Application of TOF MS Instrument in Bioanalysis – A case study

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ABSTRACT

Multiple reaction monitoring (MRM) method, with high sensitivity and selectivity on a triple quadrupole (QQQ) tandem mass spectrometry coupled with high performance liquid chromatography (LC-MS/MS) has been adopted as the most popular tool in bioanalysis field in the past two decades. However, it only allows the scientist see what he/she wants to see, the targeted analytes. The high resolution and mass accuracy on a Time-of-flight (TOF) MS could achieve high signal-to-noise ratio (S/N) by filtering out interference that is caused by isobaric compounds in the sample matrix. Besides targeted analytes signals, the rich TOF MS data can provide more information on matrix background and interference. This versatility would enable user to develop better method and provide a trouble-shooting tool. In this case study, we demonstrated the versatility of TOF data with the newly discovered interferences in Allopurinol assay.

INTRODUCTION

Quite often, in bioanalysis method development, what a user cannot see will affect the method's sensitivity (or S/N) and selectivity. These unknown factors will show up in the assay as interference that could render the method less sensitive and/or less selective. Allopurinol assay was established on a QQQ instrument previously. One interfering peak is present in the MRM transition of the analyte. The goals were to achieve comparable quantitation results on a TOF instrument as that of QQQ MS, and to characterize the interference peaks.

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MATERIALS AND METHODS

Sample Preparation:

Human plasma samples were homogenated with sodium phosphate dibasic buffer and internal standard (Allopurinol-$^{13}C,^{15}N_2$) and immediately processed by liquid-Liquid extraction with ethyl acetate. After the organic supernatant was separated and evaporated, the extracted residue was reconstituted with mobile phase and introduced into an LC/MS/MS system.

HPLC Conditions:

A Shimadzu Prominence UFLC system with a Phenomenex Kinetex XB-C18, 50x4.6mm, 5 µm column with a gradient of eluent A 2 mM ammonium acetate in water and eluent B methanol was used at a flow rate of 400 µL/min. The injection volume was set to 10 µL.

QQQ MS/MS Conditions:

QQQ MS/MS detection was performed on a SCIEX API 5000™ system with a Turbo V™ ion source and electrospray ionization (ESI) mode. The data was analyzed using Analyst® 1.6.2 software and the intelliQuan integration algorithm.

MRM transitions:

Allopurinol : 135.0 → 92.0

Allopurinol-$^{13}C,^{15}N_2$: 138.0 → 95.0

TOF MS/MS Conditions:

A SCIEX TripleTOF® 5600+ system with DuoSpray™ source and Electrospray Ionization (ESI) probe was used. Besides a general TOF Scan in the range of 70-300 amu, two product ion scans were also in the method with parent ions being Allopurinol and its labeled ^{13}C and ^{15}N internal standard (Allopurinol-$^{13}C,^{15}N_2$).

Results

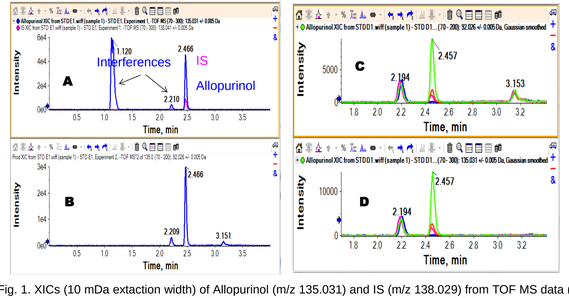

Fig. 1. XICs (10 mDa extaction width) of Allopurinol (m/z 135.031) and IS (m/z 138.029) from TOF MS data (A), and production ion m/z 92 from MS/MS data (B). XICs at various levels of blank, LLOQ, and standards are shown in C (MS/MS) and D (MS). Allopurinol elutes at 2.46 min. Peaks at other retention times are interferences. The one at 2.2 min & 3.51 min were the ones observed on QQQ instrument in MRM data.

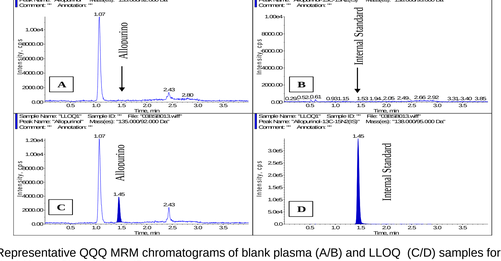

Fig. 2.Representative QQQ MRM chromatograms of blank plasma (A/B) and LLOQ (C/D) samples for Allopurinol (A and C) and its IS (B and D)

Table 1. Accuracy and Precision of Allopurinol from TOF MS Stds

Row	Compone...	Actual C...	Num. Va...	Mean	Standar...	Percent CV	Accuracy
1	093	10.00	2 of 2	1.022e1	9.239e-1	9.06	102.01
2	093	20.00	2 of 2	2.052e1	9.214e-2	0.45	102.58
3	093	100.00	2 of 2	9.744e1	4.166e0	4.27	97.44
4	093	300.00	2 of 2	2.950e2	8.826e-1	0.30	98.33
5	093	1000.00	2 of 2	9.765e2	1.991e-1	0.02	97.65
6	093	2000.00	2 of 2	2.051e3	1.379e1	0.67	102.56
7	093	3000.00	2 of 2	2.994e3	1.564e1	0.52	99.79
8	093	4000.00	2 of 2	3.985e3	9.567e1	2.40	99.64

QCs

Row	Compone...	Actual C...	Num. Va...	Mean	Standar...	Percent CV	Accuracy
1	093	10.00	6 of 6	1.032e1	5.671e-1	5.49	103.24
2	093	30.00	6 of 6	2.983e1	6.946e-1	2.33	99.43
3	093	1600.00	6 of 6	1.576e3	1.558e1	0.99	98.53
4	093	3000.00	6 of 6	3.182e3	3.421e1	1.08	106.08

Table 2. Accuracy and Precision of Allopurinol from TOF MS/MS (MRM^{HR}) Stds

Row	Compone...	Actual C...	Num. Va...	Mean	Standar...	Percent CV	Accuracy
1	135->92	10.00	2 of 2	9.940e0	2.090e-1	2.10	99.40
2	135->92	20.00	2 of 2	2.040e1	6.591e-1	3.23	101.98
3	135->92	100.00	2 of 2	1.000e2	1.068e0	1.07	100.05
4	135->92	300.00	2 of 2	3.003e2	9.874e0	3.29	100.11
5	135->92	1000.00	2 of 2	9.693e2	3.127e0	0.32	96.93
6	135->92	2000.00	2 of 2	2.025e3	7.683e1	3.79	101.27
7	135->92	3000.00	2 of 2	3.024e3	2.951e1	0.98	100.79
8	135->92	4000.00	2 of 2	3.979e3	4.295e1	1.08	99.47

QCs

Row	Compone...	Actual C...	Num. Va...	Mean	Standar...	Percent CV	Accuracy
1	135->92	10.00	6 of 6	9.210e0	4.009e-1	4.35	92.10
2	135->92	30.00	6 of 6	2.776e1	1.685e0	6.07	92.53
3	135->92	1600.00	6 of 6	1.567e3	3.344e1	2.14	97.59
4	135->92	3000.00	6 of 6	3.185e3	1.102e2	3.46	106.15

Fig. 3. Calibration curve of Allopurinol from TOF MS (A) and MS/MS (B)

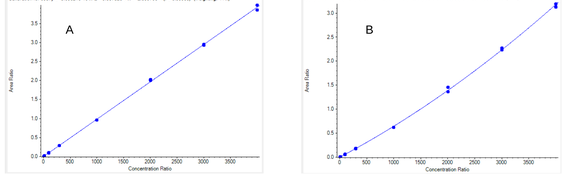

Fig. 4. Calibration curve of Allopurinol from QQQ MS/MS

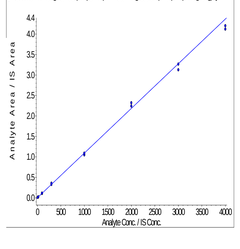

Table 3. Accuracy and Precision of Allopurinol from QQQ MS/MS (MRM)

Sample Name	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Bias	Sample Name	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Bias
LLOQ1	10.00	9.40	-6.10	QC B1	1600.00	1510.70	-5.60
LLOQ2	10.00	10.30	3.50	QC B2	1600.00	1611.60	0.70
LLOQ3	10.00	9.40	-6.00	QC B3	1600.00	1617.90	1.10
LLOQ4	10.00	10.40	4.30	QC B4	1600.00	1608.10	0.50
LLOQ5	10.00	10.40	3.90	QC B5	1600.00	1574.40	-1.60
LLOQ6	10.00	10.10	0.90	QC B6	1600.00	1583.00	-1.10
QC A1	30.00	29.60	-1.20	QC C1	3200.00	3011.60	-5.90
QC A2	30.00	30.70	2.50	QC C2	3200.00	3120.80	-2.50
QC A3	30.00	30.10	0.40	QC C3	3200.00	3102.50	-3.00
QC A4	30.00	31.10	3.60	QC C4	3200.00	3132.70	-2.10
QC A5	30.00	31.10	3.60	QC C5	3200.00	3083.30	-3.60
QC A6	30.00	31.60	5.40	QC C6	3200.00	3197.30	-0.10

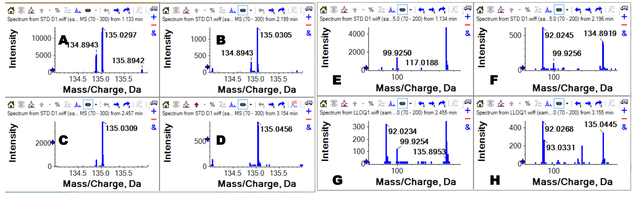

Fig. 5. MS & MS/MS spectra of Allopurinol (C&G) at t$_r$ 2.46 min and interferences at t$_r$ 1.12 (A&E), 2.19 (B&F), and 3.15 (D&H) min. Interference @ 1.12 min has isobaric ion signal, but is eliminated from MS/MS spectrum (no 92.023 production ion signal); Interference @ 2.20 has signal contributions in both MS and MS/MS spectrum; Interference at 3.15 min has MS/MS signal contribution, but in MS when extraction width is narrow enough. These data showed that specificity of analyte signal can be achieved at both MS and MS/MS levels.

Fig. 6. In the general background there is also contribution of 135.0 → 92.026 as shown on left (Top, full scan MS of general background; MS/MS of m/z 135). This could explain the concave shape of the MRM^{HR} calibration curve due to the increasing significance of the background contribution.

The above quantitative and qualitative information from HR TOF data demonstrated the versatility of TOF instrument comparing to a nominal resolution instrument.

CONCLUSIONS

In this case study, we have demonstrated that HR TOF MS instrument is able to conduct bioanalysis assay with matching precision, accuracy, and linearity to a nominal resolution triple-quad MS. User has a choice of using either MS or MS/MS data to quantify the analyte. TOF data (MS and MS/MS) will provide additional specificity in addition to the specificity from high resolution. Beyond the quantitation capability, and unlike MRM data, TOF data would also provide more information during method development phase, as well as in routine sample analysis production phase, for users to trouble-shoot unexpected things that could interfere the method and/or the results, and go beyond the phenomenon to find out the root causes.

TRADEMARKS/LICENSING

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