Accomplish outstanding quantitative performance for bioanalysis of small molecule pharmaceuticals using accurate mass spectrometry

Featuring sensitive quantification of small molecule pharmaceutical compounds using the ZenoTOF 7600 system

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This technical note demonstrates the sensitive quantification of small molecule pharmaceutical compounds in rat plasma using an accurate mass spectrometer. Low-pg/mL quantification was achieved for midazolam, imipramine and clozapine with outstanding accuracy, precision and linearity. The improved MS/MS sampling efficiency of the ZenoTOF 7600 system enhanced assay sensitivity to meet the needs of routine bioanalysis in complex matrices.

Demands for improved sensitivity in routine bioanalytical assays continue to increase as drug discovery and development programs focus on more efficacious, lower dosage compounds. Pharma development scientists supporting this work therefore require highly sensitive and selective bioanalytical methods for quantification. More recently, accurate mass spectrometers, such as time-of-flight (TOF) instruments, have been increasingly adopted for quantitative bioanalysis because they demonstrate high selectivity.[1,2] However, current TOF platforms still encounter challenges with sensitivity when used for quantification because given the loss of ion transmission between TOF pulses.

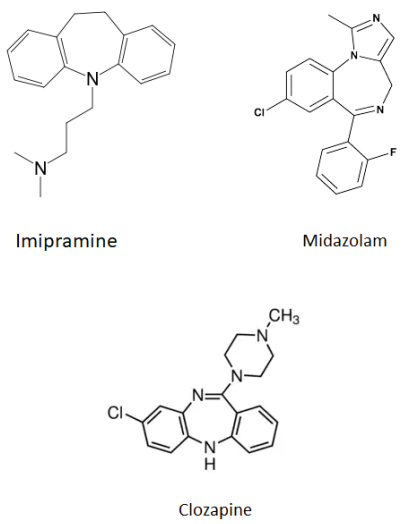

Figure 1. Structures of imipramine, midazolam and clozapine.

In this technical note, an accurate mass spectrometer with improved MS/MS sampling efficiency that offers a robust and sensitive platform to support routine bioanalysis is presented. The ZenoTOF 7600 system features a Zeno trap that controls the ion beam from the collision cell, facilitates greater ion transmission to the TOF accelerator and improves the duty cycle to ≥90% (classical TOF is below 30%). With the enhancement in the overall MS/MS sampling efficiency, the ZenoTOF 7600 system is highly advantageous for quantitative workflows that require high sensitivity.

Here, 3 small molecule pharmaceutical compounds, including imipramine, midazolam and clozapine, were quantified in rat plasma using the ZenoTOF 7600 system. Lower limits of quantification (LLOQs) of 1 pg/mL for midazolam, 1 pg/mL for imipramine and 3 pg/mL for clozapine were achieved using a protein precipitation sample preparation.

Key features of the ZenoTOF 7600 system for highly sensitive bioanalysis

- Perform sensitive quantification of small molecule pharmaceutical compounds with improvements in MS/MS sampling efficiency using the Zeno trap on a cutting-edge QTOF platform
- Achieve outstanding accuracy, precision and linearity for the analysis of small molecule pharmaceutical compounds using the ZenoTOF 7600 system
- Increase productivity with a user-friendly interface and integrated platform for data acquisition, processing and management for routine bioanalysis using SCIEX OS software

Methods

Sample preparation: Imipramine, midazolam and clozapine were spiked into 100 µL aliquots of rat plasma at concentrations ranging from 0.050 to 1000 pg/mL. Imipramine d_3 was used as an internal standard. Samples were extracted using protein precipitation with 300 µL of ice-cold acetonitrile. Samples were then vortexed and centrifuged at 10,000 rpm for 25 mins. Supernatant was collected and diluted 1:1 with water.[3]

Chromatography: Samples were analyzed using an ExionLC AC system at a flow rate of 0.6 mL/min with a Phenomenex Luna Omega Polar C18 (2.1 x 50 mm, 1.6 µm, 100 Å) column and a 7-minute gradient (Table 1). The operating column temperature was 40°C. Mobile phase A was 5mM ammonium formate with 0.2% formic acid in water and mobile phase B was acetonitrile. A 10 µL injection volume was used for analysis.

Table 1. Chromatographic gradient.

Time (min)	Flow (mL/min)	Mobile phase B (%)
1.0	*0.6*	*15*
4.0	*0.6*	*45*
4.1	*0.6*	*95*
6.0	*0.6*	*95*
6.1	*0.6*	*15*
7.0	*0.6*	*15*

Mass spectrometry: Samples were analyzed using the ZenoTOF 7600 system in positive Zeno MRM^{HR} mode. The Zeno MRM^{HR} conditions and optimized MS parameters are listed in Tables 2 and 3, respectively.

Table 2. Zeno MRM^{HR} parameters and fragments used for quantification.

ID	Q1 mass (*m/z*)	Fragment (*m/z*)	DP (V)	CE (V)
Midazolam	*326.09*	*291.12*	*40*	*37*
Imipramine	*281.20*	*86.09*	*40*	*23*
Imipramine d_3	*284.22*	*89.11*	*40*	*23*
Clozapine	*327.14*	*270.08*	*40*	*33*

Table 3. MS parameters.

Parameter	MS	MS/MS
Scan mode	*TOF-MS*	*MRM^{HR}*
Polarity	*Positive*	
Gas 1	*50 psi*	
Gas 2	*60 psi*	
Curtain gas	*35 psi*	
Source temperature	*600°C*	
Ion spray voltage	*3000 V*	
Declustering potential	*40 V*	
CAD gas	*9*	
Start mass	*200 m/z*	*70 m/z*
Stop mass	*900 m/z*	*700 m/z*
Q1 resolution	*N/A*	*Unit*
Accumulation time	*0.05 s*	*0.04 s*
Collision energy	*10 V*	*See Table 2*
CE spread	*0 V*	*0 V*
Zeno	*N/A*	*ON*
ZOD threshold (CID)	*N/A*	*20,000 cps*
Time bins to sum	*4*	*12*

Data processing: Data processing was performed using the Analytics module of SCIEX OS software.

Quantification results

Standards at concentrations ranging from 0.5 pg/mL to 3000 pg/mL were analyzed. Analysis of the calibration curve was performed using a Zeno MRM^{HR} workflow, in which each concentration level was analyzed in triplicate.

The quantitative criteria at the LLOQ included a %CV <20% and accuracy within ±20% of the nominal concentration. At all other concentrations, the %CV was required to be <15% and accuracy was required to be within ±15% of the nominal concentration.

The method provided limits of detection (LODs) of 0.5 pg/mL for midazolam, 0.5 pg/mL for imipramine and 1 pg/mL for clozapine (Figure 2). LLOQs of 1 pg/mL for midazolam, 1 pg/mL for imipramine and 3 pg/mL for clozapine were reached with 0.1 mL of rat plasma and a total run time of 7 mins (Figure 2). No interferences were observed in the matrix blank at the retention time of the compounds, as shown in Figure 2.

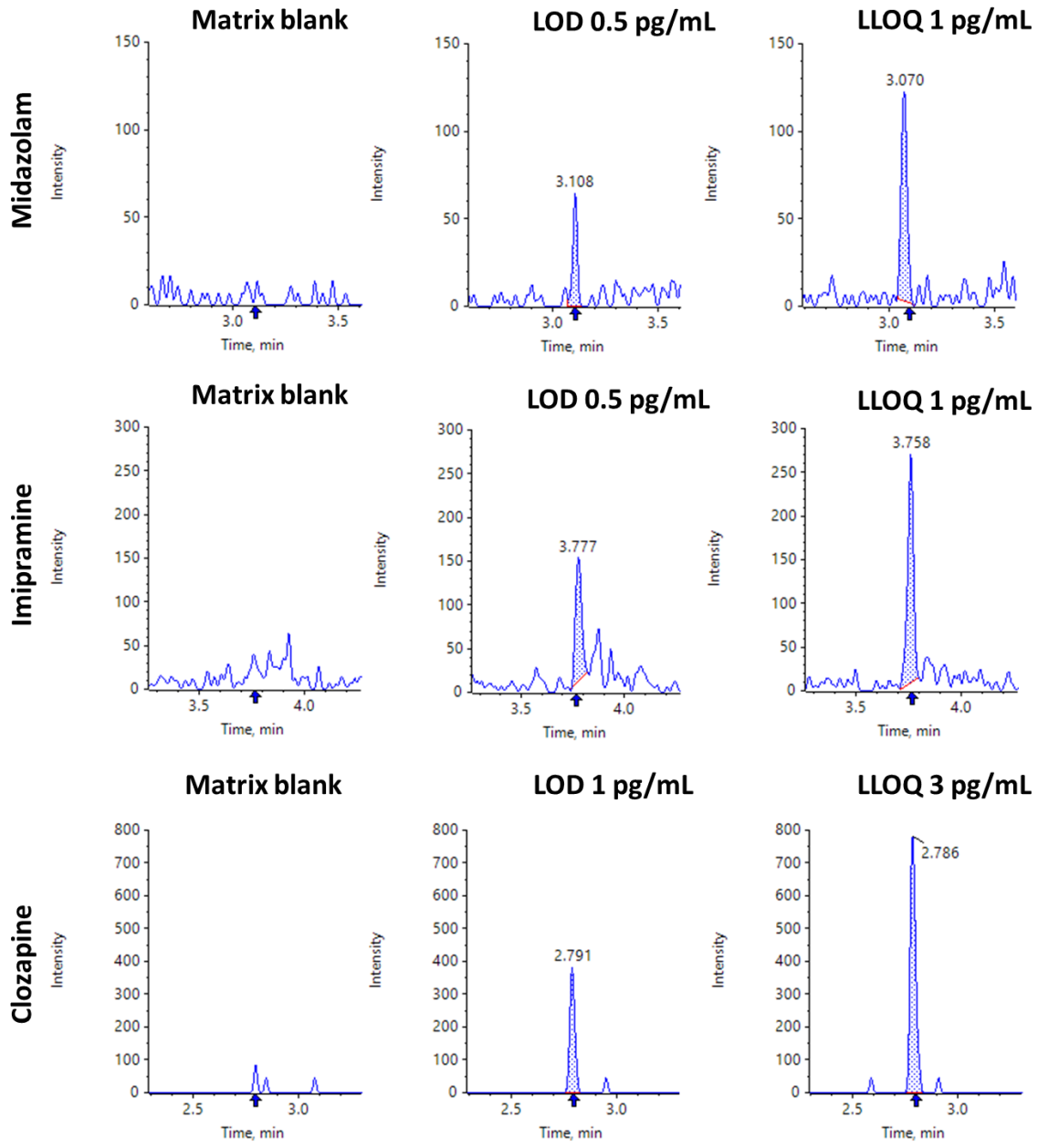

Figure 2. Representative extracted ion chromatograms (XICs) for midazolam, imipramine and clozapine in rat plasma. For each compound, XICs are shown for the matrix blank (left), LOD (middle) and LLOQ (right). No matrix interferences were observed in the matrix blank samples. LODs of 0.5 pg/mL, 0.5 pg/mL and 1 pg/mL were reached for midazolam, imipramine and clozapine, respectively. LLOQs of 1 pg/mL, 1 pg/mL and 3 pg/mL were achieved for midazolam, imipramine and clozapine, respectively.

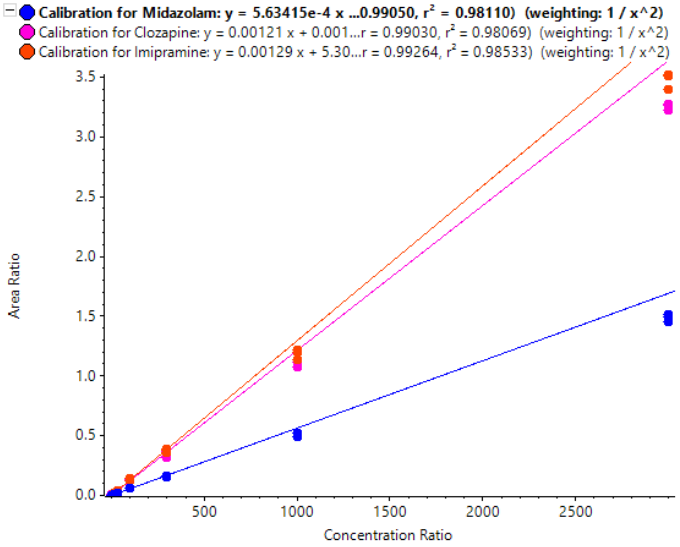

Figure 3. Calibration curves for the quantification of imipramine, midazolam and clozapine in rat plasma. A weighting of $1/x^2$ was applied for all the compounds assessed.

Final calibration curves are shown in Figure 3. Excellent linearity was observed across the concentration ranges analyzed and an overall linear dynamic range (LDR) of up to 3.5 orders of magnitude was achieved. The quantification results are summarized in Table 4. Good %CVs were achieved across all concentration levels in rat plasma for imipramine, midazolam and clozapine.

As summarized in Table 4, assay accuracy was between 87.8% and 115% for midazolam, between 88.2% and 115% for imipramine and between 89.4% and 115% for clozapine. Assay accuracy was well within acceptance criteria for all tested samples.

Table 4. Quantification summary for midazolam, imipramine and clozapine.

	Midazolam		Imipramine		Clozapine	
Nominal concentration (pg/mL)	Average accuracy (%)	CV (%)	Average accuracy (%)	CV (%)	Average accuracy (%)	CV (%)
1.0	*93.1*	*18.7*	*88.2*	*6.50*	*N/A*	*N/A*
2.0	*111*	*0.700*	*115*	*3.73*	*N/A*	*N/A*
3.0	*98.5*	*14.9*	*110*	*5.42*	*94.2*	*14.1*
10	*115*	*5.10*	*110*	*4.11*	*115*	*12.2*
30	*109*	*5.85*	*101*	*4.16*	*113*	*6.44*
100	*101*	*1.31*	*98.8*	*7.17*	*102*	*6.55*
300	*93.9*	*2.11*	*95.6*	*4.32*	*94.5*	*7.99*
1000	*89.9*	*3.50*	*91.3*	*3.23*	*91.3*	*2.69*
3000	*87.8*	*1.93*	*89.5*	*1.95*	*89.4*	*0.800*

Conclusions

- Low levels of quantification were achieved for 3 pharmaceutical compounds in rat plasma on the ZenoTOF 7600 system using the Zeno trap
- The method demonstrated excellent accuracy, precision and linearity for triplicate injections at all concentration levels
- A single platform for streamlined data acquisition, processing and management with SCIEX OS software is presented

References

1. Mike-Qingtao Huang, Zhongping (John) Lin, Naidong Weng (2013). Applications of high-resolution MS in bioanalysis. *Bioanalysis* **5**(10):1269-1276.
2. Anton Kaufmann (2020). High-resolution mass spectrometry for bioanalytical applications: Is this the new gold standard?. *Journal of Mass Spectrometry* **55**(9): e4533.
3. Achieve next level sensitivity for the evolution of routine bioanalysis. SCIEX technical note, RUO-MKT-02-14859-A.

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