Evaluation of QTOF Technology for the Quantitation of Drugs in Plasma

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MPR397

Introduction

In a typical bioanalytical laboratory, a triple quadrupole mass spectrometer is an important tool for quantitation of drugs in plasma or serum. Q-TOF technology has evolved over the years into a stable, sensitive tool. Could it be used for this application? The advantage of using the Q-TOF is higher selectivity due to good mass accuracy and product ion scan that yield greater confidence in identification. The feasiblity of using a Q-TOF instead of a triple quadrupole was evaluated for the work. Plasma extracts spiked with either midzaolam or antipyrene were used for evaluation.

Experimental

Sample Preparation:

Midazolam or antipyrine was spiked into plasma and extracted using acetonitrile. No further purification was done. The concentrations ranged from 0.1 ng/mL to 1000 ng/mL. A fast gradient method was developed that was suitable for either compound. The mobile phase contained a mixture of 10 mM ammonium acetate/0.1% acetic acid in water and 90% acetonitrile with 10% methanol. A Zorbax SB- C18, 2.1x50 mm, column was used at 45°C. This method was used on both the triple quadrupole and QTOF. The fragmentor voltage and collision energy were optimized on the triple quadrupole and confirmed to be valid for the QTOF. The ESI source conditions were the same for both instruments.

LC/MS Analysis

LC/MS/MS was performed on an Agilent 6520 Accurate-Mass Q-TOF and an Agilent 6410 Triple Quadrupole (QQQ) with the high sensitivity upgrade kit installed.

MS Conditions:

ESI Positive

Source Conditions:

Capillary Voltage 2500V

Drying gas flow (nitrogen) 12 L/min

Drying gas temperature 350°C

Nebulizer gas (nitrogen) 45 psi

Unit/unit resolution on Q1/Q2 for QQQ

QTOF Detector 2 GHz, hi dynamic range

Experimental

Compound (QQQ Transitions)	Transition	Dwell (msec)	Fragmentor Voltage	Collision Energy
Antipyrine	189.1 →131.1	200	140V	18V
Midazolam	326.1 →291.1	200	180V	25V

Compound (Q-TOF Transitions)	Transition	Acq time (msec)	Fragmentor Voltage	Collision Energy
Antipyrine	*189.1022→106.0656 → 77.0394 → 56.0506*	*400*	*175V*	*30V*
Midazolam	*326.0855 →291.1166*	*400*	*175V*	*30V*

Figure 1: MS Conditions: MRM Transitions for QQQ and Q-TOF targeted MS/MS extracted ion information. The above Q-TOF conditions were the default conditions. It is expected that sensitivity could be improved under the optimized conditions.

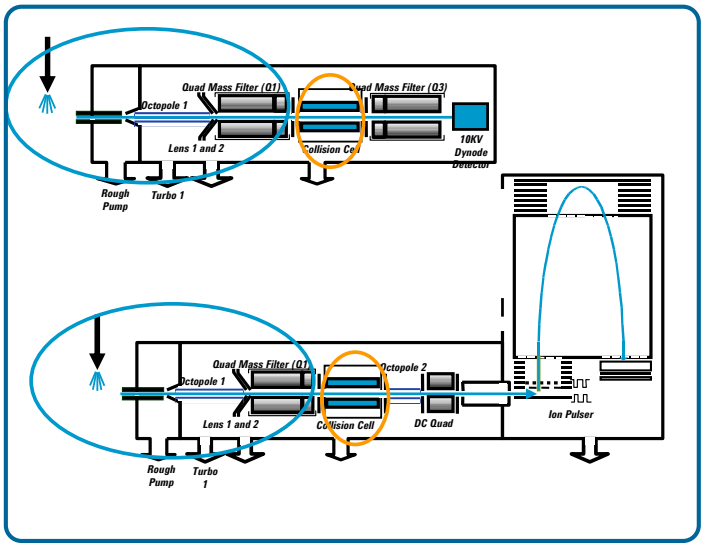

Figure 2: Comparison of the QQQ and the Q-TOF schematics. The ion optics and the collision cell are the same. Additional work has shown that the fragmentor voltage and collision energy settings are the same for either instrument. The result would be faster optimization and easier transfer between instruments.

Results and Discussion

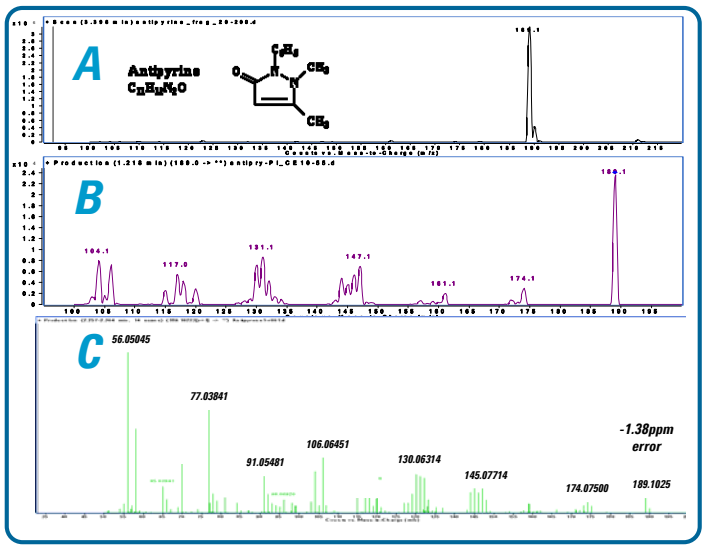

Figure 3: The (A) MS spectrum and product ion spectrum for antipyrine using the QQQ (B) and the Q-TOF (C).

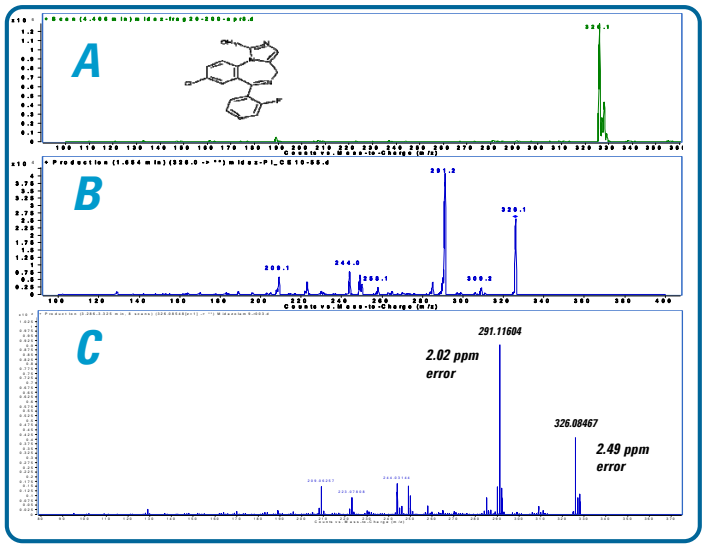

Figure 4: The (A) MS spectrum and product ion spectrum for midazolam using the QQQ (B) and the Q-TOF (C). As shown, good mass accuracy was maintained during the analysis. The above spectrum was the 26th injection.

Analysis of samples

Antipyrine was a challenging sample because of the multiple fragments that were generated. For the QQQ analysis, The relative response on m/z 104.1, 131.1, and 147.1 appear to be the same. The product ion m/z 131.1 was chosen as the quantitation ion on the QQQ because of a better signal/noise response. For the QTOF, multiple ions were summed together (m/z 56.0489, 77.0394 and 104.0856). For midazolam, the product ion m/z 291 was chosen for both QQQ and Q-TOF. All samples were done in triplicate. Due to the excellent mass accuracy of the Q-TOF, a very narrow extraction window (10 ppm) was used.

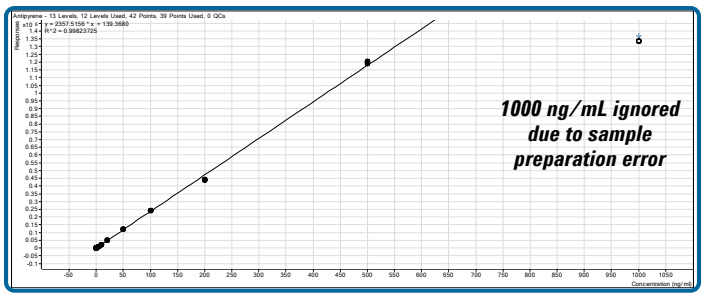

Figure 5: QQQ - Antipyrine Results: Linear fit, 1/x weighting and removal of 1000ng/mL calibrator, R^2 = 0.998, , concentration range 0.1ng/mL-500ng/mL

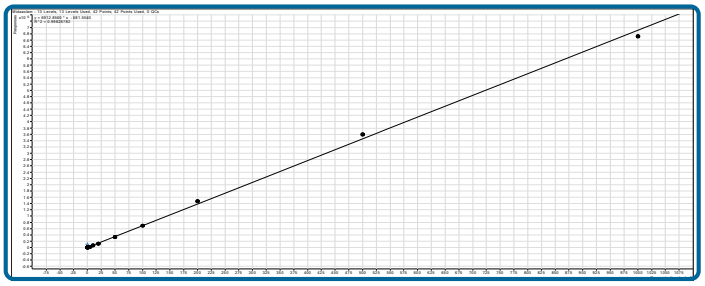

Figure 7: QQQ - Midazolam Results: Linear fit, 1/x weighting and, R^2 = 0.998, concentration range 0.1ng/mL-1000 ng/mL

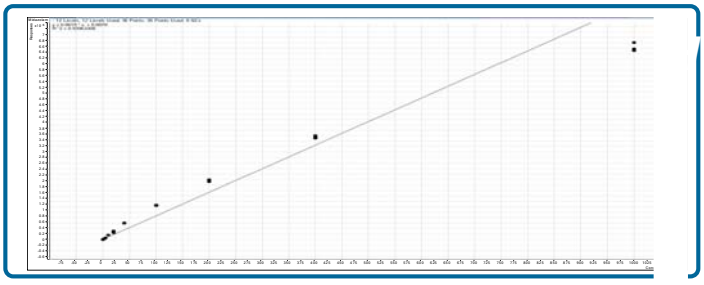

Figure 8: QTOF - Midazolam Results: Linear fit, 1/x weighting and, R^2 = 0.940, concentration range 0.1ng/mL-1000 ng/mL

Results and Discussion

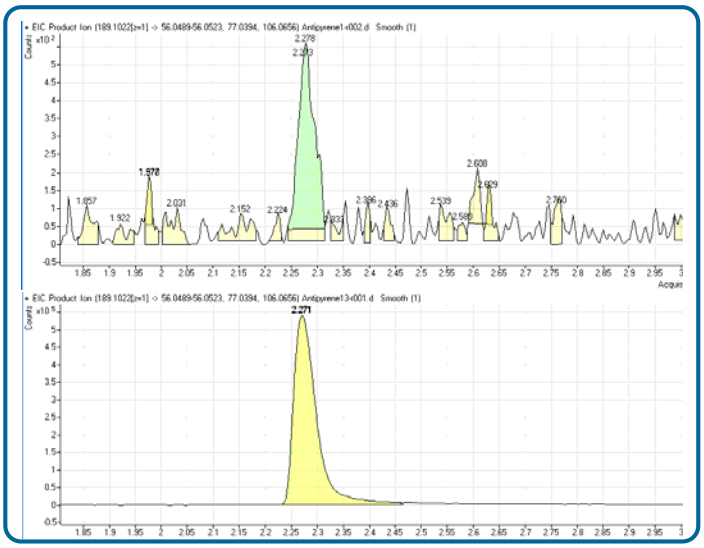

Figure 9: Q-TOF – Chromatograms of 0.1 ng/mL and 1000ng/mL antipyrine extracts. The results were obtained by the summation of 3 ions.

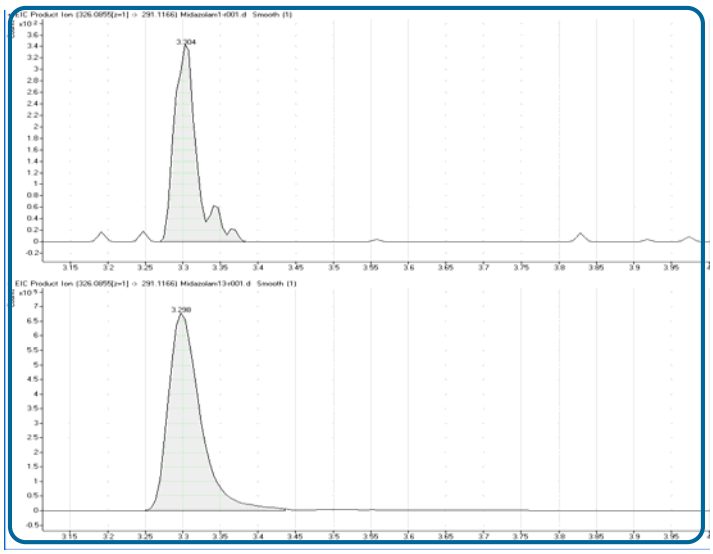

Figure 10: Q-TOF – Chromatograms of 0.1 ng/mL and 1000 ng/mL midazolam extracts. – single ion

Reproducibility of analysis

Antipyrine and midazolam were analyzed in triplicate. The reproducibility data is shown. The results show that the QQQ and the Q-TOF had very similar %RSD across the concentration range for antipyrine. The QQQ data was generated from a single ion and the QTOF data was from the summation of three ions. The QQQ data was better with midazolam. In this extract, a single ion was used for both methods.

		Exp. Conc.	QQQ	Q-TOF
Name	Level	ng/mL	%RSD	%RSD
Antipryene1	1	0.1	11.0	4.3
Antipryene2	2	0.2	7.0	n/a
Antipryene3	3	0.5	6.0	13.8
Antipryene4	4	1	3.0	6.3
Antipryene5	5	2	2.0	1.6
Antipryene6	6	5	0.8	1.0
Antipryene7	7	10	0.9	3.6
Antipryene8	8	20	1.0	0.3
Antipryene9	9	50	0.4	0.9
Antipryene10	10	100	0.8	0.8
Antipryene11	11	200	0.7	1.2
Antipryene12	12	500	0.7	0.5
Antipryene13	13	1000	0.2	0.0

		Exp. Conc.	QQQ	Q-TOF
Name	*Level*	*ng/mL*	*%RSD*	*%RSD*
Midazolam1	***1***	***0.10***	***9.0***	***10.6***
Midazolam2	***2***	***0.20***	***2.0***	***12.0***
Midazolam3	***3***	***0.50***	***2.0***	***7.8***
Midazolam4	***4***	***1.00***	***2.0***	***4.6***
Midazolam5	***5***	***2.00***	***0.6***	***12.6***
Midazolam6	***6***	***5.00***	***0.2***	***2.4***
Midazolam7	***7***	***10.00***	***0.1***	***9.3***
Midazolam8	***8***	***20.00***	***0.3***	***1.7***
Midazolam9	***9***	***50.00***	***0.2***	***1.2***
Midazolam10	***10***	***100.00***	***0.4***	***0.7***
Midazolam11	***11***	***200.00***	***0.2***	***0.2***
Midazolam12	***12***	***500.00***	***0.4***	***0.4***
Midazolam13	***13***	***1000.00***	***0.2***	***0.7***

Conclusions

Good reproducibility and sensitivity was shown across the calibration range. It was slightly better using the QQQ. The linear dynamic range was slightly better for the QQQ. Q-TOF showed better than expected sensitivity for targeted MSMS in quantitative application. The vision of using a Q-TOF for better selectivity in quantitative applications is possible