



# A versatile and sensitive approach for small molecule quantitation using an accurate mass spectrometer

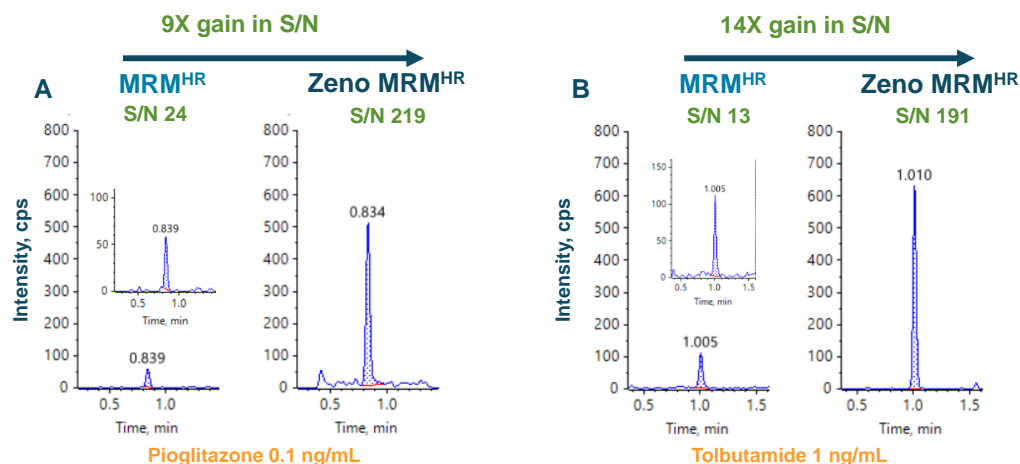
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Quantitation of small molecule therapeutics in biological matrices is critical during the drug development process. The triple quadrupole platform offers excellent sensitivity and quantitative performance and has been a crucial driver for most bioanalytical methods. However, accurate mass spectrometry has increasingly been adopted for quantitative bioanalysis.<sup>1,2</sup> With the inherent advantage of greater selectivity, improved mass resolution and the flexibility of TOF MS/MS data analysis, the ZenoTOF 7600 system provides excellent quantitative performance in multiple dimensions.<sup>3</sup>

This technical note demonstrates a versatile and sensitive approach for small molecule quantitation using the ZenoTOF 7600 system. A lower limit of quantitation (LLOQ) of 0.025 ng/mL for pioglitazone and 0.25 ng/mL for tolbutamide was achieved with an linear dynamic range (LDR) of 5 and 4.6 orders of magnitude, respectively, using a Zeno MRM<sup>HR</sup>-based quantitation workflow.

## Key benefits of using the ZenoTOF 7600 system for the quantitation workflows

- **Offering a versatile quantitation experience:** Flexibility to choose between different quantitation modes: Zeno MRM<sup>HR</sup>, MS1 and Zeno MS1-based workflows for sensitive and selective quantitation using a single MS method.
- **Achieve sub-ng/mL level quantitation using the Zeno MRM<sup>HR</sup> method in minimally treated human plasma:** Achieve a 0.025 ng/mL LLOQ for pioglitazone and a 0.5 ng/mL LLOQ for tolbutamide in human plasma using the ZenoTOF 7600 system with improved MS/MS sampling efficiency.
- **Ideal analytical performance:** Achieve accurate quantitative performance across a LDR spanning 5 orders of magnitude.
- **Streamlined data management:** Data acquisition and processing are integrated into SCIEX OS software, a 21 CFR Part 11 compliance-ready platform.



**Figure 1. Demonstration of sensitivity gain using the Zeno MRM<sup>HR</sup> mode.** A 9-fold S/N gain was achieved for pioglitazone (A) and a 14-fold S/N gain for tolbutamide (B) using Zeno MRM<sup>HR</sup> compared to conventional MRM<sup>HR</sup> using the ZenoTOF 7600 system.



## Introduction

Triple quadrupole mass spectrometers are the workhorses of quantitative assays in the drug development pipeline. As the potent modalities emerge, the need for selectivity is being addressed using methods on high-resolution accurate mass spectrometers. Despite better-resolving power and providing higher mass accuracies, achieving the required sensitivity remains a challenge on accurate mass instruments, including time-of-flight (TOF) systems. The challenge is primarily due to the typical QTOF duty cycles of 5-25%. Consequently, this results in losing up to 95% of detectable ions with each scan in the MS region. To overcome the challenge of TOF systems, improvement in the duty cycle is needed to ensure sensitive and selective quantitation of drug candidates in the pharma pipeline.<sup>1,2</sup>

The ZenoTOF 7600 system is equipped with the Zeno trap, leading up to 20-fold sensitivity gain without compromising speed, resolution, mass accuracy or dynamic range. When activated, ions are accumulated in the Zeno trap before pulsed into the TOF. Accumulating ions in the trap increases ion transmission to the TOF accelerator, improving the duty cycle to  $\geq 90\%$  across the entire mass range while performing MS/MS.<sup>1,2</sup> Unlocking higher sensitivity levels enables the detection of and quantitation of low-abundance compounds that would otherwise be undetectable. Zeno trap enables the ZenoTOF 7600 system to be highly advantageous for quantitative bioanalysis workflows.

This technical note shows different approaches for quantitation with signal-to-noise (S/N) comparison for different quantitation modes on the ZenoTOF 7600 system using small molecule analytes.

## Methods

**Sample preparation:** Pioglitazone and tolbutamide stock solutions were prepared in dimethyl sulfoxide. Both analytes were spiked into 100  $\mu\text{L}$  of human plasma at concentrations indicated in Figures 5A and 5B. A simple protein precipitation was performed with 200  $\mu\text{L}$  of acetonitrile. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 10 minutes at room temperature. The supernatant was transferred to an LC-MS vial for analysis.

**Chromatography:** Sample separation was performed using an ExionLC system at a 0.6 mL/min flow rate on a [Phenomenex Kinetex XB-C18 \(2.1 x 50 mm, 1.7  \$\mu\text{m}\$ , 100  \$\text{\AA}\$ \) column](#). A 3-minute gradient was run using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B (Table 1). The column temperature was maintained at 50°C. An injection volume of 1  $\mu\text{L}$  was used for analysis. A mixture of 1:1:1 (v/v/v) acetonitrile:methanol:water was used as a needle wash solvent.

**Table 1: Chromatographic gradient for pioglitazone and tolbutamide.**

Time (min)	Mobile phase B (%) Pioglitazone	Mobile phase B (%) Tolbutamide
0.0	20	30
0.25	20	30
1.0	50	90
1.1	95	95
2	95	95
2.1	20	30
3	20	30

**Mass spectrometry:** The optimized source and gas parameters are listed in Table 2 and the Zeno MRM<sup>HR</sup> parameters are included in Table 3.

**Table 2: Source and gas parameters on the ZenoTOF 7600 system.**

Parameter	Pioglitazone	Tolbutamide
Polarity	Positive	Negative
Ion source gas 1	60 psi	
Ion source gas 2	65 psi	
Curtain gas	45 psi	
Source temperature	600°C	
Ion spray voltage	2500	-4500V
CAD gas	10	
Column temperature	25°C	

**Table 3: Zeno MRM<sup>HR</sup> parameters used for quantitation on the ZenoTOF 7600 system.**

Parameter	Pioglitazone	Tolbutamide
TOF MS range (m/z)	200-900	200-370
TOF accumulation (ms)	75	75
Q1 resolution	Unit	Low
TOF MS/MS range (m/z)	50-500	50-300
MS/MS accumulation time (ms)	75	75
Zeno threshold (cps)	20000	
Precursor/fragment ion	357.1/134.0968	269.1/170.0270
	357.1/119.0729	269.1/106.0665
Collision energy (V)	45	-23
	60	-40

**Data processing:** Data collection and analysis were performed using SCIEX OS software, version 3.3. Peaks were automatically integrated using the MQ4 algorithm and a weighting of  $1/x^2$  was used for quantitation. An extraction window of 0.005 Da was used to generate extracted ion chromatograms (XICs) for quantitation.

## Exploring the different quantitation modes on the ZenoTOF 7600 system

Bioanalysis is imperative in characterizing critical factors in drug development, specifically for drug metabolism and pharmacokinetics (DMPK), which often requires targeted and low-level detection of pharmaceuticals in complex matrices. Both analytes achieved good sensitivity and selectivity using the Zeno MRM<sup>HR</sup> mode (Figures 2 and 3). Quantitation modes were specified in a single MS method

file, acquiring data for all modes in a single run. Automated and accurate peak integration algorithms allowed rapid analysis through streamlined and integrated data processing.

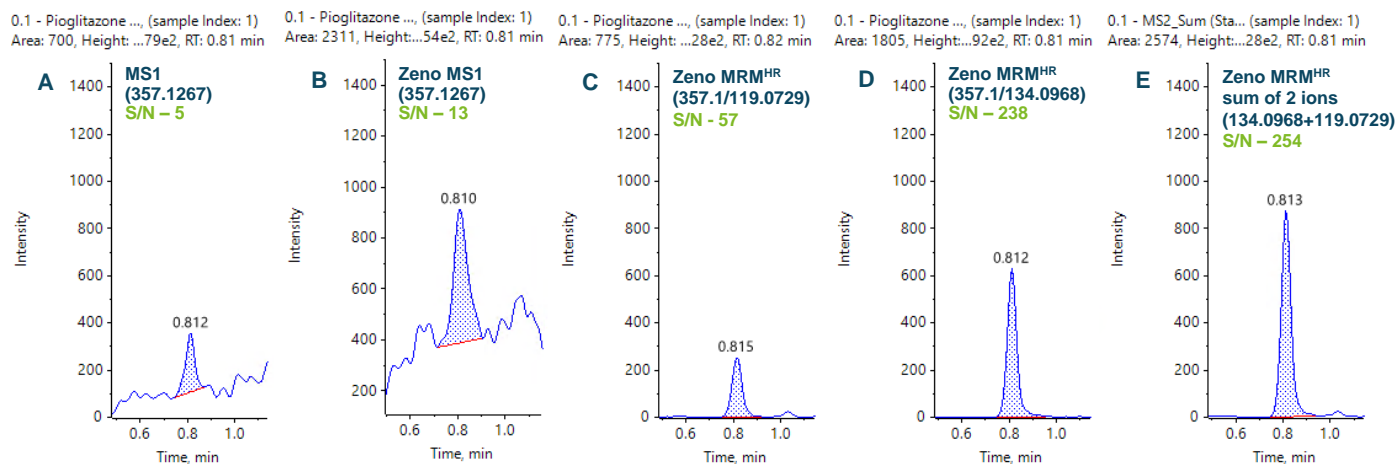
Zeno trap is a novel trapping/releasing technology integral to the ZenoTOF 7600 system and designed to overcome QTOF MS/MS duty cycle deficiencies. Figure 1 illustrates the Zeno trap in action in MRM<sup>HR</sup> mode. A 9-fold S/N gain was observed for pioglitazone (Figure 1A), and a 14-fold S/N gain was observed for tolbutamide (Figure 1B) compared to the conventional MRM<sup>HR</sup> experiment.

Figures 2 and 3 demonstrate the different flexible quantitation modes supported by the ZenoTOF 7600 system for pioglitazone and tolbutamide. MS1-based quantitation, which relies on precursor ions, offers a simple and easy-to-use method with reduced ion path tuning. With the Zeno trap enabled, a 3-fold improvement in the S/N was observed for pioglitazone based on MS1-level quantitation (Figure 2B). Using the Zeno MRM<sup>HR</sup> method, a S/N gain of 11-fold and 47-fold was achieved for the 2 most intense fragments, m/z 119.0729 (Figure 2C) and m/z 134.0968 (Figure 2D), respectively. Summation of the 2 most intense fragment ions resulted in a 51-fold improvement in S/N compared to MS1-based quantitation for pioglitazone.

Figure 3 showed a similar outcome for tolbutamide. MS1-based quantitation was improved by 3-fold using the Zeno trap (Figure 3B). An 88-fold and 94-fold improvement in S/N was achieved using the Zeno MRM<sup>HR</sup> for the 2 most intense fragments, m/z 106.0665 (Figure 3C) and m/z 170.027 (Figure 3D), respectively. Summation of the 2 most intense fragment ions resulted in a 138-fold improvement in S/N compared to MS1-based quantitation.

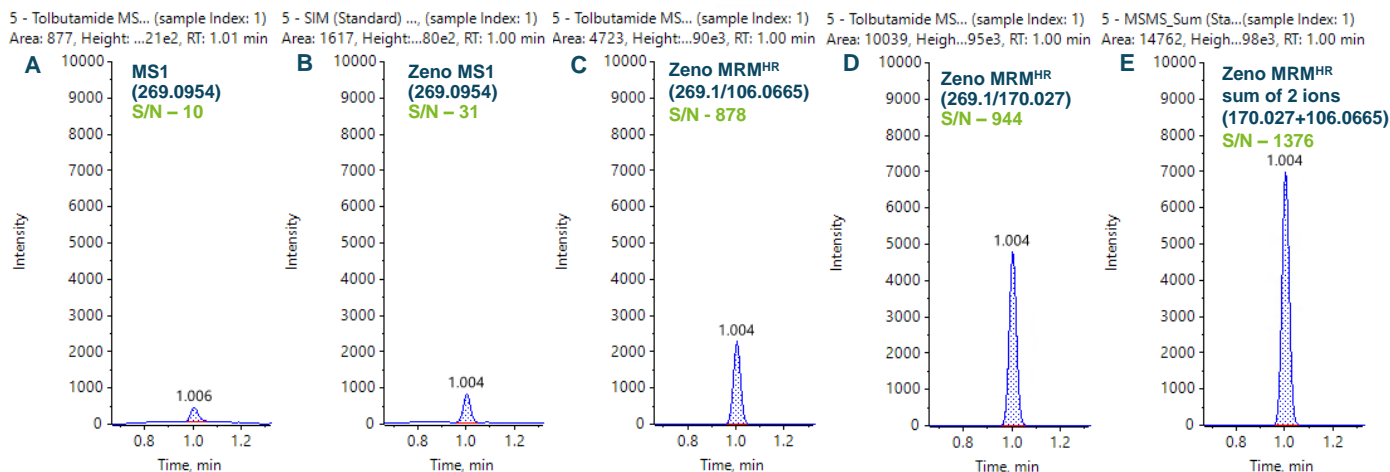


### Pioglitazone 0.1 ng/mL



**Figure 2. Versatile quantitation modes on the ZenoTOF 7600 system.** S/N gains illustrated for pioglitazone in human plasma were calculated using peak-to-peak. An extraction window of 0.005 Da was used to generate XICs.

### Tolbutamide 5 ng/mL



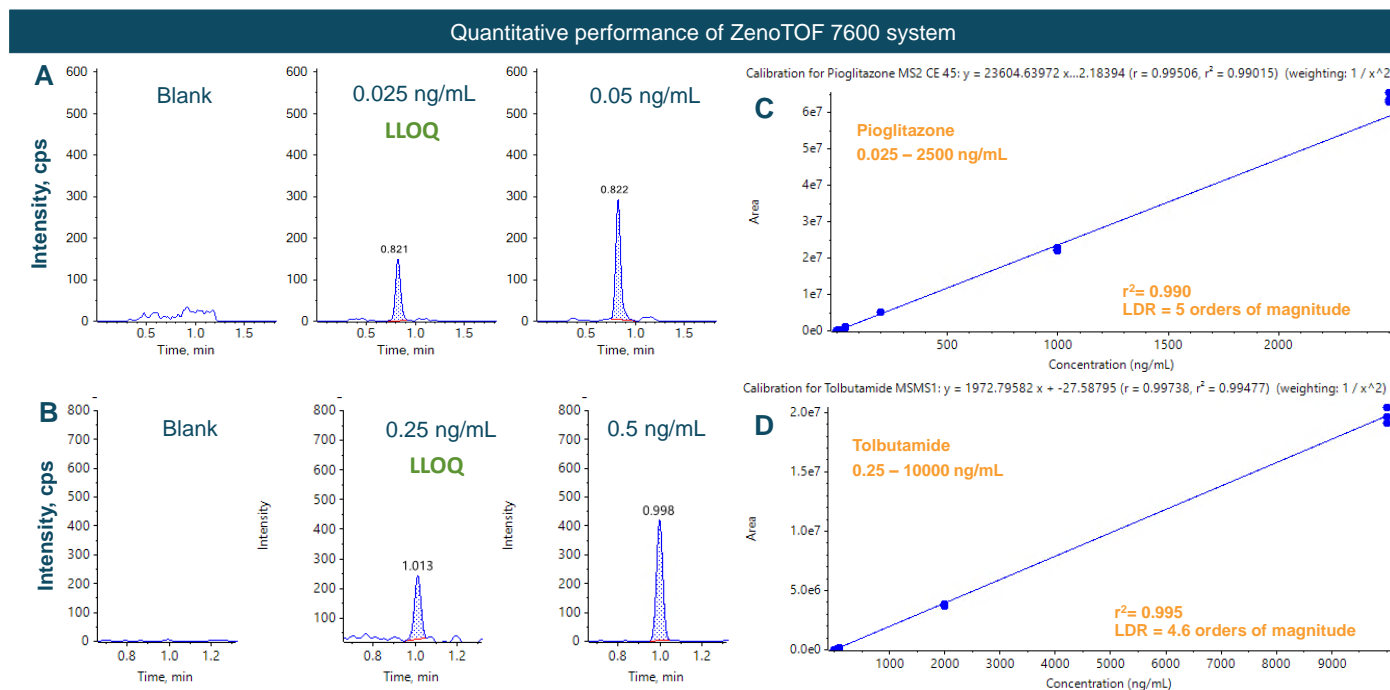
**Figure 3. Versatile quantitation modes on the ZenoTOF 7600 system.** S/N gains illustrated for tolbutamide in human plasma were calculated using peak-to-peak. An extraction window of 0.005 Da was used to generate XICs.

### Quantitation performance using Zeno MRM<sup>HR</sup>

Linearity was achieved between 0.025 ng/mL and 2500 ng/mL with an  $r^2$  of 0.995 (Figure 4A) for pioglitazone and between 0.25 ng/mL and 10000 ng/mL with an  $r^2$  of 0.995

(Figure 4B) for tolbutamide. An LDR spanning 5 orders of magnitude was achieved for pioglitazone and 4.6 orders for tolbutamide.





**Figure 4. Quantitative performance using the Zeno MRM<sup>HR</sup> method.** Representative extracted ion chromatograms (XICs) of the analytes at the Blank, LLOQ, along with 2x of LLOQ levels for pioglitazone (A) and tolbutamide (B). Linearity was achieved between 0.025 ng/mL and 2500 ng/mL and spanned an LDR of 5 orders of magnitude with an  $r^2$  of 0.995 for pioglitazone (C) and between 0.25 ng/mL and 10000 ng/mL and spanned an LDR of 4.6 orders of magnitude with an  $r^2$  of 0.995 for tolbutamide (D). Each concentration level was analyzed in triplicate.

Analytical performance was evaluated for accuracy and precision. The accuracy of the calculated mean was expected to be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean for each concentration was expected to be <20% at the LLOQ and <15% at higher

concentrations. Accuracy was within  $\pm 10\%$  for pioglitazone and  $\pm 7\%$  for tolbutamide of the nominal concentrations and the %CV was <14% for pioglitazone and tolbutamide (Figure 5A and 5B). Calculated accuracy and %CV values met the acceptance criteria at each concentration level.

A	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates	Value #1	Value #2	Value #3
	Pioglitazone MS2 CE 45	0.0250	3 of 3	0.0268	0.00352	13.1	107.	0.0268	0.0233	0.0304
	Pioglitazone MS2 CE 45	0.0500	3 of 3	0.0462	0.00137	2.97	92.3	0.0477	0.0452	0.0456
	Pioglitazone MS2 CE 45	0.100	3 of 3	0.0880	0.00304	3.45	88.0	0.0846	0.0890	0.0904
	Pioglitazone MS2 CE 45	0.400	3 of 3	0.374	0.0113	3.01	93.4	0.386	0.364	0.371
	Pioglitazone MS2 CE 45	1.60	3 of 3	1.52	0.0248	1.63	95.3	1.50	1.54	1.54
	Pioglitazone MS2 CE 45	8.00	3 of 3	7.88	0.204	2.59	98.5	7.68	8.08	7.89
	Pioglitazone MS2 CE 45	40.0	3 of 3	43.8	2.21	5.04	109.	43.6	46.1	41.7
	Pioglitazone MS2 CE 45	200.	3 of 3	221.	0.381	0.173	110.	221.	220.	221.
	Pioglitazone MS2 CE 45	1000.	3 of 3	957.	17.6	1.84	95.7	943.	952.	977.
	Pioglitazone MS2 CE 45	2500.	3 of 3	2740.	58.9	2.15	110.	2800.	2690.	2720.
B	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates	Value #1	Value #2	Value #3
	Tolbutamide MSMS1	0.250	3 of 3	0.256	0.0104	4.07	102.	0.267	0.252	0.247
	Tolbutamide MSMS1	0.500	3 of 3	0.491	0.0644	13.1	98.3	0.461	0.565	0.448
	Tolbutamide MSMS1	1.00	3 of 3	0.944	0.0215	2.27	94.4	0.926	0.968	0.936
	Tolbutamide MSMS1	5.00	3 of 3	4.94	0.230	4.65	98.8	5.10	5.04	4.68
	Tolbutamide MSMS1	50.0	3 of 3	51.6	0.175	0.340	103.	51.6	51.7	51.4
	Tolbutamide MSMS1	100.	3 of 3	107.	4.64	4.32	107.	102.	109.	111.
	Tolbutamide MSMS1	2000.	3 of 3	1920.	43.5	2.27	95.8	1960.	1900.	1880.
	Tolbutamide MSMS1	10000.	3 of 3	9990.	344.	3.45	99.9	9950.	9670.	10400.

**Figure 5. Quantitative performance for analysis of small molecule analytes.** Pioglitazone (A) and tolbutamide (B). Reproducibility and accuracy results were determined from the calibration curve across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

## Compliance-ready SCIEX OS software

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations. Figure 6 illustrates the features of SCIEX OS software used to monitor the audit trail, acquire and

process data, and configure user access. The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards. The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer.

**Audit Trail**

Easily search and filter for specific high-risk events in audit trail viewer. Built-in data integrity features allow you to tailor each functionality specifically to meet compliance needs and data security requirements.

**Central Administration**

Users can manage groups, role definitions, workstations and projects across all systems using the SCIEX OS software Central Administrator Console (CAC). It supports all regulated and non-regulated compliance standards.

**Filter Audit Trail**

Find instances where

Sample Name: [v] Is: [v] Contains: Standard [v]

Reason: [v] Is: [v] Contains: manual integration [v]

<No Filter> [v] Is: [v] Contains: [v]

And where time and date are: From: 3/3/2023 12:00 AM To: 3/3/2023 12:00 AM

**Centralized Management**

User Management  
User Pool  
User Roles and Permissions  
Workstation Management  
Project Management  
Workgroup Management

**Manage Workgroups and Assignments**

Method Developers

Reviewers

Users

Workstations

Projects

Use Central Settings for Projects

Assign or Unassign Project Root from Project Pool

Project Root Directory

Subtree: \\server.allocation.local\Orp\Users\jane.konda\CAC Demo

CAC Demo

**Confirm Change Events**

Change Events

Timestamp	Event Name	Event Description
3/3/2023 4:48:49 PM	Project settings enabled/disabled in a workgroup	Project based central settings enabled for workgroup Reviewers
3/3/2023 4:48:49 PM	Projects assigned/unassigned to a workgroup	Projects assigned/unassigned to a workgroup CAC Demo Default assigned to Workgroup Reviewers

E-Signature Timestamp: 3/3/2023 4:48:49 PM  
User Name: ABCDEFGHIJ\BCT Project  
Full User Name: BCT Project3

Reason: Project assigned

Password: [masked]

**Configuration**

Devices  
Projects  
User Management  
Queue  
Licenses  
LIMS Communication  
General  
Software Updates  
CAC  
About

**Users Roles and Permission Categories**

Administrator Method Developer Analyst Reviewer

Permission	Administrator	Method Developer	Analyst	Reviewer
<b>Batch</b>				
Submit/unlocked methods	[x]	[x]	[x]	[x]
Open	[x]	[x]	[x]	[x]
Save as	[x]	[x]	[x]	[x]
Submit	[x]	[x]	[x]	[x]
Save	[x]	[x]	[x]	[x]
Save ion reference table	[x]	[x]	[x]	[x]
Add data sub-folders	[x]	[x]	[x]	[x]
Configure Decision Rules	[x]	[x]	[x]	[x]
<b>Configuration</b>				
General tab	[x]	[x]	[x]	[x]
General: change regional setting	[x]	[x]	[x]	[x]
General: full screen mode	[x]	[x]	[x]	[x]
LIMS communication tab	[x]	[x]	[x]	[x]
Audit maps tab	[x]	[x]	[x]	[x]
Queue tab	[x]	[x]	[x]	[x]
Queue: instrument idle time	[x]	[x]	[x]	[x]
Queue: max number of acquired samples	[x]	[x]	[x]	[x]
Queue: other queue settings	[x]	[x]	[x]	[x]

**Duplicate a User Role**

Create a new user role based on an existing role.

Existing user role: Administrator

New role name: [Type a name]

New role description: [Type a description]

**Figure 6: Features on the SCIEX OS software for monitoring user access and evaluating the audit trail.** The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst and reviewer levels.

## Conclusions

- A single MS method with various approaches for small molecule quantitation using Zeno TOF 7600 was employed and assessed to achieve the best sensitivity and selectivity for the quantitation of small molecules in a complex matrix
- A 9-fold and 14-fold gain in sensitivity (based on S/N) was achieved in the positive and negative modes using the Zeno MRM<sup>HR</sup> mode, respectively, compared to conventional MRM<sup>HR</sup>
- An LDR of up to 5 orders of magnitude was achieved for pioglitazone and 4.6 orders of magnitude for tolbutamide using Zeno MRM<sup>HR</sup> mode on positive and negative polarity
- A rapid analysis with minimal sample preparation was used to highlight the selective and sensitive quantitation of small molecules in human plasma
- SCIEX OS software is compliance-ready to support 21 CFR Part 11 and integrates with an accurate mass spectrometer to support data acquisition, processing and management on a single platform

## References

1. SCIEX Zeno trap whitepaper, [RUO-MKT-19-13373-C](#)
2. Accomplish outstanding quantitative performance for bioanalysis of small molecule pharmaceuticals using accurate mass spectrometry. SCIEX technical note, [RUO-MKT-02-15026-A](#)

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