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Quantitation by High Resolution Mass Spectrometry: Case Study of TOF MS for the Quantitation of Allopurinol from Human Plasma

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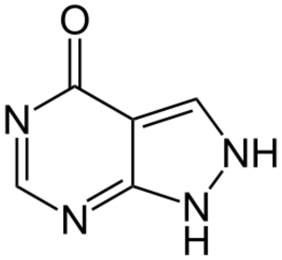
Introduction

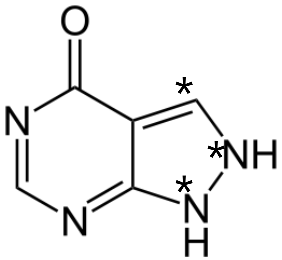
- Multiple reaction monitoring (MRM) methods has been the most popular tool in bioanalysis for the past two decades.
- Isobaric interferences present in complex matrices can be a challenge when they elute at or near the retention time of the analyte of interest in MRM chromatograms.
- The high resolution and mass accuracy on a Time-of-flight (TOF) MS provides better selectivity and can achieve higher signal-to-noise ratios (S/N) by filtering out interferences caused by isobaric compounds in the sample matrix.
- In addition to the targeted analyte signals, the rich TOF MS data can provide more information on matrix background, metabolites and interferences.
- This versatility provides users a valuable trouble-shooting tool and enables users to develop better methods

Allopurinol Assay

- In this study, we demonstrate the versatility of TOF data acquisition using the SCIEX TripleTOF® 5600+ system in an assay of allopurinol
- Allopurinol is a medication used primarily to treat excess uric acid in the blood and its complications, including chronic gout. It is a xanthine oxidase inhibitor and is administered orally.
- An allopurinol assay was previously established on a SCIEX API 5000^{TM} system and 2 isobaric peaks were present in the MRM chromatogram of the analyte



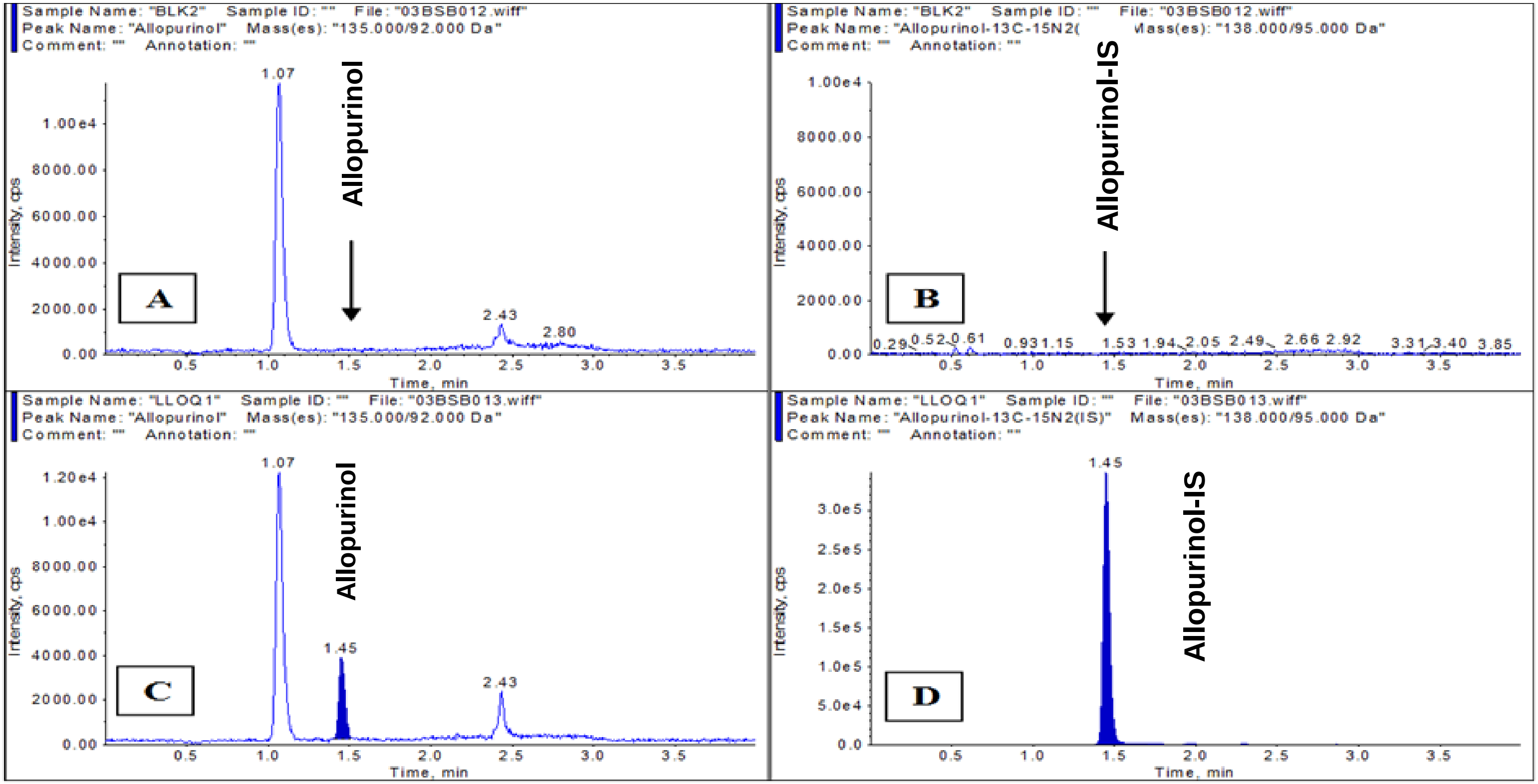
Allopurinol



Allopurinol-$^{13}C,^{15}N_2$

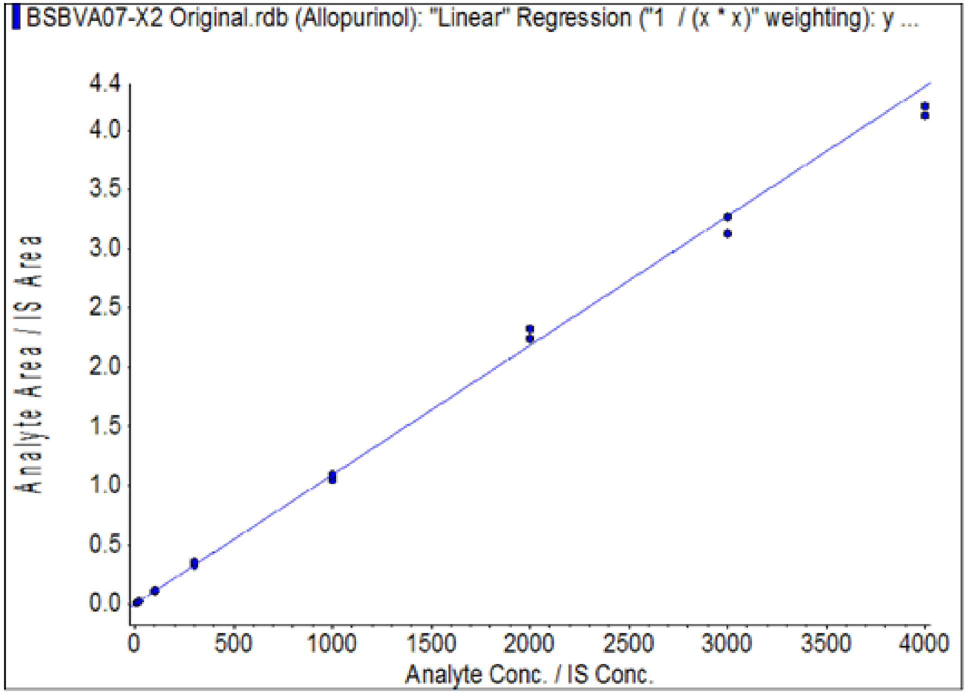
Allopurinol MRM Chromatograms

Representative MRM chromatograms of blank plasma (A/B) and LLOQ (C/D) samples for Allopurinol 1.45 min (A and C) and its internal standard (B and D) 1.45 min.



- Isobaric endogenous compounds seen at 1.07 min and 2.43 min in the Allopurinol MRM chromatogram

Calibration Curve and Statistics of MRM Quantitation



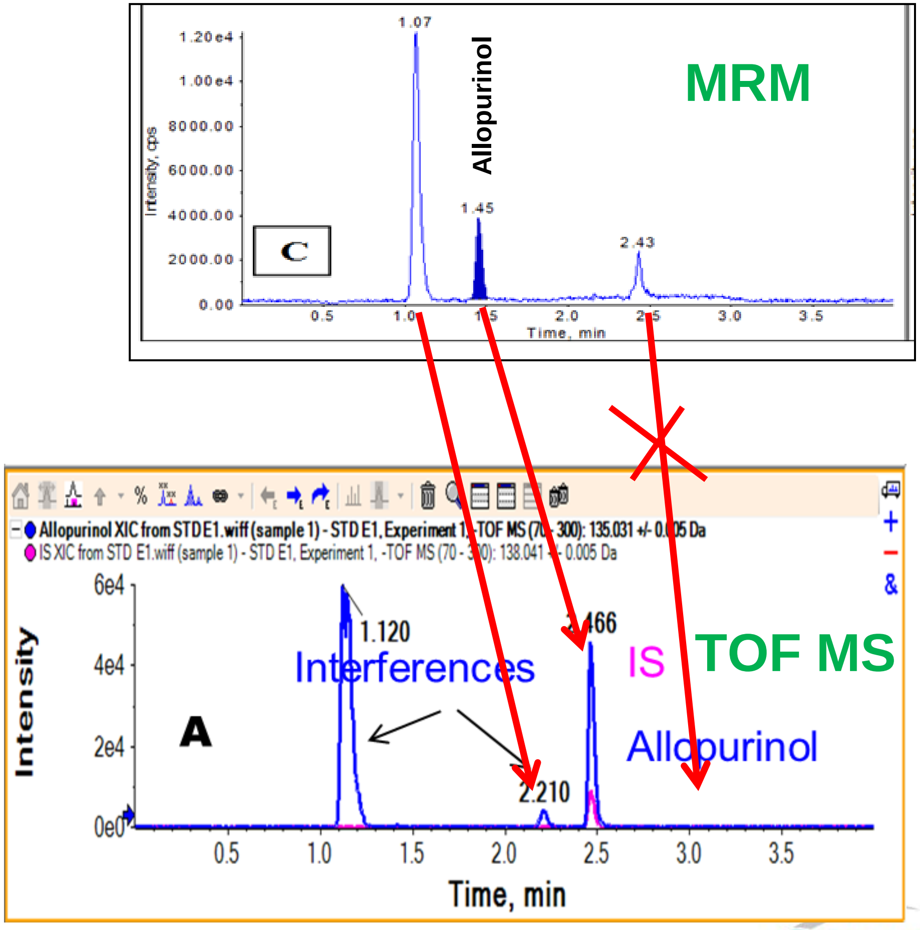
- Calibration curve linear
- Quantitation range was 10 to 3200 ng/mL
- QC Statistics are shown below

Sample Name	Actual Concentration	Mean Calculated Concentration (ng/mL)	%CV	Accuracy
LLOQ	10.0	10.0	4.77%	100.00%
QC A	30.0	30.7	2.39%	102.33%
QC B	1600	1584	2.52%	99.02%
QC C	3200	3108	1.97%	97.13%

Allopurinol TOF MS Chromatograms

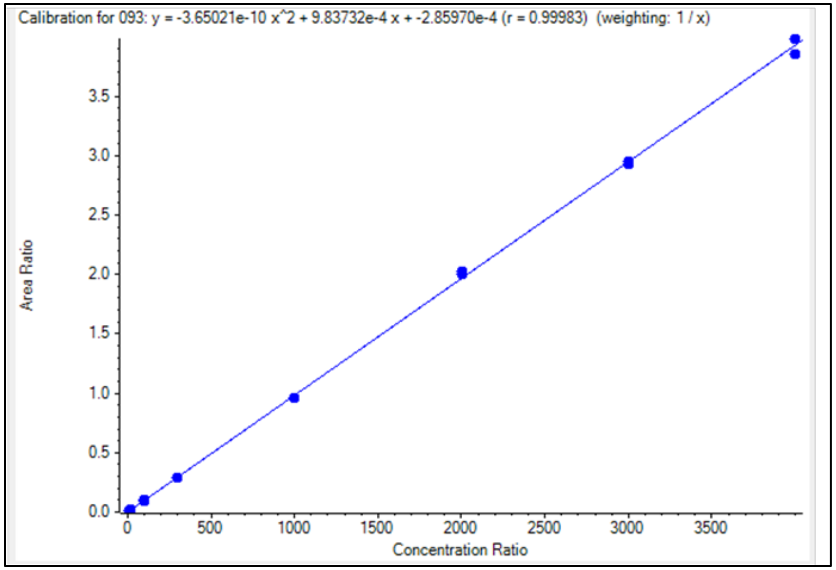
- A different LC system was used for analysis on the SCIEX TripleTOF® 5600+ system and retention times are shifted
- The peaks in the TOF MS chromatogram (A) were generated from an XIC (10 mDa extraction width) of the parent ion m/z 135.031 and IS 138.029.

- Allopurinol elutes at 2.47 min (1.45 min in MRM) and the isobaric compounds at 1.45 min in the MRM at 2.21 min in the TOF MS chromatogram
- The isobaric compound at 2.43 min in the MRM chromatogram is not present in the TOF MS chromatogram
- A new compound at 1.12 min is revealed in the TOF MS XIC

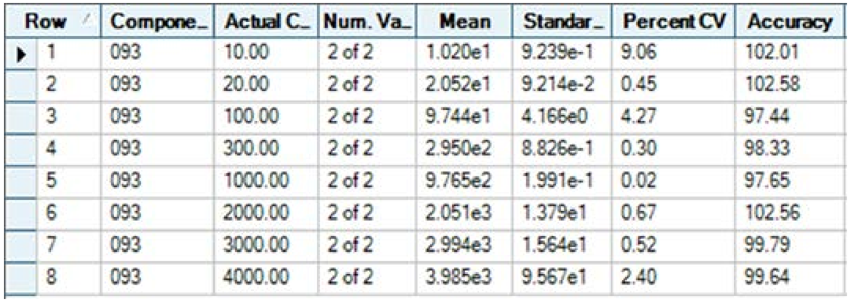


Allopurinol TOF MS Quantitation

- A calibration curve and QC samples were run on the SCIEX TripleTOF® 5600+ system and the TOF MS XIC of Allopurinol and Allopurinol-IS were used to build the calibration curve

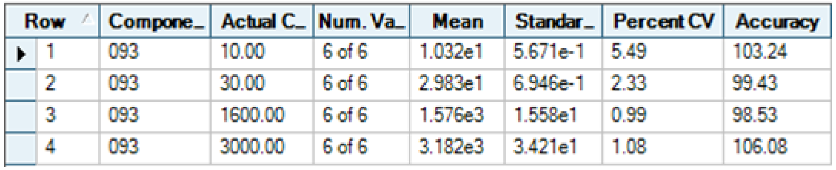


Calibration Standard Statistics



Row	Compone…	Actual C…	Num. Va…	Mean	Standar…	Percent CV	Accuracy
▶ 1	093	10.00	2 of 2	1.020e1	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

QC Sample Statistics



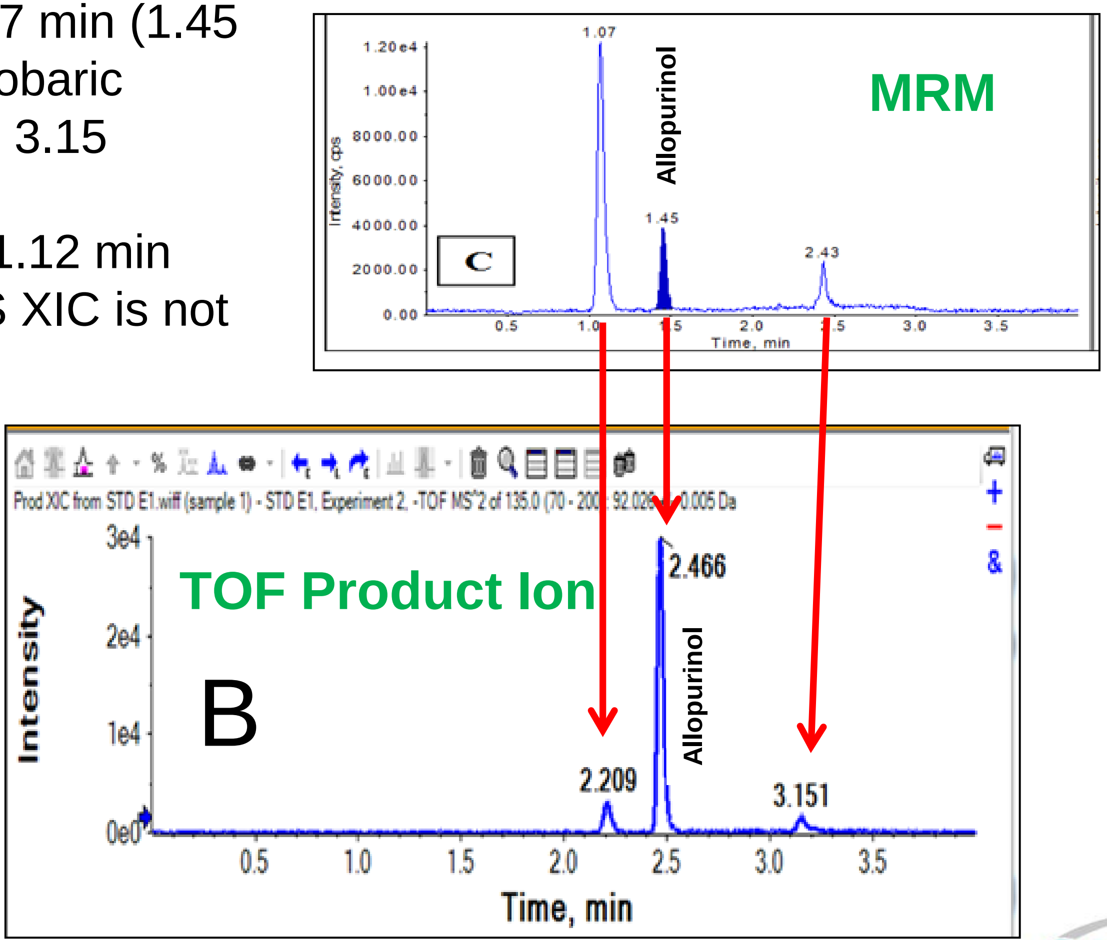
Row	Compone…	Actual C…	Num. Va…	Mean	Standar…	Percent CV	Accuracy
▶ 1	093	10.00	6 of 6	1.032e1	5.671e-1	5.49	103.24
2	093	30.00	6 of 6	2.983e1	6.946e-1	2.33	99.43
3	093	1600.00	6 of 6	1.576e3	1.558e1	0.99	98.53
4	093	3000.00	6 of 6	3.182e3	3.421e1	1.08	106.08

- Quantitation using TOF MS gave matching precision, accuracy, and linearity to the nominal resolution triple-quad MS.

Allopurinol TOF Product Ion Chromatograms (MRM^{HR})

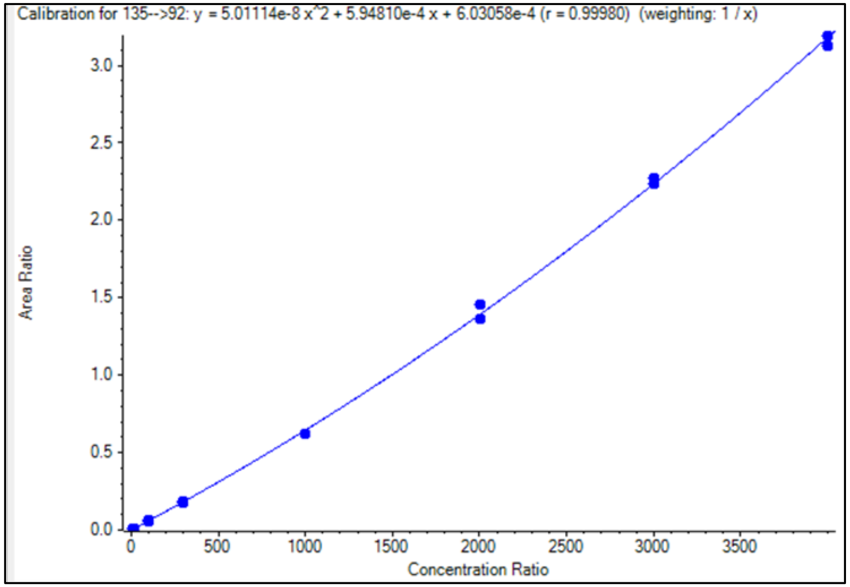
- The peaks in the TOF product ion (MRM^{HR}) chromatogram (B) were generated from an XIC (10 mDa extraction width) of the product ion m/z 92.026

- Allopurinol elutes at 2.47 min (1.45 min in MRM) and the isobaric compounds at 2.21 and 3.15 minutes
- The new compound at 1.12 min revealed in the TOF MS XIC is not present in the MRM^{HR} chromatogram

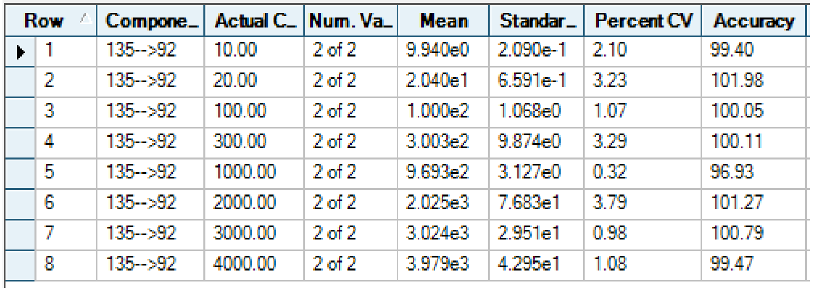


Allopurinol MRM^{HR} Quantitation

- The TOF Product Ion XIC (MRM^{HR}) of Allopurinol 92.026 and Allopurinol-IS were used to build the calibration curve

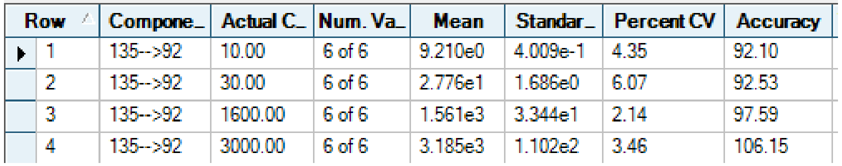


Calibration Standard Statistics



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

QC Sample Statistics

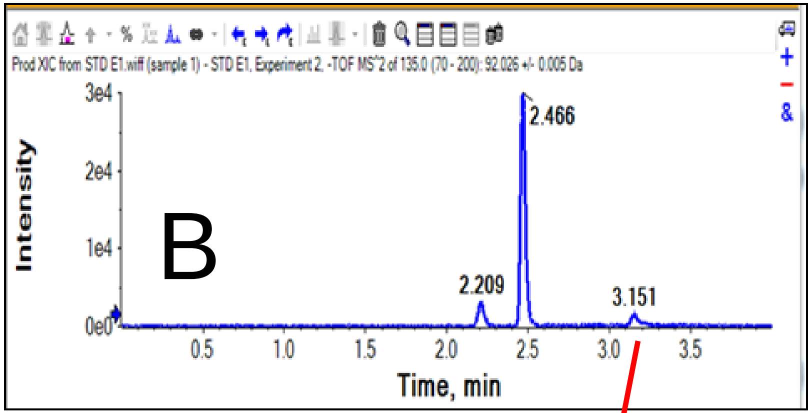


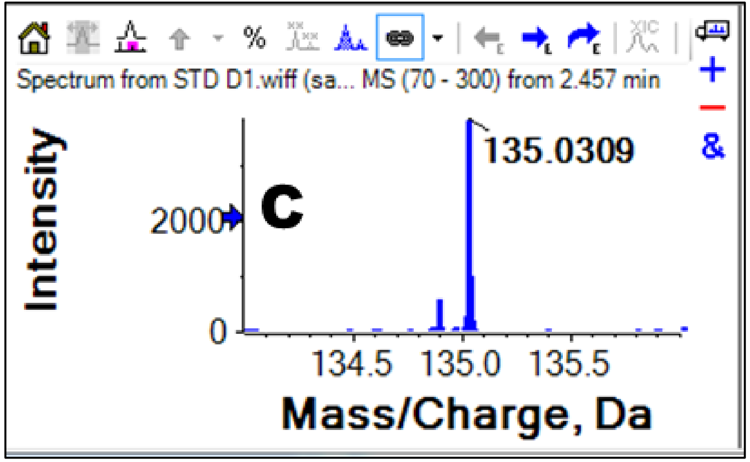
Row	Compone…	Actual C…	Num. Va…	Mean	Standar…	Percent CV	Accuracy
1	135-->92	10.00	6 of 6	9.210e0	4.009e-1	4.35	92.10
2	135-->92	30.00	6 of 6	2.776e1	1.686e0	6.07	92.53
3	135-->92	1600.00	6 of 6	1.561e3	3.344e1	2.14	97.59
4	135-->92	3000.00	6 of 6	3.185e3	1.102e2	3.46	106.15

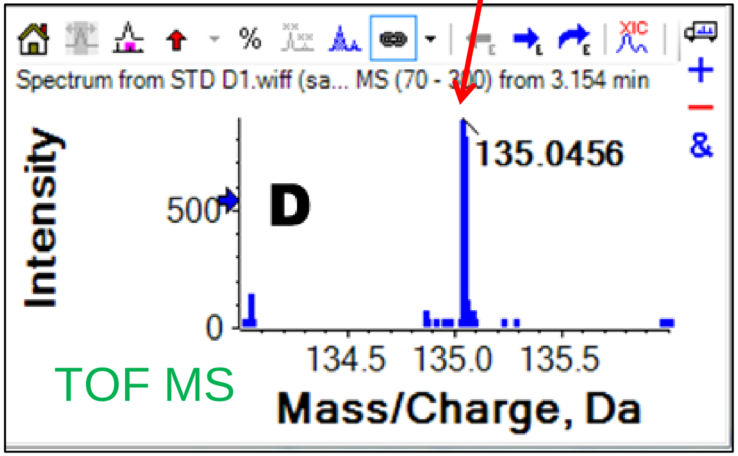
- The calibration curve for quantitation using TOF Product Ion XIC (MRM^{HR}) was concave in shape and required a polynomial fit
- The polynomial fit gave acceptable precision and accuracy to the TOF MS and nominal resolution triple-quad MS.

TOF MS Spectra of Allopurinol

- The 3.15 min isobaric peak (B) was eliminated from the TOFMS XIC due to its molecular ion (135.0456) larger-than ±0.005 Da (D)
- The Allopurinol molecular ion has an m/z of 135.0309 (C).



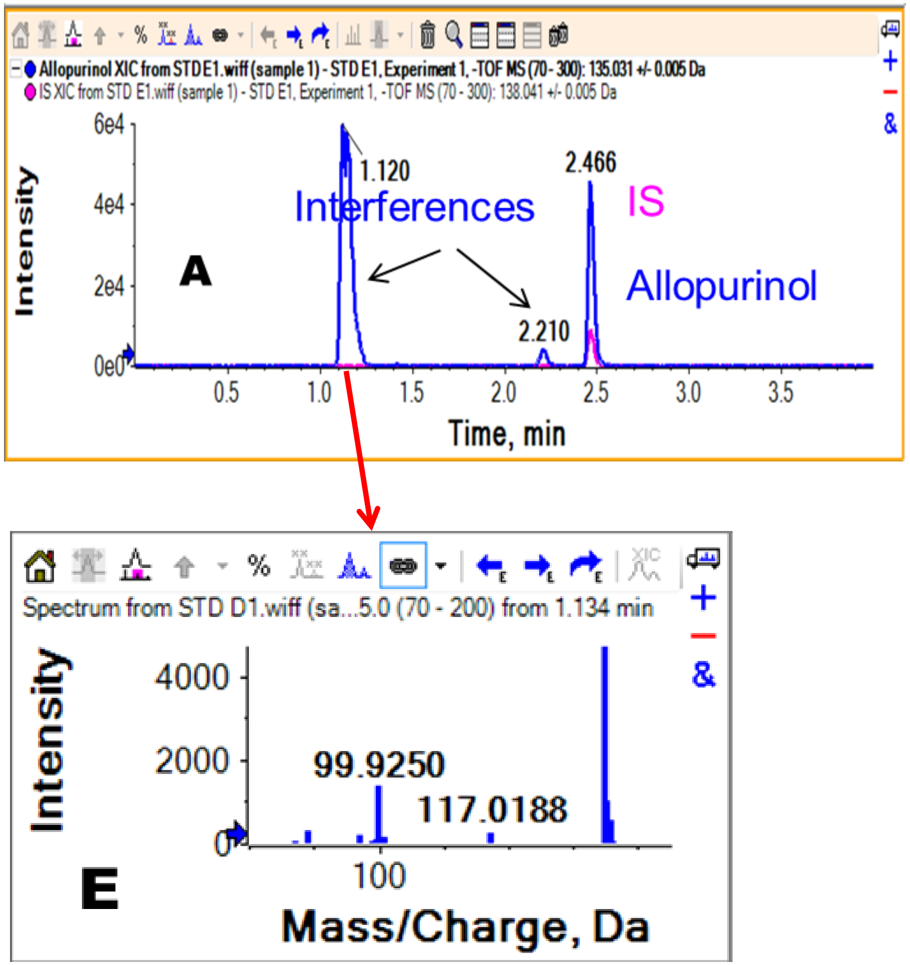




Allopurinol TOF MS

TOF Product Ion Spectra of Allopurinol

- The compound at 1.12 min revealed in the TOF MS XIC (A) is not present in the MRM^{HR} chromatogram because there is no product ion signal at 92.023 (E).



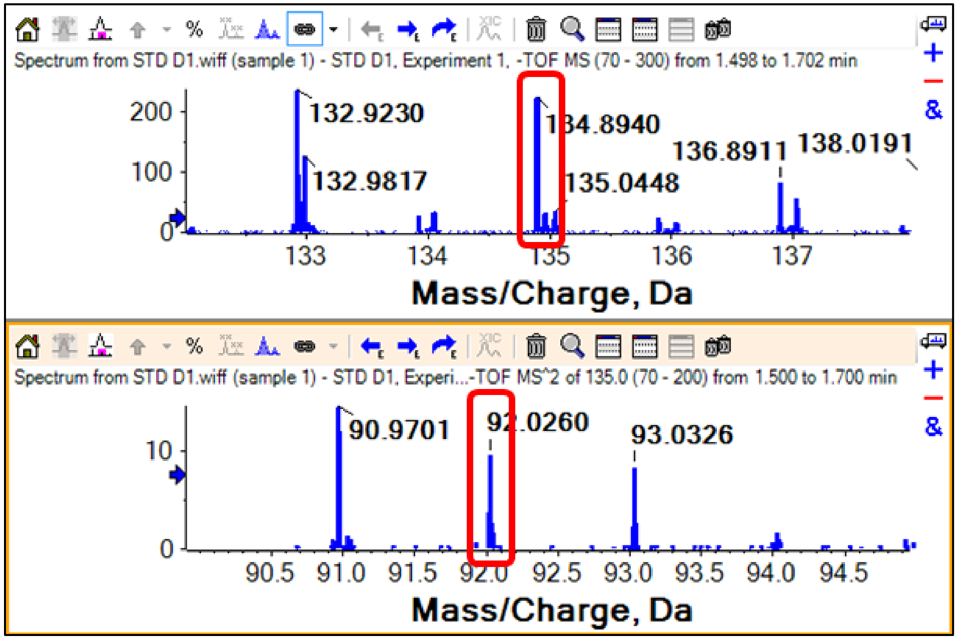
Allopurinol XIC from STD E1.wiff (sample 1) - STD E1, Experiment 1, -TOF MS (70 - 300): 135.031 +/- 0.005 Da
IS XIC from STD E1.wiff (sample 1) - STD E1, Experiment 1, -TOF MS (70 - 300): 138.041 +/- 0.005 Da

Spectrum from STD D1.wiff (sa...5.0 (70 - 200) from 1.134 min

TOF Product Ion

MRM^{HR} Quantitation

- The MRM^{HR} standard curve was concave in shape, which is characteristic of a constant systematic contribution of intensity (background) in the chromatogram



Background intensity in MRM^{HR} (1.5 to 1.7 min)

- In the background there is also a contribution of 135.0 → 92.026 as shown on left
- This background intensity in MRM^{HR} affects the linearity of the calibration curve at low concentrations and results in the concave shape
- This low mass background contribution was not significant on the SCIEX API 5000™ system

Summary

- In this case study, we have demonstrated that the TOF MS instrument is able to conduct a bioanalytical assay with matching precision, accuracy, and linearity to a nominal resolution triple-quad MS.
- Analysts have 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 also provides more information during method development for users to trouble-shoot issues that could interfere with the method and/or the results

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