Comparison of Full Scan High Resolution MS and Triple Quadrupole SRM in Quantitative Bioanalysis

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INTRODUCTION

Triple quadrupole MS has been the workhorse for quantification of drugs and their metabolites in biological samples. Recently, there is a growing interest in the application of full scan high resolution MS (HRMS) to this area. Besides quantitative information, it also has the potential to provide additional information related drug metabolites or biomarkers. Before LC-HRMS can be fully integrated into quantitative bioanalysis, there is a need to build a knowledge base regarding the sensitivity, specificity, linearity, accuracy/precision and assay performance in the quantitation of drugs in biological matrices.

Herein, we present our evaluation and comparison of the performance of HRMS and triple quadrupole MS for the quantification of drugs in plasma.

Can high resolution MS-detection be used in quantitative bioanalysis?

Triple quadrupole MS (SRM)

- Pros
  - High degree of selectivity
  - Good sensitivity
  - Longer duty cycle time
- Cons
  - Time-consuming method development for SRM
  - Sometimes compounds don't fragment or have not a specific fragment
  - Detect only analytes included in SRM method
  - Post acquisition data mining is not possible if the transitions are not present in the method file.

High resolution full scan MS detection

- Pros
  - High degree of mass resolving power
  - High degree of mass accuracy (<5 ppm)
  - Generic method for all analytes
  - Retrospective data analysis to look for metabolites, biomarkers, and endogenous components without sample re-injection
- Cons
  - Needs evaluation on method selectivity, sensitivity, linear dynamic range, ruggedness
  - Significant increase in data storage and processing needs

METHOD

Sample Preparation

• Twenty-eight model drug compounds, proprietary and marketed, including buspirone, nefazodone, prednisolone and reserpine were used. These compounds were selected due to their diverse physico-chemical properties.

• Human plasma samples was precipitated with acetonitrile, followed by spiking with neat solutions of these compounds. The resulting extracts were injected onto LC-MS systems. For compounds for which stable-isotope labeled (SIL) analogs were available, the SIL analogs were used as internal standards. For other compounds, structure analogs were used as IS.

• Calibration curves range: 0.2 to 1000 ng/mL. Samples were analyzed in six replicates for SRM and seven replicates for HRMS method

LC and Mass Spectrometry Conditions

LC conditions

- Column: Waters Acquity UPLC HSS T3, 1.8 µm, 2.1 x 50 mm
- Mobile phases: (A) – 0.005% formic acid in water
  (B) – acetonitrile
- Gradient elution:
  0 - 2 min from 20%B to 95%B; 2 - 2.8 min 95%B; 2.8 – 2.9 min from 95%B to 20%B
  - Flow rate: 0.5 mL/min; Column temperature: 40 ºC;
  - Injection volume: 5 µL

HRMS (TOF)

- Agilent QTOF 6530 equipped with ESI source.
- Agilent 1200 UHPLC system
- Resolution setting = 10,000
- Scan range: *m/z* 100 - *m/z* 1600, in positive or negative ESI mode
- Samples were analyzed using one MS method

SRM (triple quadrupole)

- Thermo-Fisher TSQ Quantum with ESI source
- Accela UHPLC system
- Unit resolutions for Q1 and Q3
- Dwell time was 25 ms for each transition
- CE of each compound was optimized
- Samples were analyzed using three separate MS methods in order to achieve sufficient data points across a chromatographic peak

Evaluation Criteria

• **LLOQ determination:**
  - ✓ Analyte signal-to-background ratio > 5
  - ✓ %Dev < 20% for at least 3 replicates
  - ✓ %CV < 20% for at least 3 replicates
  - ✓ Peak responses showed proportional increase with concentrations

• **Linearity determination:**
  - ✓ Regression model: Linear with 1/x2 weighing
  - ✓ %Dev < 15% for all levels except LLOQ

Method Development

Table 1. Summary of lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) of the model compounds from human plasma obtained using HRMS (Agilent QTOF) and SRM (Thermo-Quantum).

				LLOQ	LLOQ	ULOQ	ULOQ
	Elemental Composition	[M+H]+	SRM	HRMS	SRM	HRMS	SRM
Compound 1	C29H28Cl2F2N2O4S	609.1188	609>591	0.2	0.2	500	1000
Compound 2	C27H24FN5O3S	518.1657	518>347	2.0	0.2	1000	1000
Compound 3	C23H27Cl2N3O2	448.1553	448>285	1.0	0.5	1000	1000
Compound 4	C23H27Cl2N3O3	464.1502	464>301	2.0	0.5	1000	1000
Compound 5	C23H25Cl2N3O2	446.1397	446>285	2.0	0.5	1000	500
Compound 6	C19H19FN4O3	371.1514	371>313	1.0	0.5	1000	1000
Compound 7	C18H24N6O	341.2084	341>285	2.0	0.5	1000	1000
Compound 8	C19H21F2N5O3	406.1685	406>240	0.5	0.5	1000	1000
Compound 9	C18H19F2N5O3	392.1529	392>240	2.0	0.5	1000	1000
Compound 10	C18H18ClN3O	328.1211	328>118	0.5	0.5	500	1000
Compound 11	C21H25NO3	340.1907	340>225	1.0	0.5	1000	1000
Compound 12	C21H27NO3	342.2064	342>227	1.0	0.5	1000	1000
Compound 13	C28H29ClN2O3S	509.1660	509>260	1.0	0.5	500	1000
Compound 14	C28H26FN5O3S	532.1813	532>361	5.0	0.5	1000	1000
Compound 15	C10H12Cl2N2	231.0450	231>188	1.0	1.0	1000	1000
Compound 16	C36H45N5O5S	660.3214	660>535	0.2	1.0	1000	1000
Compound 17	C28H26Cl2F2N2O4S	595.1031	595>497	10.0	2.0	1000	500
Compound 18	C24H19ClN2O4S	467.0827	467>383	1.0	2.0	1000	1000
Compound 19	C24H21ClN2O4S	469.0983	469>385	5.0	20.0	1000	1000
Compound 20	C40H50N8O6	739.3926	739>565	5.0	50.0	1000	1000
Compound 21	C35H43N5O5S	646.3058	646>553	1.0	20.0	1000	1000
Compound 22	C37H46N4O5S	659.3262	659>536	2.0	10.0	500	1000
Compound 23	C35H46ClN5O9S	748.2778	748>648	1.0	ND	1000	ND
Prednisolone	C21H28O5	361.2010	361>147	10.0	5.0	1000	1000
Prednisone	C21H26O5	359.1853	359>147	5.0	5.0	1000	1000
Nefazodone	C25H32ClN5O2	470.2317	470>274	0.5	0.2	1000	1000
Buspirone	C21H31N5O2	386.2551	386>122	0.2	0.2	1000	1000
Reserpine	C33H40N2O9	609.2807	609>195	0.5	0.5	1000	1000

ND – Not Determined

Table 2. Sensitivity comparison of HRMS and SRM in quantification of model compounds

LLOQ	Percentage of compounds achieving specified LLOQ	
(ng/mL)	HRMS*	SRM**
1	57%	70%
2	79%	78%
5	93%	85%
10	100%	89%

*N = 28; **N = 27

Table 3. Accuracy and precision of Buspirone standard curves

SRM method Quantum				HRMS method QTOF 6530			
STD (ng/mL)	Mean Conc (ng/mL)	Precision %CV	Accuracy (%)	STD (ng/mL)	Mean Conc (ng/mL)	Precision %CV	Accuracy (%)
0.2	0.20	8.3	98.3	0.2	0.20	15.5	99.3
0.5	0.52	2.8	104.5	0.5	0.53	6.6	105.7
1	1.01	4.8	101.1	1	0.98	3.1	98.2
2	1.96	3.1	98.1	2	1.79	3.1	89.5
5	4.88	1.9	97.5	5	4.90	1.7	97.9
10	9.79	2.9	97.9	10	9.82	2.0	98.2
20	19.67	2.0	98.4	20	18.41	1.8	92.1
50	49.38	1.4	98.8	50	52.47	1.8	104.9
100	98.48	2.2	98.5	100	109.40	2.1	109.4
200	210.04	2.3	105.0	200	201.61	2.7	100.8
500	519.96	2.1	104.0	500	524.27	1.3	104.9
1000	979.03	1.1	97.9	1000	991.70	2.4	99.2
Linear dynamic range of 0.20 -1000; N = 6				Linear dynamic range of 0.20 - 1000; N = 7.			

Table 4. Mass Accuracy of Buspirone at different concentrations (Exact mass of Buspirone: *m/z* 386.2551)

STD (ng/mL)	Mean Peak Area Ratio	%CV Peak Area	Measured *m/z*	Mass Accuracy (ppm)
0.2	0.0103	13.9	386.2558	1.89
0.5	0.0245	6.1	386.2544	-1.85
1	0.0441	19.3	386.2540	-2.77
2	0.0789	3.4	386.2542	-2.40
5	0.2128	1.2	386.2543	-2.18
10	0.4248	2.0	386.2545	-1.48
20	0.7951	0.9	386.2548	-0.85
50	2.2627	0.5	386.2551	-0.11
100	4.7155	1.1	386.2548	-0.78
200	8.6885	0.5	386.2538	-3.25
500	22.5915	0.6	386.2536	-3.77
1000	42.7322	0.3	386.2538	-3.25

N = 7

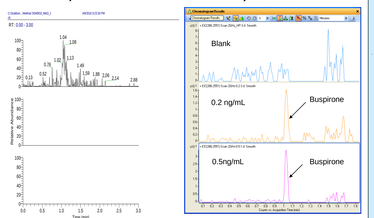

Figure 1. LC-MS and LC-MS/MS chromatograms of buspirone a) SRM of *m/z* 386>122 obtained using Quantum-SRM, and b) EIC of *m/z* 386.2551 obtained using QTOF-MS.

RESULTS AND DISCUSSION

➢ **Comparison of LLOQ's and linear dynamic ranges**

– Table 1 summarizes the results on LLOQ and dynamic range of the 28 model compounds. In general, the two techniques yielded comparable results in terms of LLOQ and dynamic range, with triple quadrupole MS being slightly more sensitive and wider dynamic range for some selected compounds.

– Using HRMS, 57% of the compounds achieved 1 ng/mL; 75% achieved 2 ng/mL; 93% achieved 5 ng/mL; 100% achieved 10 ng/mL (Table 2).

– HRMS provides a good dynamic range of 500-5000 for at least 75% of the tested model compounds (Table 1).

➢ **Comparison of assay performance**

– Table 3 shows the results of accuracy and precision for quantitation of buspirone in human plasma standard curve extracts. HRMS achieved an excellent linear dynamic range of 0.2 - 1000 ng/mL. The same LLOQ and linear calibration curve were established in both HRMS and SRM methods (Table 3 and Figure 1).

– HRMS gave excellent mass accuracy over the calibration range for buspirone (Table 4).

➢ **Post-acquisition data-mining**

– Since data from a wide range of m/z's are acquired by HRMS (instead of selected SRM), the data can be mined post-acquisition to obtain additional information. This is illustrated with phospholipids elution profile (Figure 2) that is used to verify chromatographic resolution.

➢ **Additional advantages of HRMS**

– Moreover, multiple analytes can be analyzed by HRMS in one single run, while triple-quadrupole SRM is limited by the dwell time and peak width of the chromatographic peak. In addition, no MS method development (e.g. CE, SRM transitions) is required for HRMS which simplifies the method development.

CONCLUSIONS

- Based on the results from the 28 model compounds evaluated, QTOF HRMS provides comparable results with those of SRM using triple quadrupole MS for the quantification of drugs in plasma extract.
- HRMS achieved excellent selectivity, accuracy/precision, mass accuracy and ruggedness.
- One generic LC-HRMS method was used to analyze all of the compounds in a single injection, while optimization of parameters (e.g., CE, SRM transitions) was needed for SRM.
- HRMS can be used for the quantification of target analytes. It also allows for post-acquisition data mining to obtain additional information in the sample, such as phospholipids, metabolites, biomarkers in a single injection.

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