Quantitative performance of a next-generation, highly robust triple quadrupole mass spectrometer

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Bioanalytical laboratories are constantly challenged by the need for reliable triple quadrupole mass spectrometers to ensure the delivery of proper quantitative performance. In order to effectively meet the required drug discovery and development timelines, bioanalytical workflows need robust mass spectrometers to minimize instrument downtime.

Enhanced robustness to support long-term routine bioanalysis is now feasible on the SCIEX 7500+ system with Mass Guard technology.[1] Established bioanalytical workflows on the legacy SCIEX 7500 system can be seamlessly transferred to the SCIEX 7500+ system to capitalize on increased robustness while maintaining system sensitivity. In this technical note, a quantitative method for a commercially available glucagon-like peptide-1 (GLP-1) analog, liraglutide, in rat plasma was applied to demonstrate method transferability from the SCIEX 7500 system to the more robust SCIEX 7500+ system (**Figure 1**).

Key benefits of quantitative workflows on the SCIEX 7500+ system

- **Easy method transfer with equivalent quantitative performance:** A comparable LLOQ of 0.05 ng/mL and an accurate quantitative performance with a %CV <5% were achieved for the quantitation of liraglutide in rat plasma
- **Wide dynamic range retained following method transfer:** A wide linear dynamic range (LDR) between 0.05 ng/mL and 500 ng/mL (r^2 >0.996) spanning 4 orders of magnitude was reached on the SCIEX 7500+ system
- **User accessibility to the DJet+ assembly:** Easily perform front-end cleaning needed to maintain system performance
- **Built-in contamination check procedures in SCIEX OS software:** Enables easy monitoring of instrument performance for quick troubleshooting
- **Equivalent software capabilities for bioanalysis:** Similar data management and compliance (21 CFR Part 11) features are available on SCIEX OS software

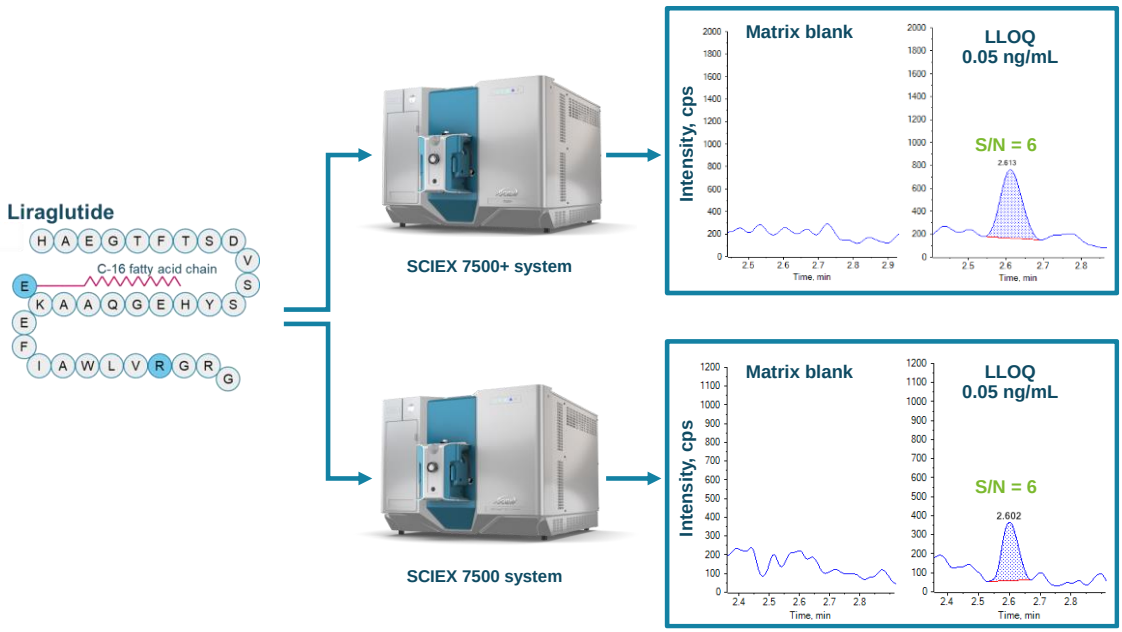

Figure 1: High fidelity method integration onto the SCIEX 7500+ system without compromising assay sensitivity. Liraglutide was analyzed using the SCIEX 7500+ system and the SCIEX 7500 system. Representative extracted ion chromatograms (XICs) of liraglutide in the matrix blank and at 0.05 ng/mL are shown on the SCIEX 7500+ system and the SCIEX 7500 system. An equivalent LLOQ at 0.05 ng/mL with a signal-to-noise (S/N) of 6 (peak-to-peak calculation) was reached on the SCIEX 7500+ system, highlighting comparable system sensitivity during method transfer. No interferences were observed in the rat plasma matrix blank.

Introduction

LC-MS/MS assay development for bioanalysis relies on an accurate, precise and robust methodology that can withstand long-term routine runs without substantial instrument downtime. Thus, instrument reliability is imperative in meeting the arduous timelines during drug discovery and development.

The SCIEX 7500+ system features enhanced robustness using Mass Guard technology[1] while maintaining unwavering quantitative comparability to the legacy SCIEX 7500 system. The presented method demonstrates quantitative method transferability from the SCIEX 7500 system to the SCIEX 7500+ system for a commercially available GLP-1 analog, liraglutide.

Both platforms demonstrated the same level of sensitivity at an LLOQ of 0.05 ng/mL for liraglutide in rat plasma (**Figure 1**). Accurate quantitative performance with a %CV <5% at all concentration levels was achieved across an LDR spanning 4 orders of magnitude on the SCIEX 7500+ system and the SCIEX 7500 system. Instrument uptime was further maintained for long-term and routine bioanalysis of GLP-1 analogs on the SCIEX 7500+ system with the user-accessible DJet+ assembly for front-end cleaning.[2]

Methods

The same LC conditions and MS parameters were used on the SCIEX 7500+ system and the SCIEX 7500 system. The same samples were used for analysis on both platforms.

Samples and reagents: The GLP-1 analogs liraglutide and semaglutide (IS) were purchased from Cayman Chemical Company. Liraglutide and semaglutide were reconstituted in 3% and 6% formic acid in methanol, respectively.

Sample preparation: Liraglutide was spiked into 100 µL rat plasma at concentrations ranging from 0.05 to 500 ng/mL. Semaglutide was used as an internal standard (IS) and spiked at 4 ng. Protein precipitation was performed with 300 µL of methanol. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 12 minutes at room temperature. A 300 µL aliquot of 10% aqueous ammonia was added to the supernatant and gently mixed. A 600 µL aliquot of the sample volume was transferred to an anion exchange Oasis MAX µElution plate. Samples were washed with 5% ammonia in 1:1 (v/v) methanol/water. A second wash was performed using 1:2:2 (v/v/v) water:methanol:acetonitrile. Finally, elution was performed using 6% formic acid in 1:2:2 (v/v/v) water:methanol:acetonitrile mixture. The final elution volume was 100 µL. An injection volume of 2 µL was used for analysis.

Chromatography: Analytes were separated using a Phenomenex Kinetex C18 (2.1 x 50 mm, 2.6 µm, 100 Å) column at a temperature of 50°C. The ExionLC AE system was operated at a 0.6 mL/min flow rate (**Table 1**). Analysis was performed on a nominal mass spectrometer in positive mode. Collision energy, source and MS parameters were optimized for MRM-based quantitation.

Table 1: LC gradient.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	70	30
0.2	70	30
1.5	55	45
2.9	40	60
3.0	5	95
4.0	5	95
4.1	70	30
5.0	70	30

Mass spectrometry: The optimized source and gas parameters are listed in **Table 2** and the MRM parameters are included in **Table 3**.

Table 2: Source and gas parameters.

Parameter	Value
Polarity	Positive
Ion source gas 1	50 psi
Ion source gas 2	60 psi
Curtain gas	45 psi
Source temperature	550°C
Ion spray voltage	5000 V
CAD gas	9

Table 3: MRM parameters used for quantitation.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	CXP (V)	Q0D (V)
Liraglutide	939.0	1064.0	40	15	70
Semaglutide (IS)	1029.3	135.9	40	15	-10

Data processing: Data collection and analysis were performed using SCIEX OS software, version 3.3.1. Peaks were automatically integrated using the MQ4 algorithm and a weighting of $1/x^2$ was used for quantitation.

Quantitative performance on the SCIEX 7500+ system

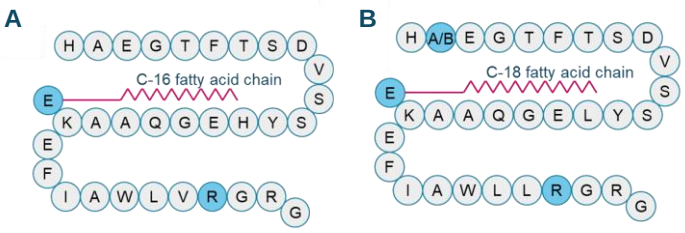

Figure 2: Structures of GLP-1 analogs. The left panel shows the structure of the target analyte, liraglutide (A) and the right panel shows the structure of the IS, semaglutide (B).

Liraglutide (**Figure 2A**) was quantified in rat plasma with the presence of semaglutide (**Figure 2B**) as an IS. Method transfer to the SCIEX 7500+ system was performed with the same LC conditions and MS parameters as on the legacy SCIEX 7500 system.

An LLOQ of 0.05 ng/mL was achieved for liraglutide on the SCIEX 7500+ system and the SCIEX 7500 system (**Figure 1**). No interferences were observed in the rat plasma matrix blank. Representative XICs of liraglutide in rat plasma at 0.1 ng/mL, 5 ng/mL and 500 ng/mL were also assessed between the MS platforms (**Figure 3**). S/N (peak-to-peak calculation) was applied to evaluate the sensitivity comparability of the SCIEX 7500+ system to the SCIEX 7500 system. At 0.1 ng/mL, a S/N of 9 was achieved on both MS systems. For liraglutide at 5 ng/mL, a S/N of 299 was reached on the SCIEX 7500+ system, and a S/N of 289 was reached on the SCIEX 7500 system. At a concentration of 500 ng/mL, a S/N of 2777 was acquired on the SCIEX 7500+ system, and a S/N of 2718 was acquired on the SCIEX 7500 system (**Figure 3**). Therefore, equivalent quantitative sensitivity was demonstrated on the SCIEX 7500+ system compared to the previous generation SCIEX 7500 system.

An r^2 >0.996 was achieved across the 0.05 to 500 ng/mL concentration range with an LDR spanning 4 orders of magnitude on both MS platforms (**Figure 4**). As a result, a comparable wide quantitative range can be analyzed using the SCIEX 7500+ system.

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.

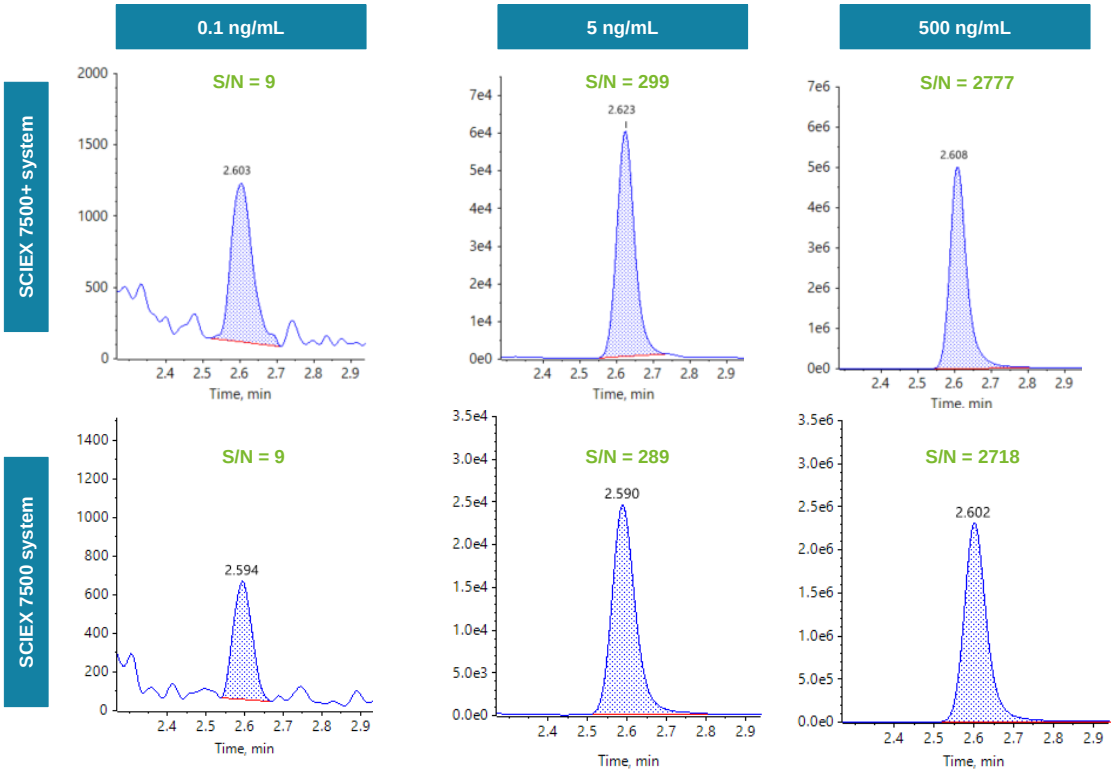

Figure 3: Representative XICs of liraglutide at 0.1 ng/mL, 5 ng/mL and 500 ng/mL in rat plasma using the SCIEX 7500+ system (top) and the SCIEX 7500 system (bottom). S/N was measured using peak-to-peak calculations to assess system sensitivity. Similar S/N was observed across the various concentrations, highlighting that the quantitative sensitivity was equivalent on the SCIEX 7500+ system.

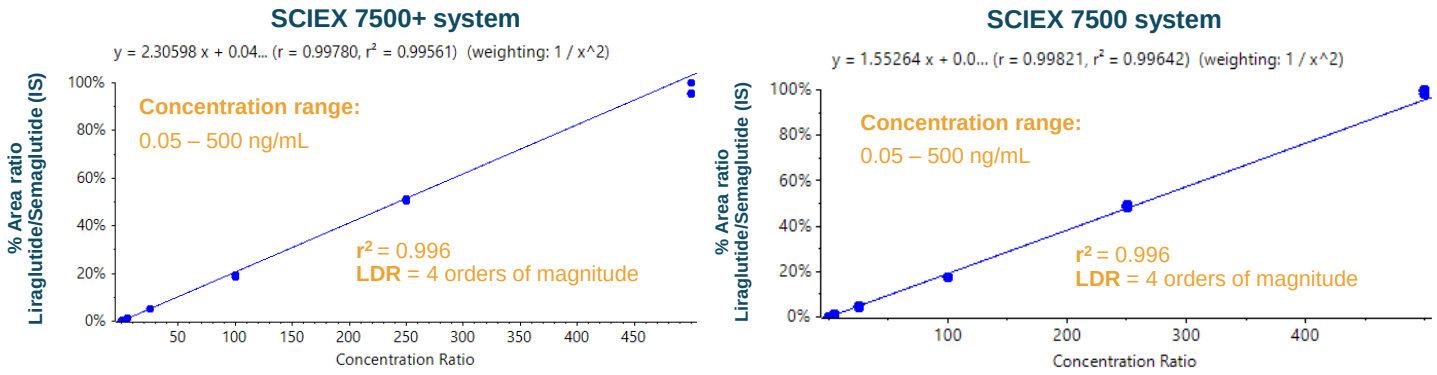

Figure 4: Calibration curve for liraglutide in rat plasma using the SCIEX 7500+ system (left) and the SCIEX 7500 system (right). The area ratio between liraglutide and semaglutide (IS) was used to generate each calibration curve. Each concentration level was run in triplicate. Linearity was achieved between 0.05 ng/mL and 500 ng/mL and spanned an LDR of 4 orders of magnitude with an r^2 >0.996 on the SCIEX 7500+ system and the SCIEX 7500 system. Therefore, a wide quantitative range was retained on the SCIEX 7500+ system.

SCIEX 7500+ system

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates	Value #1	Value #2	Value #3
0.05	3 of 3	0.047	0.001	2.89	94.5	0.047	0.046	0.049
0.10	3 of 3	0.107	0.003	2.76	107.	0.105	0.106	0.110
0.20	3 of 3	0.212	0.001	0.692	106.	0.214	0.212	0.211
0.50	3 of 3	0.511	0.007	1.37	102.	0.519	0.509	0.506
1.00	3 of 3	1.032	0.031	3.05	103.	1.045	0.996	1.055
5.00	3 of 3	5.253	0.012	0.232	105.	5.265	5.240	5.254
25.00	3 of 3	24.620	0.590	2.39	98.5	24.146	25.280	24.435
100.00	3 of 3	91.223	2.095	2.30	91.2	90.143	93.637	89.889
250.00	3 of 3	245.727	2.735	1.11	98.3	245.155	248.702	243.324
500.00	3 of 3	469.129	12.630	2.69	93.8	460.322	483.599	463.465

SCIEX 7500 system

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates	Value #1	Value #2	Value #3
0.05	3 of 3	0.052	0.002	3.32	103.	0.052	0.053	0.050
0.10	3 of 3	0.091	0.003	3.52	91.3	0.093	0.094	0.088
0.20	3 of 3	0.204	0.005	2.58	102.	0.208	0.198	0.207
0.50	3 of 3	0.517	0.026	5.11	103.	0.524	0.539	0.488
1.00	3 of 3	1.007	0.008	0.756	101.	0.998	1.012	1.011
5.00	3 of 3	5.205	0.115	2.21	104.	5.088	5.318	5.208
25.00	3 of 3	24.554	0.850	3.46	98.2	23.579	25.138	24.947
100.00	3 of 3	90.924	1.039	1.14	90.9	90.241	92.120	90.411
250.00	3 of 3	254.953	3.620	1.42	102.	257.826	250.888	256.145
500.00	3 of 3	518.866	4.469	0.861	104.	519.109	514.280	523.209

Figure 5: Quantitative performance for liraglutide analysis on the SCIEX 7500+ system (top) compared to the SCIEX 7500 system (bottom). 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. Final results showed a %CV <5% with an average accuracy of ±9% based on the nominal concentration on both platforms. As a result, equivalent quantitative performance was observed on the SCIEX 7500+ system compared to the SCIEX 7500 system.

The %CV of the calculated mean for each concentration was expected to be <20% at the LLOQ and <15% at higher concentrations. The assay accuracy was within ±9% of the nominal concentration with a %CV <5% for liraglutide in rat plasma on both MS platforms (**Figure 5**). As a result, comparable quantitative performance was reached on the SCIEX 7500+ system with the assurance of meeting the bioanalytical guideline requisites at the LLOQ and higher concentration levels.

Enhanced software tools for monitoring system performance

The SCIEX OS software provides a built-in automated workflow that enables the user to monitor the detector performance and system charging events with minimal manual intervention (**Figure 6**). The contamination check procedure enables system

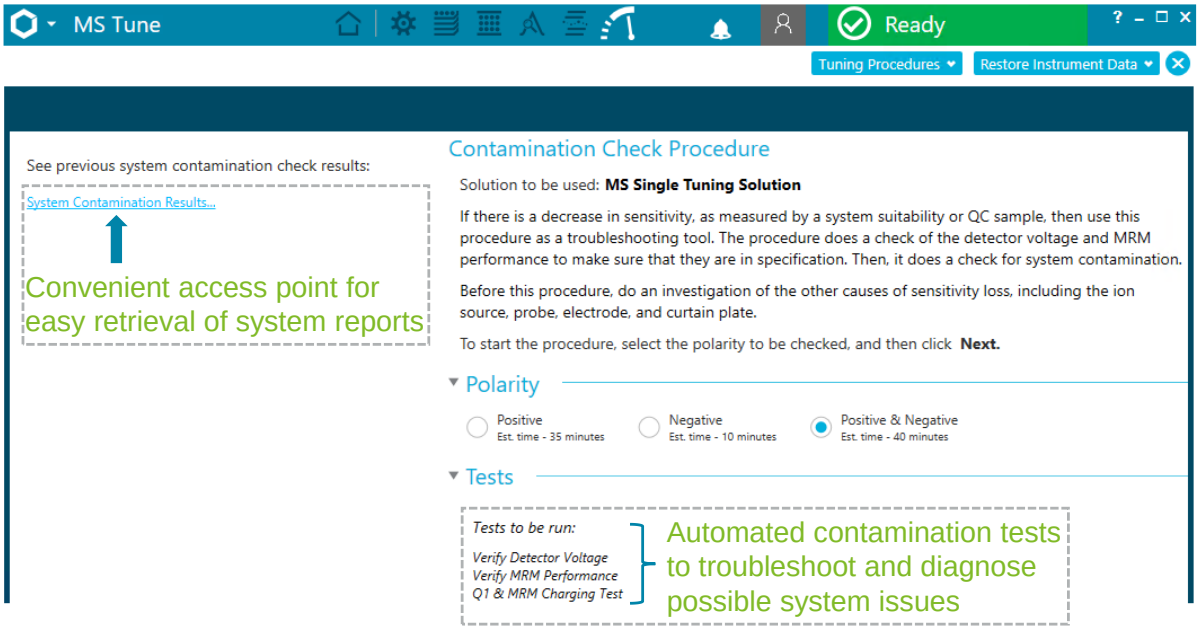

Figure 6. Built-in contamination check procedures in SCIEX OS software for easy troubleshooting. The MS Tune module in SCIEX OS software provides an automated contamination check procedure that allows the user to troubleshoot and monitor instrument performance during sensitivity loss. At the end of the procedure, the software generates a summary report of the instrument performance based on the tests ran.

tests to be run in both the positive and negative polarities using the MS single tuning solution.

System tests for the contamination check procedure include verification of the detector voltage, MRM performance and Q1 and MRM charging tests. System reports are then generated and can be easily compared against previous contamination check results using the SCIEX OS software.

Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for regulated bioanalysis can be executed on the SCIEX 7500+ system, ensuring high fidelity when performing method transfers while retaining critical compliance features.

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 7** illustrates the features of SCIEX OS software that are 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.

Figure 7: Features of 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

- Equivalent LLOQ (0.05 ng/mL) was achieved for quantitation of liraglutide on the SCIEX 7500+ system compared to the SCIEX 7500 system, highlighting retention of assay sensitivity over a method transfer
- An identical quantitative range with a linear range between 0.05 ng/mL and 500 ng/mL (r^2 >0.996) and an LDR spanning 4 orders of magnitude was achieved on the SCIEX 7500+ system compared to the SCIEX 7500 system
- Comparable quantitative performance was demonstrated with accurate and highly reproducible (%CV <5%) results on the SCIEX 7500+ system compared to the SCIEX 7500 system
- The combination of SCIEX OS software enhancements for system performance tracking and the extractable DJet+ assembly offers increased flexibility for user-initiated management of system maintenance and uptime
- Retain data management and compliance-readiness (21 CFR Part 11) features using SCIEX OS software to support non-regulated and regulated bioanalysis on the SCIEX 7500+ system

References

1. Redefine bioanalysis with enhanced robustness on the SCIEX 7500+ system. SCIEX technical note, MKT-31350-A.
2. Build resilience with the SCIEX 7500+ system. SCIEX brochure, MKT-31468-A.

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