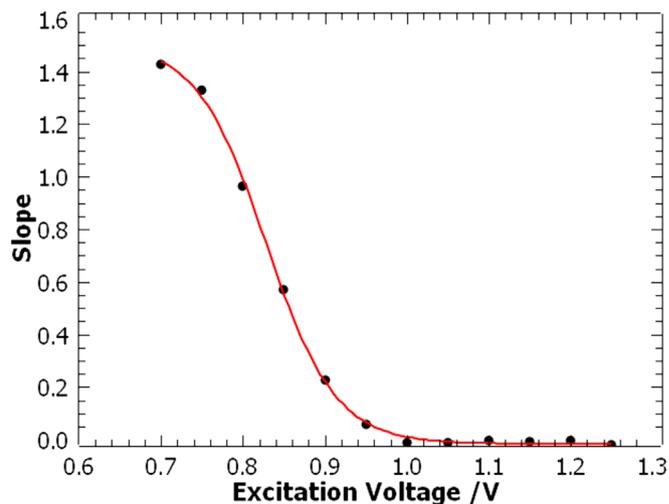


FIGURE 5 Slope of the linear regression of R against the concentration of *IntPept* plotted as a function of the excitation voltage (regression models shown in Figure 4 and slope values shown in Table S4). A sigmoidal decrease of the slope with the excitation voltage can be observed.

confirmed by plotting a linear regression for R against the concentration of *IntPept* at several excitation voltages (from 0.70 to 1.25 V; see Figure 4). Table S4 shows the slope and intercept for each regression model. The regression models show good coefficients of determination before the total CID fragmentation of *IntPept* (i.e., excitation voltages lower than 0.95 V).

The intercepts are very similar and correspond to the ratio R of the sample without *IntPept* (i.e., mixture 1:1:0), as expected. The average value of these intercepts was calculated and compared with the average value of $R = 0.83$ obtained for the sample without *IntPept*. The t test confirms that the mean value of the intercepts does not differ significantly from the mean value of $R = 0.83$ obtained for the sample without *IntPept*. The calculated t value of 0.62 is below the two-sided t value for $\alpha = 0.05$ and 11 degrees of freedom, which is 2.20. The p value is 0.54.

Figure 4 shows that the calibration line slopes decrease with the excitation voltage due to the fragmentation of *IntPept* by CID. Figure 5 shows the plot of the latter slopes as a function of the excitation voltage. It is noteworthy that the sigmoidal decrease of the slope with excitation voltage is very similar to the SY curve of *IntPept* (Figure 1) and also to the R curve for the mixture with only *IntPept*

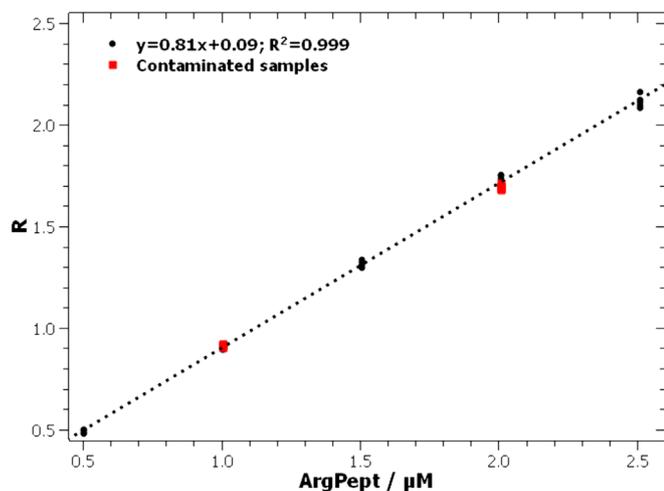


FIGURE 6 Internal standard calibration line (black line and black circles) obtained from the ratio, R , of mass spectrometry (MS) peaks intensities of the tryptic peptide and the internal standard. All the calibration standards have the same concentration of IS ($1 \mu\text{M}$) and were analysed at six excitation voltages under gas-phase collisional purification (GPCP) conditions. Two standards (i.e., at 1 and $2 \mu\text{M}$ of $ArgPept$) were intentionally contaminated with $2 \mu\text{M}$ of $IntPept$. These contaminated samples (red squares) were also analysed at the same excitation voltages following the same procedure as for the calibration standards. The trapping gas pressure was set at $2.02 \cdot 10^{-5}$ mbar.

and IS (mixture 1:0:2, pink triangles in Figure 3). The highest slope is obtained at 0.70 V, denoting the lowest fragmentation of $IntPept$, where SY is almost 1. For excitation voltages higher than 0.95 V, $IntPept$ undergoes complete fragmentation due to CID, resulting in slopes very near to 0.

3.3 | Internal standard calibration for the accurate quantification of the tryptic peptide by pseudo-MRM

MS/MS spectra of five calibration standards (prepared as described in the experimental section) were acquired with a broadband isolation window ($8 m/z$ centred at m/z 783) at six excitation voltages: 1 , 1.05 , 1.1 , 1.15 , 1.2 and 1.25 V. All these excitation voltages are higher than 0.95 V. As stated previously, this means that the calibration standards are analysed under conditions of GPCP: the isobaric interference $IntPept$ (if present in the sample) will be fragmented by CID. Pseudo-MRM can then be applied at these voltages to accurately quantify $ArgPept$ despite the presence of $IntPept$.

Each calibration standard was analysed four times at each excitation voltage. The mean value of the four replicates was calculated for each excitation voltage and plotted in the calibration model (Figure 6). Each calibration standard has six values (i.e., one for each excitation voltage). The calibration line exhibits a good coefficient of determination ($R^2 = 0.999$) for the concentration interval under study (i.e., from 0.5 to $2.5 \mu\text{M}$ of $ArgPept$). The regression model's linearity was evaluated using ANOVA (see Table 2). The high F value obtained for the

regression model ($F = 28\,712$) and the very low p value of $1.04 \cdot 10^{-43}$ confirmed the high correlation of the ratio, R , with the $ArgPept$ concentration; that is, the correlation is much higher than the residuals of the calibration model. This result is consistent with the good $R^2 = 0.999$ of the calibration model. The F -statistic for lack of fit was obtained by dividing the lack of fit mean square ($MS_{LoF} = 4.82 \cdot 10^{-4}$) by the pure error mean square ($MS_{pE} = 3.29 \cdot 10^{-4}$). The resulting F value of 1.46 was lower than the tabulated one-sided F value (i.e., $F_{0.95,3,25} = 2.99$, for $\alpha = 0.05$ and 3 and 25 degrees of freedom). The p value was found to be 0.2483 , which is higher than the significance level $\alpha = 0.05$. The F and p values therefore indicate that the model's lack of fit is not statistically significant in comparison with experimental variability, and thus, the calibration model is validated.

Two calibration standards were deliberately contaminated with $2 \mu\text{M}$ $IntPept$ and subsequently analysed four times at each excitation voltage following the same procedure as the non-contaminated samples. Figure 6 presents the six values of R calculated for the two spiked contaminated standards (1 - and $2 \mu\text{M}$ $ArgPept$, depicted as filled red squares). In both instances, the ratio R is highly similar to the one obtained from the calibration standards that are free from $IntPept$.

The predictive ability of the calibration model was evaluated by calculating the proportional bias of the concentrations estimated with the calibration model, $C_{\text{calibration}}$, for each calibration standard at each excitation voltage:

$$\text{bias}(\%) = \frac{C_{\text{calibration}} - C_{\text{standard}}}{C_{\text{standard}}} \cdot 100$$

where C_{standard} corresponds to the concentration of the calibration standard. The proportional bias of all the calibration standards is within the 95% confidence interval of the calibration model.³² This interval is calculated for each concentration level and is expressed as a percentage relative to concentration. Figure 7 shows that all the standards are correctly predicted. The proportional bias of the standards is always lower than 5%. The concentration of $ArgPept$ was calculated for the two contaminated standards according to the linear calibration model. The proportional bias of the two contaminated standards is also lower than 5%. The 95% confidence interval³² for these contaminated standards is $(1.01 \pm 0.05) \mu\text{M}$ (for the standard at $1 \mu\text{M}$) and $(1.98 \pm 0.05) \mu\text{M}$ (for the standard at $2 \mu\text{M}$). Both confidence intervals contain the spiked concentrations of $ArgPept$, which confirms that the tryptic peptide is accurately quantified by pseudo-MRM despite the presence of the isobaric interference $IntPept$.

The limit of detection ($LD = 0.08 \mu\text{M}$) was calculated using the internal standard calibration model's information and the following formula:

$$LD = 3.3 \cdot \frac{s_e}{b_1}$$

where s_e is the standard error of the calibration model ($s_e = 1.92 \cdot 10^{-2}$) and b_1 corresponds to the slope ($b_1 = 0.81$). This

TABLE 2 ANOVA table of the regression model with replicates shown in Figure 6.

Source of variations	Sum of squares (SS)	Degrees of freedom	Mean squares (MS)	F	p value
Regression model	9.93	1	9.93	28712.5	$1.04 \cdot 10^{-43}$
Residual	$9.68 \cdot 10^{-3}$	28	$3.46 \cdot 10^{-4}$		
Lack of fit	$1.45 \cdot 10^{-3}$	3	$4.82 \cdot 10^{-4}$	1.46	0.2483
Pure error	$8.23 \cdot 10^{-3}$	25	$3.29 \cdot 10^{-4}$		
Total	9.93843	29			

Abbreviation: ANOVA, analysis of variance.

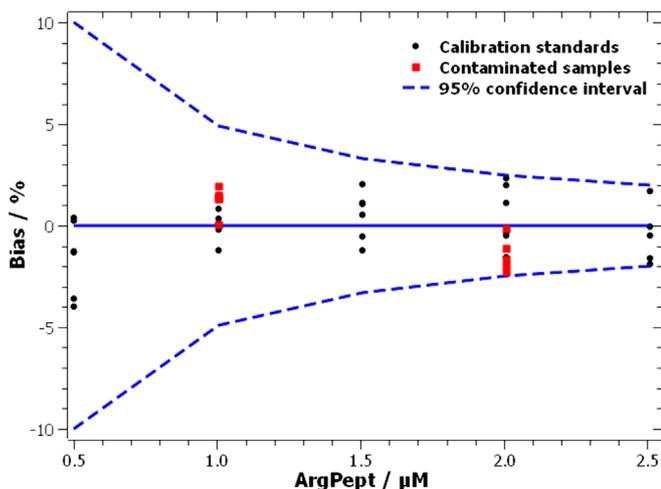


FIGURE 7 Proportional bias of the calibration standards calculated at each excitation voltage for both the calibration standards (depicted by black circles) and for the contaminated standards (depicted by red squares). All the standards fall within the 95% confidence interval of the calibration model that was calculated at each concentration level.

approach is recommended in LC-MS methods as it gives conservative estimates when determining LD from calibration lines.^{33,34}

3.4 | Quantification of the tryptic peptide and its isobaric interference in a reference sample

To evaluate the accuracy of the developed parallel pseudo-MRM quantification method, we used a reference sample spiked with 0.40- μM *ArgPept*, 0.425- μM *IS* and 0.20- μM *IntPept*. The spiked sample was analysed by MS/MS (8 m/z isolation window centred at m/z 783) at an excitation voltage before GPCP occurs (at 0.70 V) and after GPCP occurs (at 1.00, 1.05, 1.10, 1.15, 1.20 and 1.25 V). The ratio, R , was calculated at each excitation voltage, and the average value obtained after GPCP (from 1.00–1.25 V) was 0.844. The *ArgPept*/*IS* ratio was also calculated from the internal standard calibration curve (Figure 6). A value of 0.93 was obtained. The concentration of *ArgPept* was then calculated by multiplying 0.93 by the concentration of *IS* (0.425 μM). Considering a 95% confidence interval, the concentration of *ArgPept* was determined at (0.395 ± 0.042) μM . This confidence interval was calculated from the uncertainty of the calibration model³²

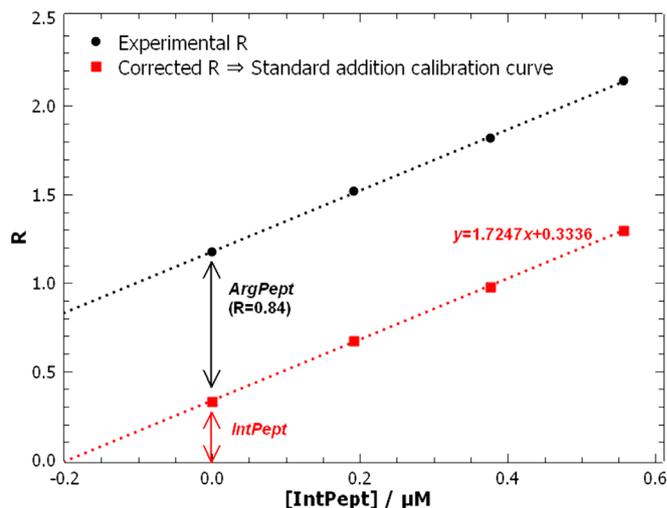


FIGURE 8 Standard addition calibration curve (red squares) calculated from the experimental R values (black squares) obtained from the tandem mass spectrometry (MS/MS) spectra of the spiked samples at 0.70 V excitation voltage. The corrected R values (red squares) were calculated by subtracting $R = 0.84$ to all experimental values (that corresponds to the contribution of *ArgPept*). The concentration of *IntPept* (~ 0.2 μM) is shown graphically by extrapolating the standard addition calibration curve to $y = 0$.

and multiplied by the concentration of *IS*. The accuracy is then assessed as the confidence interval includes the reference value of 0.40 μM .

We have also calculated the concentration of *IntPept*. In this case, we used the R value obtained at 0.70 V corresponding to an excitation voltage at which *IntPept* does not fragment by CID in order to achieve the highest sensitivity (as shown in Figures 4 and 5). The reference sample was spiked with *IntPept* at three concentration levels: 0.19, 0.38 and 0.56 μM . The experimental values of R are shown in Figure 8 (black circles). As shown in Figure 3, the ratio before GPCP is the sum of the ratio due to *ArgPept* and *IntPept*. The contribution of *ArgPept* is easily known as it corresponds to the ratio, R , calculated at the plateau (at excitation voltages higher than 0.95 V). To plot the standard addition curve, the average value $R = 0.84$ (obtained for the reference sample after GPCP) was subtracted from each of the experimental R values (black circles). The corrected R values (red squares) were then obtained. A standard addition calibration model was calculated from these values ($y = 1.7247x + 0.3336$,

$R^2 = 0.9996$). As expected, the intercept is different from 0 and is related to the *IntPept* concentration, which is calculated by extrapolating the calibration model to $y = 0$. The concentration of *IntPept*, calculated as the ratio of the intercept and the slope, was $0.1934 \mu\text{M}$. The 95% confidence interval obtained for the extrapolated value was $(0.193 \pm 0.029 \mu\text{M})$. The accuracy for the quantification of *IntPept* is also assessed as the confidence interval includes the reference value of *IntPept* ($0.20 \mu\text{M}$) with an absolute error lower than $0.01 \mu\text{M}$ ($<3.5\%$ relative error). This means that this method can also be used to quantify the isobaric interference if a standard of the isobaric interference is available to perform a standard addition calibration model at an excitation voltage prior to GPCP.

3.5 | Internal standard quantification using LC ERMS

All the results shown in the previous sections were obtained at medium trapping gas pressure (i.e., $2.02 \cdot 10^{-5}$ mbar). The aim of this section is to show that internal standard quantification can also be applied using LC ERMS for the analysis of complex samples using a high trapping gas pressure (i.e., $3.00 \cdot 10^{-5}$ mbar).

SY curves for the three protonated peptides separately (*IntPept*, *ArgPept* and *IS*) and the 1:1 mixture of *ArgPept/IntPept* were obtained at high pressure (Figure 9). Similarly, to the lower pressure, SY curves of *ArgPept* and *IS* overlap. Figure 9 shows that the SY curves are shifted to higher excitation voltages compared to those obtained at medium pressure. A more detailed analysis of this phenomenon is actually on track in our lab, but a tentative explanation for this observation may be as follows: as the trapping/collision gas pressure is increased, the number of ion/neutral collisions is greater, with a greater number of potential cooling collisions. Collisional cooling may then be more efficient, and the excitation voltage required to achieve the same degree of

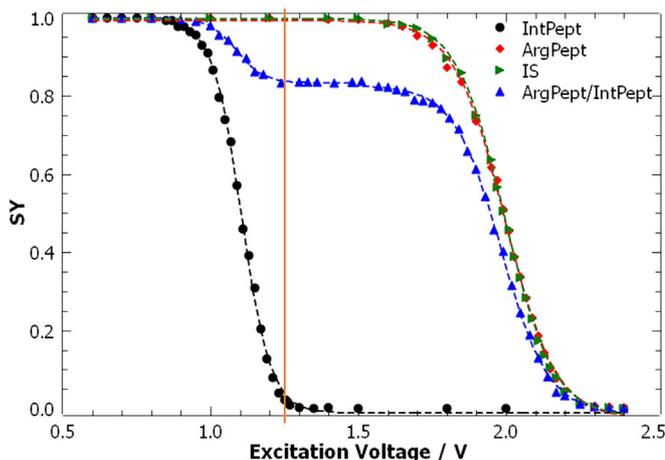


FIGURE 9 Survival yield (SY) curves for the pure protonated *IntPept* (black circles), *ArgPept* (red diamonds) and *IS* (green triangles). The SY curve of a mixture with a molar ratio 1:1 of *ArgPept* and *IntPept* (blue vertical triangles) is also shown. The ion trap pressure was $3.00 \cdot 10^{-5}$ mbar.

fragmentation (1-SY) in the same time window (excitation time) must therefore be increased. At medium pressure, the plateau for the *ArgPept/IntPept* mixture is observed from 0.92 to 1.18 V (Figure 1), whereas at high pressure, the plateau becomes almost twice as wide and is shifted to higher excitation voltages, that is, from 1.24 to 1.65 V (Figure 9). This effect was already observed for a mixture of three isobaric compounds: the high trapping pressure showing a better differentiation of the isobaric compounds with the appearance of two plateaus that were not visible at lower pressures.²² In our case, the plateau is observed at both pressures, eliminating the need to increase the trapping gas pressure for pseudo-MRM quantification of the tryptic peptide. Nevertheless, it may become necessary to increase the trapping gas pressure in case the isobaric interferences fragment at excitation voltages that are too similar to those of the analyte, which could complicate the removal of the interference by GPCP.

The horizontal position of the plateau indicates that *IntPept* is fully fragmented at 1.24 V (orange vertical line, Figure 9), and GPCP must be performed at high trapping pressure at a minimum excitation voltage of 1.24 V.

To show that the same information can also be obtained with an internal standard, we plotted the ratio, R , of MS peaks intensities for 'analyte/IS' as a function of CID excitation voltage. The MS/MS spectra were also acquired using a broadband isolation window of 8 m/z centred at m/z 783.00, as previously done for medium pressure. Three distinct peptide mixtures were analysed. The first mixture contained only *ArgPept* and *IS*, the second one only *IntPept* and *IS* and the third one contained all three peptides. The R ratios were plotted against the excitation voltage for the three mixtures (Figure 10).

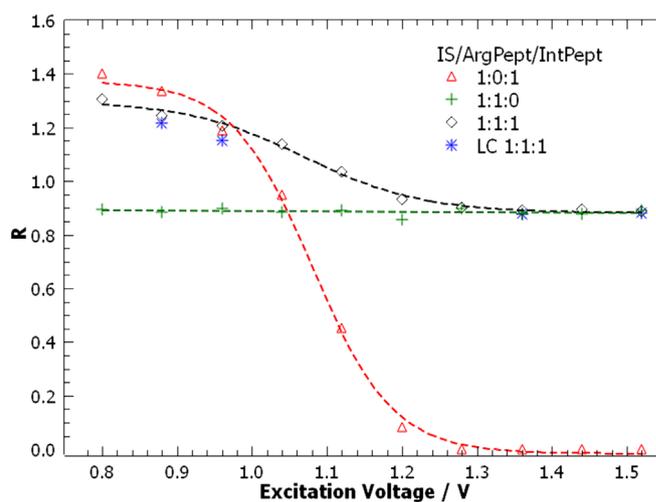


FIGURE 10 Ratio, R , of mass spectrometry (MS) peak intensities for m/z 781.4 and m/z 784.4. R is plotted as a function of the collision-induced dissociation (CID) excitation voltage for three mixtures of *IS*, *ArgPept* and *IntPept*. All MS/MS spectra were acquired with a broadband isolation window of 8 m/z centred at m/z 743. The mixture with the three peptides was also measured by liquid chromatography (LC) at four excitation voltages (depicted as blue stars). The R ratios calculated for LC were very similar to those calculated with direct infusion.

The behaviour of R with the excitation voltage is in accordance with the results obtained at medium gas trapping pressure (Figure 3). GPCP is also clearly observed for the *IntPept/IS* mixture (i.e., 1:0:1, red triangles in Figure 10). The ratio R decreases monotonously as the excitation voltage is increased to reach a value of 0 at 1.28 V (as shown in Figure 9 for the SY curve of *IntPept*). As previously observed for medium pressure, R remains almost constant at all excitation voltages ($R \approx 0.9$) for the mixture of *ArgPept* and *IS* (i.e., 1:1:0, green cross in Figure 10). The ratios R curve obtained for the 1:1:1 mixture of the three peptides (black diamonds) is also a linear combination of the same curves obtained for the *IntPept/IS* and *ArgPept/IS* mixtures showing a monotonous decrease with increasing the excitation voltage. However, it does not reach $R = 0$ at 1.28 V. Instead, a plateau at $R \approx 0.9$ is observed from 1.28 up to 1.55 V, which is very close to the ratio R obtained for the *ArgPept/IS* mixture (green cross, Figure 10). These results therefore confirm that the plateau R is a reliable indicator of GPCP even at high trapping gas pressure.

LC was coupled to the ion trap mass spectrometer. The LC conditions were tuned to minimise the analysis time. As with direct infusion, MS/MS chromatograms were measured with a wide isolation window of 8 m/z centred at m/z 783.00. *ArgPept* and *IntPept* were injected separately. The retention times were 0.7 min for *ArgPept* and 0.8 min for *IntPept* (see Figure S3). Therefore, under these conditions, the tryptic peptide and its isobaric interference partially co-elute.

Figure 11 shows the LC-MS/MS chromatograms for the mixture of the three peptides (1:1:1). These chromatograms were obtained at two excitation voltages: before GPCP (at 0.88 V, left-hand side of Figure 11) and after GPCP (at 1.36 V, right-hand side of Figure 11). Two EICs were obtained: at m/z 781.4 (for *ArgPept*, in blue) and at m/z 784.4 (for *IS*, in magenta).

The peak height for *ArgPept* at 1.36 V decreased significantly due to the elimination of the co-eluted isobaric interference, whereas the peak height for *IS* remains almost unaffected. The complete elimination of the co-eluted interference is also confirmed by the chromatograms obtained for the mixture of *IntPept* and *IS* at the same excitation voltages (see Figure S4). The EIC for *IntPept* shows no chromatographic peak at 1.36 V, whereas the *IS* chromatographic peak appears almost unaffected. Furthermore, it can be observed that the chromatographic peaks of *ArgPept* and *IS* are perfectly overlapping at 1.36 V, which was not the case at 0.88 V, also showing that the co-eluted interference can be discarded by GPCP. The perfect co-elution of *ArgPept* and *IS* can be observed from the chromatograms obtained for the mixture of *ArgPept* and *IS* (see Figure S5). The EICs obtained at both excitation voltages in Figure S5 show perfect co-elution of both compounds. Moreover, the chromatograms are almost unaffected at 1.36 V, confirming that neither *ArgPept* nor *IS* show fragment ions at 1.36 V.

The ratio, R , was calculated from both chromatograms as the ratio of the peak areas obtained for *ArgPept* (EIC at m/z 781.4) and *IS* (EIC at m/z 784.4). At 0.88 V, we obtained a ratio $R = 1.22$ that, as shown in Figure 10 with blue stars, is very similar to the ratio obtained for the same sample by direct infusion (black diamonds). LC was also performed at 0.96 V, and the ratio of peak areas, R , decreases as observed for direct infusion. At 1.36 V, R decreased to 0.89 due to the complete fragmentation of the co-eluted *IntPept* by pseudo-MRM. Figure 10 shows that almost the same ratio of peak areas was obtained at 1.52 V, indicating that in LC, this ratio, R , remains almost constant after GPCP and is very similar to the R values obtained by direct infusion. Therefore, the proposed method could be used not only in direct infusion but also in combination with LC for the analysis of complex samples.

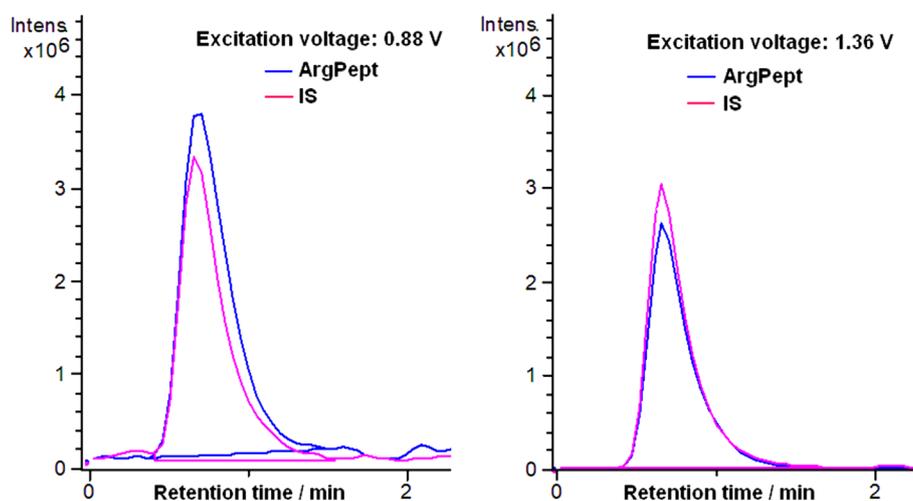


FIGURE 11 Liquid chromatography tandem mass spectrometry (LC-MS/MS) chromatograms obtained by pseudo-multiple reaction monitoring (MRM) for the 1:1:1 mixture of the three peptides. The chromatograms are shown before GPCP (at 0.88 V, left-hand side) and after gas-phase collisional purification (GPCP, right-hand side). Two extracted ion chromatograms (EICs) are shown: one for m/z 781.4 (blue curve, *ArgPept*) and another for m/z 784.4 (magenta curve, *IS*). The chromatographic peak of *ArgPept* decreases significantly at 1.36 V due to the fragmentation of the interference, whereas the chromatographic peak of *IS* remains quite similar at both excitation voltages. As with direct infusion, the chromatograms were acquired at high trapping gas pressure ($3.00 \cdot 10^{-5}$ mbar) and with a wide isolation window (8 m/z centred at m/z 783).

4 | CONCLUSIONS

We successfully quantified a tryptic peptide by using an isotopically labelled internal standard and energy-resolved tandem mass spectrometry strategy (ERMS), despite the presence of an isobaric peptide. This was achieved using CID ERMS on an ion trap mass analyser, with the use of an unconventional broadband isolation window to simultaneously isolate and excite both the analyte and its internal standard. This method can be considered an alternative way to performing parallel pseudo-MRM.^{30,31} Few years ago, we presented this strategy as a proof-of-concept and by employing a sodium-cationised poly(ethylene)-glycol as a dummy internal standard with a similar m/z .²⁵ In this paper, we have gone a step further by substituting the dummy internal standard with a conventional isotopically labelled internal standard, which is frequently employed in MS for quantification purposes. Herein, the methodology has been assessed and validated through statistical tools such as ANOVA and a reference sample that is entirely independent from the calibration standards. In addition to quantifying the tryptic peptide using an Internal Standard (IS) calibration model, we have also demonstrated that it is also feasible to quantify the isobaric peptide by standard additions.

In the present case, monitoring the *analyte/IS* ratio as a function of the excitation voltage showed a gradual decrease in the presence of isobaric contamination. This is due to differences in the fragmentation energetics of the tryptic and non-tryptic (interferent) peptides. The appearance of a plateau above a certain excitation voltage indeed indicates complete fragmentation of the isobaric non-tryptic peptide interferent. The ratio *R* at the plateau remains unaffected by the concentration of interferent, meaning that the tryptic peptide was purified from its isobaric contaminant, as confirmed by the one-way ANOVA. Thus, this plateau is a reliable indicator for determining the excitation voltage to be applied in pseudo-MRM.

We applied pseudo-MRM above this excitation voltage to quantify the tryptic peptide with an internal standard calibration model. The calibration model showed no lack of fit and a linear dynamic range from 0.5 up to 2.5 μM . The detection limit was 0.08 μM . Two contaminated standards, intentionally contaminated with the isobaric peptide, were correctly quantified by pseudo-MRM with 1% deviation. Similar results were obtained with lower concentrations of IS and tryptic peptide. The accuracy of the method for quantifying the tryptic peptide was also assessed and yielded $\sim 1.2\%$ deviation and a confidence interval of $0.395 \pm 0.039 \mu\text{M}$ (prepared at 0.40 μM). The interferent was also quantified by standard additions down to 0.2 μM with $\sim 3\%$ deviation. Finally, comparable results were obtained by LC ERMS experiments, suggesting the capability of analysing complex samples with the proposed methodology.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Alicia Maroto  <https://orcid.org/0000-0001-7821-7726>

Dany Jeanne dit Fouque  <https://orcid.org/0000-0001-6525-4632>

Rémy Lartia  <https://orcid.org/0000-0002-0049-6726>

Antony Memboeuf  <https://orcid.org/0000-0001-8540-2188>

REFERENCES

- Sargent M (Ed). *Guide to Achieving Reliable Quantitative LC-MS Measurements*. LGC; 2013.
- Galmiche M, Rodrigues A, Motsch E, Delhomme O, François YN, Millet M. The use of pseudo-MRM for a sensitive and selective detection and quantification of polycyclic aromatic compounds by tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2022;36(13):e9307. doi:10.1002/rcm.9307
- Shang D, Kim M, Haberl M. Rapid and sensitive method for the determination of polycyclic aromatic hydrocarbons in soils using pseudo multiple reaction monitoring gas chromatography/tandem mass spectrometry. *J Chromatogr A*. 2014;1334:118-125. doi:10.1016/j.chroma.2014.01.074
- Yan J, Kim M, Haberl M, et al. Determination of polycyclic aromatic hydrocarbons in surface water using simplified liquid-liquid micro-extraction and pseudo-MRM GC/MS/MS. *Anal Methods*. 2018;10(4):405-416. doi:10.1039/C7AY01902E
- An N, Zhu Q-F, Yu L, Chen Y-T, Chen S-L, Feng Y-Q. Derivatization assisted LC-p-MRM-MS with high CID voltage for rapid analysis of brassinosteroids. *Talanta*. 2020;217:121058. doi:10.1016/j.talanta.2020.121058
- Dit Fouque DJ, Maroto A, Memboeuf A. Purification and quantification of an isomeric compound in a mixture by collisional excitation in multistage mass spectrometry experiments. *Anal Chem*. 2016;88(22):10821-10825. doi:10.1021/acs.analchem.6b03490
- Majumdar TK, Clairet F, Tabet JC, Cooks RG. Epimer distinction and structural effects on gas-phase acidities of alcohols measured using the kinetic method. *J Am Chem Soc*. 1992;114(8):2897-2903. doi:10.1021/ja00034a021
- Tao WA, Wu L, Cooks RG. Differentiation and quantitation of isomeric dipeptides by low-energy dissociation of copper (II)-bound complexes. *J Am Soc Mass Spectrom*. 2001;12(5):490-496. doi:10.1016/S1044-0305(01)00237-9
- Crotti S, Menicatti M, Pallecchi M, Bartolucci G. Tandem mass spectrometry approaches for recognition of isomeric compounds mixtures. *Mass Spectrom Rev*. e21757.
- Tao WA, Cooks RG. Chiral analysis by MS. *Anal Chem*. 2003;75(1):25-31. doi:10.1021/ac0312110
- Laphorn C, Pullen F, Chowdhry BZ. Ion mobility spectrometry-mass spectrometry (IMS-MS) of small molecules: separating and assigning structures to ions. *Mass Spectrom Rev*. 2013;32(1):43-71. doi:10.1002/mas.21349
- Hanozin E, Morsa D, de Pauw E. Energetics and structural characterization of isomers using ion mobility and gas-phase H/D exchange: learning from lasso peptides. *Proteomics*. 2015;15(16):2823-2834. doi:10.1002/pmic.201400534
- Morsa D, Defize T, Dehareng D, Jérôme C, de Pauw E. Polymer topology revealed by ion mobility coupled with mass spectrometry. *Anal Chem*. 2014;86(19):9693-9700. doi:10.1021/ac502246g
- Wu Q, Wang J-Y, Han D-Q, Yao Z-P. Recent advances in differentiation of isomers by ion mobility mass spectrometry. *Trends Anal Chem*. 2020;124:115801. doi:10.1016/j.trac.2019.115801
- Memboeuf A, Jullien L, Lartia R, Brasme B, Gimbert Y. Tandem mass spectrometric analysis of a mixture of isobars using the survival yield technique. *J Am Soc Mass Spectrom*. 2011;22(10):1744-1752. doi:10.1007/s13361-011-0195-8

16. Dit Fouque DJ, Lartia R, Maroto A, Memboeuf A. Quantification of intramolecular click chemistry modified synthetic peptide isomers in mixtures using tandem mass spectrometry and the survival yield technique. *Anal Bioanal Chem*. 2018;410(23):5765-5777. doi:10.1007/s00216-018-1258-5
17. Josse T, Winter JD, Dubois P, Coulembier O, Gerbaux P, Memboeuf A. A tandem mass spectrometry-based method to assess the architectural purity of synthetic polymers: a case of a cyclic Polylactide obtained by click chemistry. *Polym Chem*. 2014;6(1):64-69. doi:10.1039/C4PY01087F
18. Menicatti M, Guandalini L, Dei S, et al. The power of energy-resolved tandem mass spectrometry experiments for resolution of isomers: the case of drug plasma stability investigation of multidrug resistance inhibitors. *Rapid Commun Mass Spectrom*. 2016;30(3):423-432. doi:10.1002/rcm.7453
19. Menicatti M, Guandalini L, Dei S, et al. Energy resolved tandem mass spectrometry experiments for resolution of isobaric compounds: a case of cis/trans isomerism. *Eur J Mass Spectrom*. 2016;22(5):235-243. doi:10.1255/ejms.1446
20. Pallecchi M, Lucio L, Braconi L, et al. Isomers recognition in HPLC-MS/MS analysis of human plasma samples by using an ion trap supported by a linear equations-based algorithm. *Int J Mol Sci*. 2023;24(13):11155. doi:10.3390/ijms241311155
21. Menicatti M, Pallecchi M, Bua S, et al. Resolution of co-eluting isomers of anti-inflammatory drugs conjugated to carbonic anhydrase inhibitors from plasma in liquid chromatography by energy-resolved tandem mass spectrometry. *J Enzyme Inhib Med Chem*. 2018;33(1):671-679. doi:10.1080/14756366.2018.1445737
22. Maroto A, Dit Fouque DJ, Memboeuf A. Ion trap MS using high trapping gas pressure enables unequivocal structural analysis of three isobaric compounds in a mixture by using energy-resolved mass spectrometry and the survival yield technique. *J Mass Spectrom*. 2020;55(7):e4478. doi:10.1002/jms.4478
23. Dit Fouque DJ, Maroto A, Memboeuf A. Structural analysis of a compound despite the presence of an isobaric interference by using in-source collision induced dissociation and tandem mass spectrometry. *J Mass Spectrom*. 2021;56(2):e4698. doi:10.1002/jms.4698
24. Maroto A, dit Fouque DJ, Lartia R, Memboeuf A. LC-MS accurate quantification of a tryptic peptide co-eluted with an isobaric interference by using in-source collisional purification. *Anal Bioanal Chem*. 2023;415(29):7211-7221. doi:10.1007/s00216-023-04989-w
25. Dit Fouque DJ, Maroto A, Memboeuf A. Internal standard quantification using tandem mass spectrometry of a tryptic peptide in the presence of an isobaric interference. *Anal Chem*. 2018;90(24):14126-14130. doi:10.1021/acs.analchem.8b05016
26. Download LibreOffice|LibreOffice - Free Office Suite - Based on OpenOffice - Compatible with Microsoft. <https://www.libreoffice.org/download/download-libreoffice/> (accessed 2023-12-03).
27. SciDAVis. <https://scidavis.sourceforge.net/> (accessed 2023-12-03).
28. Dongré AR, Jones JL, Somogyi Á, Wysocki VH. Influence of peptide composition, gas-phase basicity, and chemical modification on fragmentation efficiency: evidence for the mobile proton model. *J Am Chem Soc*. 1996;118(35):8365-8374. doi:10.1021/ja9542193
29. Paizs B, Suhai S. Fragmentation pathways of protonated peptides. *Mass Spectrom Rev*. 2005;24(4):508-548. doi:10.1002/mas.20024
30. Zhang H, Jia H, Gao Z, Xiang Y, Jiang T, Xu W. Parallel pseudo-MRM on the "brick" miniature mass spectrometer for high throughput multi-target screening. *Talanta*. 2023;252:123866. doi:10.1016/j.talanta.2022.123866
31. Xu Z, Jiang T, Xu Q, Zhai Y, Li D, Xu W. Pseudo-multiple reaction monitoring (pseudo-MRM) mode on the "brick" mass spectrometer. *Using the Grid-SWIFT Waveform Anal Chem*. 2019;91(21):13838-13846. doi:10.1021/acs.analchem.9b03315
32. Massart DL, Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J. *Handbook of Chemometrics and Qualimetrics: Part A*. Elsevier; 1997.
33. Krueve A, Rebane R, Kipper K, et al. Tutorial review on validation of liquid chromatography-mass spectrometry methods: part I. *Anal Chim Acta*. 2015;870:29-44. doi:10.1016/j.aca.2015.02.017
34. Evard H, Krueve A, Leito I. Tutorial on estimating the limit of detection using LC-MS analysis, part I: theoretical review. *Anal Chim Acta*. 2016;942:23-39. doi:10.1016/j.aca.2016.08.043

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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