Parallel Reaction Monitoring and Selected Reaction Monitoring Exhibit Comparable Analytical Performance

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ABSTRACT

Purpose

To evaluate the analytical performance of the parallel reaction monitoring (PRM) method on a Thermo Scientific™ benchtop high resolution quadrupole-Orbitrap™ mass spectrometer and draw a performance comparison to the selected reaction monitoring (SRM) method on a triple quadrupole mass spectrometer.

Methods

Linearity range, precision, and accuracy were compared on two applications, epi-vitamin D quantitation in plasma and barbiturate measurement in urine.

Results

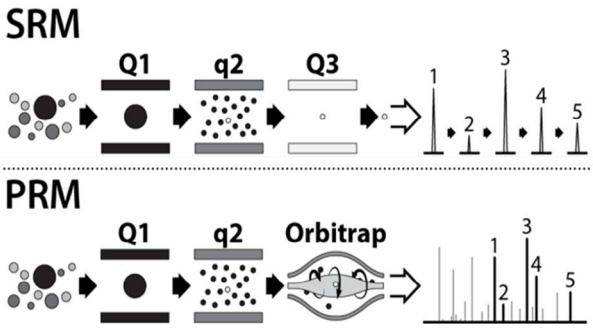
We demonstrated comparable quantitative performance between PRM and SRM across multiple differing parameters including matrix, polarity, and ionization mode.

INTRODUCTION

SRM, performed on triple quadrupole (QqQ) mass spectrometers (MS), has emerged as the MS "gold-standard" for targeted quantification in clinical research and forensic toxicology applications.

With the development of the hybrid quadrupole-Orbitrap MS instruments, an alternative quantitative method is PRM. In PRM, the third quadruple of a triple quadrupole is essentially substituted with a high resolution accurate mass Thermo Scientific™ Orbitrap™ mass analyzer to permit the parallel detection of all target product ions in one, concerted high resolution mass analysis.

Figure 1. Schematic view of SRM and PRM



MATERIALS AND METHODS

Sample Preparation

For the application of epi-vitamin D quantitation (25-OH vitamin D3, epi-25-OH vitamin D3, 25-OH vitamin D2), plasma samples were processed by protein precipitation followed by solid phase extraction.

For the application of barbiturate measurement, urine samples were diluted 20 fold. The five barbiturates are amobarbital, butalbital, phenobarbital, pentobarbital, secobarbital.

After preparation, the same samples were divided equally to two aliquots.

Liquid Chromatography

Identical front ends (autosampler, LC pump, and column heater) were used for both methods. They are Thermo Scientific™ Dionex™ UltiMate™ 3000RS LC system with an OAS autosampler.

For epi-vitamin D application, five minutes LC separation was run on Thermo Scientific™ Hypersil GOLD™ PFP column.

For barbiturates application, the LC column used was a Thermo Scientific™ Accucore™ C18 column. The total run time was six minutes.

Mass Spectrometry

PRM was conducted on a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap mass spectrometer (FIGURE 1).

SRM was conducted on Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer (FIGURE 1).

In PRM, a single precursor ion was selected in the quadrupole with an isolation width of 2.0 m/z and fragmented in the HCD cell using an optimized, compound-specific collision energy. The resulting MS/MS product ion spectrum was detected in the Orbitrap detector at a resolution of 35,000 (FWHM at m/z of 200). In SRM, the cycle time was 0.5 second. Q1 and Q3 resolution was set as 0.7 Da (FWHM).

Figure 2. Two mass spectrometers used in this study.

Q Exactive Focus hybrid quadrupole-Orbitrap MS

TSQ Endura triple quadrupole MS

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RESULTS

For epi-vitamin D analysis, both PRM and SRM were conducted in positive polarity with APCI probes.

For barbiturates analysis, both PRM and SRM were conducted in negative polarity with heated ESI probes.

For the PRM analysis of barbiturates, for each analyte and internal standard (IS), the precursor extract mass in the MS/MS spectrum was used for quantification and the most abundant fragment was used for confirmation. For epi-vitamin D analysis, a specific fragment from the MS/MS spectrum was selected as the quantifying ion. In both cases, the chromatograms were reconstructed with a mass accuracy of 5 ppm for quantification.

In SRM analysis of both sets of compound, two products ions were collected. One was for quantification and another one was for ion ratio confirmation. To ensure confident quantification, at least 15 scans were collected across each peak for both PRM and SRM analysis.

Figure 3. Chromatographic separation of epi-vitamin D (left) and barbiturates (right).

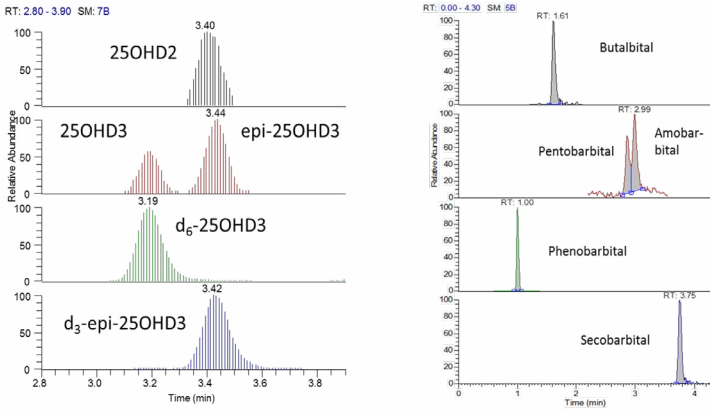
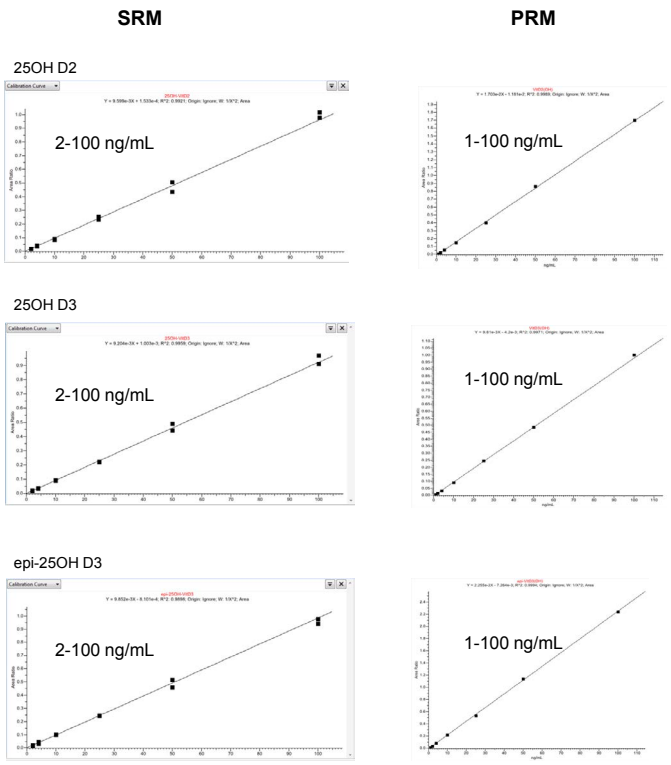


Figure 4. Representative calibration curves



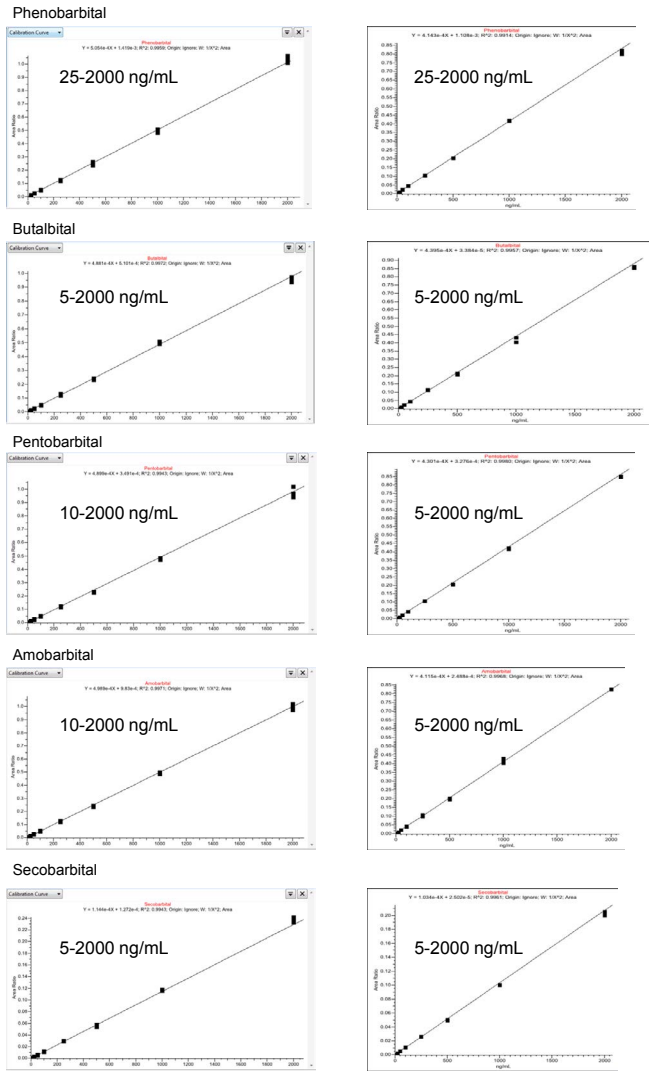


Table 1. Linearity ranges (ng/mL) using either SRM or PRM method.

	25OH D2	25OH D3	epi-25OH D3	Amobarbital	Butalbital	Pentobarbital	Phenobarbital	Secobarbital
PRM	1-100	1-100	1-100	5-2000	5-2000	5-2000	25-2000	5-2000
SRM	2-100	2-100	2-100	10-2000	5-2000	10-2000	25-2000	5-2000

Table 2. Inter-assay precision (% RSD (concentration ng/mL)) for Vitamin D using PRM method (n=15). BQL, below quantitation limit.

QC Level	25OH D2	25OH D3	epi-25OH D3
QC0	BQL	6.4 (8.4)	BQL
QC1	5.3 (6.0)	4.1 (14.4)	4.4 (6.6)
QC2	3.1 (15.0)	3.2 (23.4)	2.9 (15.6)
QC3	4.2 (50.0)	3.5 (58.4)	2.5 (50.6)

TABLE 3. Inter-assay precision (% RSD (concentration ng/mL)) using SRM method (n=15).
BQL, below quantitation limit.

QC Level	25OH D2	25OH D3	epi-25OH D3
QC0	BQL	7.6	BQL
QC1	7.6	8.6	6.9
QC2	6.4	4.8	9.4
QC3	5.2	3.4	6.9

TABLE 4. Inter-assay precision (% RSD) for barbiturates using PRM method (n=15).

QC Level	Amobarbital	Butalbital	Pentobarbital	Phenobarbital	Secobarbital
Low QC (25 ng/mL)	4.6	4.5	6.5	5.5	5.1
Medium QC (100 ng/mL)	9.7	6.1	6.2	5.6	5.7
High QC (1000 ng/mL)	7.0	5.4	6.1	4.1	5.1

TABLE 5. Inter-assay precision (% RSD) for barbiturates using SRM method (n=15).

QC Level	Amobarbital	Butalbital	Pentobarbital	Phenobarbital	Secobarbital
Low QC (25 ng/mL)	4.6	4.5	5.4	8.4	6.3
Medium QC (100 ng/mL)	1.8	2.4	1.8	5.0	3.8
High QC (1000 ng/mL)	1.4	1.9	1.9	2.1	0.9

TABLE 6. Accuracy of PRM method demonstrated with NIST standards. % Recovery (Concentration of NIST standard in ng/mL)

	25OH D2	25OH D3	epi-25OH D3
Level 1	BQL (0.54)	108 (28.8)	103 (1.84)
Level 2	BQL (0.81)	110 (18.1)	90.7 (1.29)
Level 3	97.0 (13.3)	104 (19.8)	114 (1.18)
Level 4	BQL (0.55)	106 (29.4)	90.9 (26.4)

TABLE 7. Accuracy of SRM method demonstrated with NIST standards. % Recovery (Concentration of NIST standard in ng/mL)

	25OH D2	25OH D3	epi-25OH D3
Level 1	BQL (0.54)	93.4 (28.8)	BQL (1.84)
Level 2	BQL (0.81)	102 (18.1)	BQL (1.29)
Level 3	92.7 (13.3)	105 (19.8)	BQL (1.18)
Level 4	BQL (0.55)	98.0 (29.4)	100 (26.4)

CONCLUSIONS

We demonstrated comparable quantitative performance between PRM (Q Exactive Focus quadrupole-Orbitrap MS) and SRM (TSQ Endura triple quadrupole MS) in terms of linearity range, precision, and accuracy.

Q Exactive Focus MS is a versatile instrument. In addition to quantitation, it can also be used for screening, structure elucidation and bulk substance identification in clinical research and forensic toxicology applications.