Recent Development in Improving the Precision of Quantitative Analysis for Linear Ion Trap (LIT) and LIT-Orbitrap Tandem Mass Spectrometry

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ABSTRACT

Purpose: Improve the quantitative precision of both LTQ and LTQ-Orbitrap mass spectrometers.

Methods: Utilize the novel quantitative ion trap tandem mass spectrometry.

Results: The precision of quantitative analysis are improved with both continuous and pulsed ion sources.

INTRODUCTION

Recently, a novel method for precise quantitative analysis for linear ion trap tandem mass spectrometry has been developed. Compared to existing conventional methods that require the analyte and corresponding internal standard ions to be injected and analyzed using two successive mass analysis scans, each with its own ion injection event, the new methodology allows the linear ion trap mass spectrometer to perform tandem mass analyses of the analyte and the internal standard, but utilizes one ion injection event. This minimizes the quantitation inaccuracies introduced by ionization fluctuations and leads to significant improvements in the precision of quantitative analyses. In this research study, a series of applications is explored utilizing this novel technology and demonstrates the quantitative improvements for various applications.

MATERIALS AND METHODS

Sample Preparation

Five perfluorooctanoic acid (PFOA) solutions, ranging from 0.02 to 0.4 μM, were prepared in MeOH with spiked internal standard (0.12 μM, $^{13}C_8$ PFOA). Five Glutamic acid(Glu) solutions, ranging from 2 to 40 μM, were prepared in MeOH with spiked internal standard (13 μM, $^{13}C_4{}^{15}N$-Glu).

Methods and Systems

A modified Thermo Scientific™ Velos Pro Ion Trap Mass Spectrometer, Figure 1&2, and Thermo Scientific™ Orbitrap Velos Pro mass spectrometer were used in this study.

Figure 1. Velos Pro Ion Trap Mass Spectrometer

Figure 2. The configuration of the modified Velos Pro linear ion trap mass spectrometer used in this study.

Both continuous and pulsed ion sources were tested. Precursor ions of the analyte and internal standard are simultaneously isolated from the background using a dual-notch (2 amu q=0.86) waveform during ion injection into the linear ion trap, where the isolated precursor ions can then be separately fragmented by collision-induced dissociation and individually analyzed (mode 1 shown in Figure 3). In another method, precursor ions of the analyte and internal standard can be concurrently fragmented by collision-induced-dissociation (CID) or high-energy collisional dissociation (HCD), with product ions of both precursor species being analyzed by the linear ion trap (mode 2 shown in Figure 4) or orbitrap in the hybrid instrument in one scan (mode 3).

Figure 3. Mode 1, the method of quantitative mass analysis using an ion trap mass spectrometer. (Ref 1)

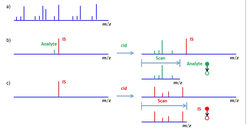
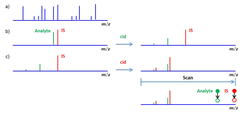


Figure 4. Mode 2, the method of quantitative mass analysis using an ion trap mass spectrometer. (Ref 2)



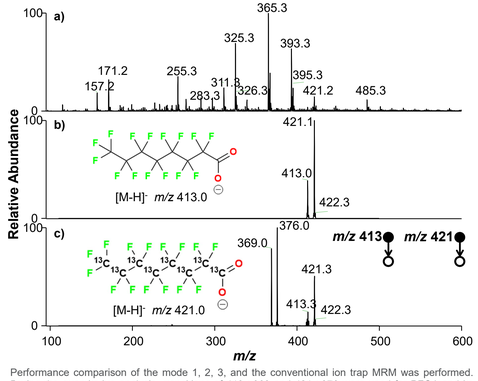
Data Analysis

For mode 1, 2, and 3 described above, the characteristic product ions of the analyte and internal standard were used to construct the calibration curve. The samples were analyzed using both a conventional continuous nanoESI, as well as a home-built pulsed nanoESI source. Data were collected using the same samples when comparing the scan modes.

RESULTS

Continuous Ion Source

Figure 5. a) Full scan of 0.1 μM PFOA sample. Analyte and IS being b) isolated and c) fragmented concurrently.



Performance comparison of the mode 1, 2, 3, and the conventional ion trap MRM was performed. During the quantitative analysis, transitions of 413->369 and 421->376 were used for PFOA and its IS, respectively, when the ions were analyzed by the ion trap. A 5 ppm mass window was used in the peak selection when the ions were analyzed by the orbitrap. The intensities of the selected product ions' peaks were used to calculate the relative intensity ratios of analyte/IS. The results of ten consecutive scans were recorded and used to calculate the relative standard deviation (RSD), as a measure of the precision comparison.

Table 1. The results of 10 consecutive scans of 5 different scan modes with 0.1 μM PFOA (IS: 0.12 μM). The theoretical ratio of PFOA to IS was 0.83.

# scan	Scan type				
	Full Scan (Orbitrap)	Mode 1	Mode 2	Mode 3	MRM
1	0.81	2.8	0.67	0.72	0.88
2	0.81	2.8	0.67	0.79	0.65
3	0.81	3.2	0.70	0.74	0.65
4	0.82	2.7	0.70	0.78	0.86
5	0.67	3.6	0.57	0.82	1.02
6	0.76	3.6	0.70	0.80	0.64
7	0.73	3.1	0.62	0.76	1.11
8	0.68	3.1	0.67	0.65	0.73
9	0.83	2.9	0.63	0.77	0.68
10	0.96	2.8	0.71	0.75	1.15
Mean	0.79	3.0	0.66	0.76	0.84
Std.	0.08	0.3	0.05	0.05	0.20
RSD	11%	11%	7%	6%	24%

Figure 6. The calibration curves, corresponding to each scan method, for the analysis of PFOA in the concentration range of 0.02 to 1 μM. The error bars are standard deviations obtained from 10 consecutive scans at each data point.

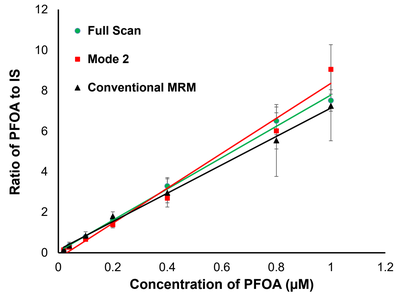


Table 2. Comparison of the RSDs of the mode 2 vs the full scan and conventional MRM scan modes at various concentration points of PFOA.

Scan Type		Concentration of PFOA (μM)						
		0.02	0.04	0.1	0.2	0.40	0.8	1
Full Scan Conventional MRM	RSD	15%	12%	11%	13%	13%	10%	7%
		17%	48%	24%	12%	24%	32%	24%
Mode 2		12%	11%	7%	11%	9%	15%	13%

Figure 7. The calibration curves, corresponding to each scan method, for the analysis of Glu in the concentration range of 2 to 80 μM. The error bars are standard deviations obtained from 10 consecutive scans at each data point.

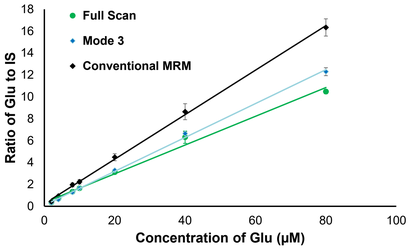


Table 3. Comparison of the RSDs of the mode 3 vs the full scan and conventional MRM scan modes at various concentration points of Glu.

Scan Type		Concentration of Glu (μM)						
		2	4	8	10	20	40	80
Full Scan	RSD	1%	2%	1%	3%	9%	2%	2%
Conventional MRM		8%	9%	9%	7%	8%	5%	7%
Mode 3		3%	2%	1%	2%	2%	3%	3%

Pulsed Ion Source

With the novel ion trap scan mode, the product ion intensities of the internal standard matched the variations of the product ion intensities of the analyte much better than with the conventional MRM mode. This suggested that novel ion trap scan mode would allow more accurate quantitation analysis, especially for pulsed ion sources.

Figure 8. Mode 2 MS analysis of 50 μg/L PFOA and $^{13}C_8$ PFOA (1:1) in MeOH with (pulsed) Relay ESI; Characteristic ions of m/z 369 and m/z 376 were used for PFOA and $^{13}C_8$ PFOA respectively. a) Total ion chromatograph, b) Extracted ion chromatograph of m/z 369, c) Extracted ion chromatograph of m/z 376.

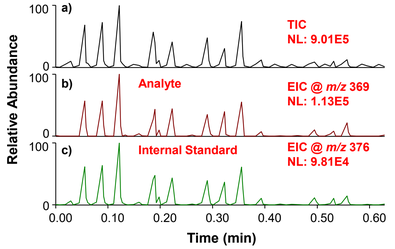


Figure 9. Conventional MRM analysis of 50 μg/L PFOA and $^{13}C_8$ PFOA (1:1) in MeOH with (pulsed) Relay ESI; Characteristic ions of m/z 369 and m/z 376 were used for PFOA and $^{13}C_8$ PFOA respectively. a) Total ion chromatograph, b) Extracted ion chromatograph of m/z 369, c) Extracted ion chromatograph of m/z 376.

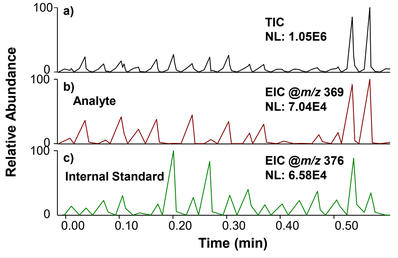


Figure 10. Comparation of the novel scan mode 2 and the conventional MRM method when analyzing 50 μg/L PFOA and $^{13}C_8$ PFOA with pulsed ion source. The theoretical ratio of Analyte/IS was 1.0. The measured ratios were obtained by calculating the intensities of selected peaks; herein characteristic transitions of 413->369 and 421->376 were used for PFOA and its IS, respectively.

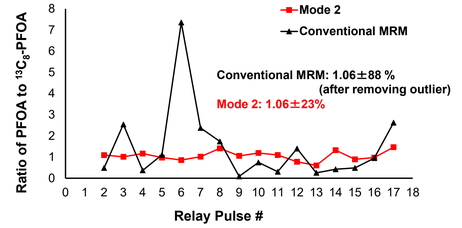
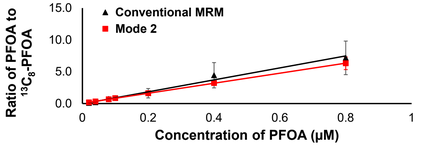


Figure 11. Calibration curves of the conventional MRM method and the novel scan mode 2 when coupled with pulsed ion source. The PFOA concentration range was from 0.02 to 0.8 μM. The error bars are standard deviations calculated with 5 consecutive spectra at each data point.



CONCLUSIONS

- The novel ion trap tandem mass spectrometry improved the precision for quantitative analysis for LIT and LIT-Orbitrap mass spectrometry with both continuous and pulsed ionization sources.

REFERENCES

1. L. Li, Methods and Systems for Quantitative Mass Analysis, US Patent 9911588
2. L. Li, etc. Methods and Systems for Quantitative Mass Analysis, US Patent 9911587

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TRADEMARKS/LICENSING

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