Forensic

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High Sensitivity and Dynamic Range for 93-Compound Forensic Panel Analysis in Urine

Using the SCIEX Triple Quad™ 5500+ – QTRAP® Ready System

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The ability to screen and accurately quantify novel drugs of abuse and other emerging compounds present in a variety of complex biological matrices is critical for any forensic laboratory. One of the challenges associated with the analysis of such samples is the wide range of concentrations observed for the compounds of interest. This can result in analytes being detected at concentrations outside the calibration range which often requires sample dilution. In order to limit further sample preparation and re-analysis, it is highly desirable to use an instrument with a wide linear dynamic range capable of accurately quantitate across a wide concentration range.

In this technical note, a highly selective and sensitive LC-MS/MS method for the analysis of a comprehensive forensic panel consisting of 93 compounds in urine is described using the SCIEX Triple Quad 5500+ System. The addition of the High Energy Dynode (HED) detection system is shown to increase the linear dynamic range by an additional order of magnitude over the SCIEX Triple Quad 5500 System while maintaining the sensitivity and robustness of the system. In addition, the use of the rapid 5 msec polarity switching allows detection of ions in positive and negative mode in a single run without compromising data quality.

Figure 1. High Linear Dynamic Range Observed in Both Positive and Negative Modes Using the SCIEX Triple Quad 5500+ System. Calibration curves for A) MDMA in positive mode and B) THC-COOH in negative mode. Excellent linear response and sensitivity were observed for all analytes, even with fast (5msec) polarity switching time.

Key Features of the SCIEX Triple Quad 5500+ – QTRAP Ready System for Forensic Studies

- The SCIEX Triple Quad 5500+ System is an easy to use, rugged and versatile instrument for higher throughput screening applications

- HED detector technology allows expanded linear dynamic range (LDR) for broad quantitative coverage

- Rapid 5 msec polarity switching enables ion detection in positive and negative mode, negating the need for the two injection workflow typically performed

- Reduced analysis time and wider LDR mean lab results can be delivered quicker with minimized re-analysis needed

- QTRAP functionality can be enabled, allowing analyte identification using the ion trap to collect MS/MS spectra that can be matched to library spectra for added confirmation

- Turbo V™ source with Curtain Gas™ interface reduces chemical noise even when running high flow rates

Methods

Analytes and Solutions: A total of 93 target analytes plus internal standards were purchased from Cerilliant Corporation (Round Rock, TX). Two solutions were prepared in methanol: one for the 93 analytes (analyte standard stock solution) and the other for the internal standards (IS standard stock solution). The list of target analytes and internal standards is the same as the one used in a previously-published technical note.[1]

Calibrator Preparation: A 10 µg/mL analyte stock standard solution was prepared in MeOH. Six levels of calibrators ranging from 10 µg/mL to 100 pg/mL were prepared, along with a 1 ng/mL IS standard stock solution.

Sample Preparation: 90 µL of blank urine sample and 10 µL of each calibrator solution were mixed with 25 µL of ICMS Rapid Hydrolysis Buffer, 20 µL IMCSzyme and 10 µL of the 1 ng/mL IS standard stock solution. IMCS Rapid Hydrolysis Buffer and IMCSzyme solutions were purchased from IMCS (Columbia, SC). Hydrolysis time was 60 min at 55˚C. Following hydrolysis, 0.2 mL of methanol and 0.625 mL of water were added to the mixture. The resulting solution was centrifuged at 21,000 g for 10 min. 50 µL of the supernatant was transferred to a glass vial with insert for analysis by LC-MS/MS.

Liquid Chromatography: HPLC separation was performed on an ExionLC™ System using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6µm, 00B-4495-E0). The separation conditions were identical to those used in the aforementioned technical note.[1] Mobile phases were ammonium formate in water (MPA) and formic acid in methanol (MPB). The injection volume was 5 µL and the LC runtime was 6.5 min.

Mass Spectrometry: Both the SCIEX QTRAP 5500 System and SCIEX Triple Quad 5500+ – QTRAP Ready System were run using the same method parameters and the same Turbo V™ source, with generic settings appropriate for the flow rate. The same LC stack was used and moved between the instruments in an attempt to reduce variability and to allow direct comparison of the MS instruments performance. An acquisition method consisting of 196 MRM transitions was created using the *Scheduled* MRM™ Algorithm in Analyst® Software 1.7.1 to ensure the highest data quality was acquired. The MRM transitions monitored and the source parameters were based on the previously-published technical note.[1] Each standard mixture was injected in triplicate. Data was processed in MultiQuant™ Software 3.0.3.

Generation of Calibration Curves Using MRM

The optimized *Scheduled* MRM Algorithm acquisition method used in this study allows quantification of the target analytes through the detection of two MRM transitions per analyte. Since data was acquired on both the QTRAP 5500 and the SCIEX Triple Quad 5500+ System, direct comparison can be made between the sensitivity, linear dynamic range and overall performance of the two systems.

First, calibration curves were generated using the two MRM transitions for each analyte. As seen in Figure 2, good linearity was observed for both instruments for methedrone from 1 pg/mL to 0.1 µg/mL (Figure 2, A and B). However, the SCIEX Triple Quad 5500+ System was the only system able to maintain a linear response up to 1 µg/mL (Figure 2, C and D). This is mainly due to the HED detector system that is capable of providing up to six orders of linear dynamic range (LDR), hence allowing broader quantitative coverage for demanding forensic workflows.
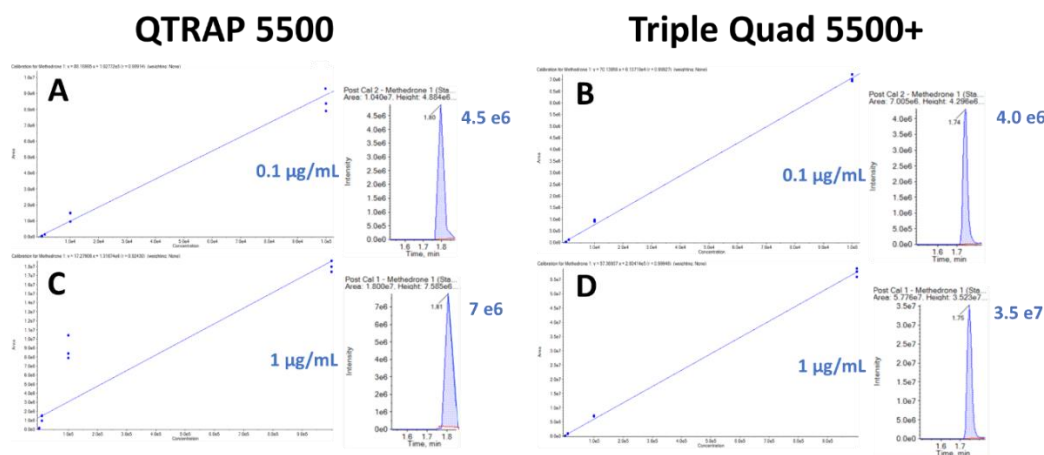

Figure 2: Improved Linear Dynamic Range (LDR) Using the SCIEX Triple Quad 5500+ – QTRAP Ready System. Similar calibration curves were observed for both instruments for methedrone from 1 pg/mL to 0.1 µg/mL on the SCIEX QTRAP 5500 (A) and the SCIEX Triple Quad 5500+ (B). Linearity was maintained only on the SCIEX Triple Quad 5500+ System (D) when the concentration range was increased by one order of magnitude.

Early detector saturation can also be observed on the SCIEX QTRAP 5500 System by comparing the analyte peak areas for concentrations above 0.1 µg/mL. The signal response for methedrone when the concentration was increased from 0.1 to 1 µg/mL should increase by 10x. The peak area increased by an order of magnitude from 4.0 e6 to 3.5 e7 on the SCIEX Triple Quad 5500+ System (inset, Figure 2 B and D) but only increased from 4.5 e6 to 7.0 e6 on the SCIEX QTRAP 5500 System (inset, Figure 2 A and C). This creates the observed non-linearity in the standard concentration curves, typical of detector saturation. This demonstrates that the HED detection system on the SCIEX Triple Quad 5500+ System is providing a substantial improvement for confident analyte quantification at the higher analyte concentrations. This is a significant advantage for toxicology laboratories performing targeted quantification on large analyte panels where the possible unknown sample concentrations can vary widely.

Analytical Sensitivity of the SCIEX Triple Quad 5500+ – QTRAP Ready System

Achieving high sensitivity and linearity of calibration is essential to any toxicology laboratory needing to screen and quantitate large panels of target analytes on a daily basis. Figure 3 shows the extracted ion chromatogram (XIC) traces for the two transitions (290.1 Da → 168 Da, Figure 3B, and 290.1 Da → 105 Da, Figure 3C) of benzoylecgonine and the two resulting calibration curves (Figure 3A). The two series of XIC traces show a high level of consistency and precision across the calibration series for concentrations ranging from over 5 orders of magnitude. In addition, excellent linearity was observed for both transitions of benzoylecgonine with R^2 values of 0.9996 and 0.9975, respectively.

Using an analytical method that consistently delivers reproducible and accurate results for every injection of every batch is essential to attaining reliable results. In this experiment, a series of three replicate injections were run to evaluate the reproducibility and robustness of the method using the SCIEX Triple Quad 5500+ System. The Percent Coefficient of Variance (%CV) and the Accuracy were calculated for each concentration used in the calibration range. The statistical results for the wide dynamic range calibration curve for benzoylecgonine on the SCIEX Triple Quad 5500+ System are summarized in Table 1. Excellent reproducibility and accuracy were observed across the tested concentration range (5 orders of magnitude) using generic settings and without extensive assay optimization. Similar trends were observed for the majority of the target analytes in this study.
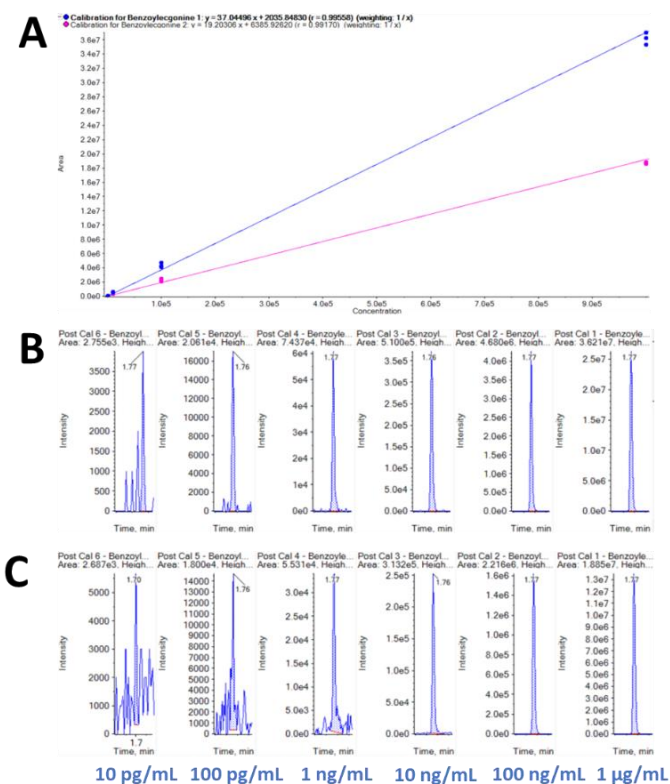

Figure 3. High Sensitivity and Linearity for Benzoylecgonine.
A) Calibration curves resulting from the calibration series for the two transitions of benzoylecgonine from 10 pg/mL to 1 µg/mL. XIC traces and peak integration for benzoylecgonine's B) 290.1 Da → 168 Da and C) 290.1 Da → 105 Da transitions. The calibration curves and XIC traces demonstrate reliable linearity and excellent sensitivity.

Table 1. Statistical Results From the Concentration Curve for Benzoylecgonine Using the SCIEX Triple Quad 5500+ System.

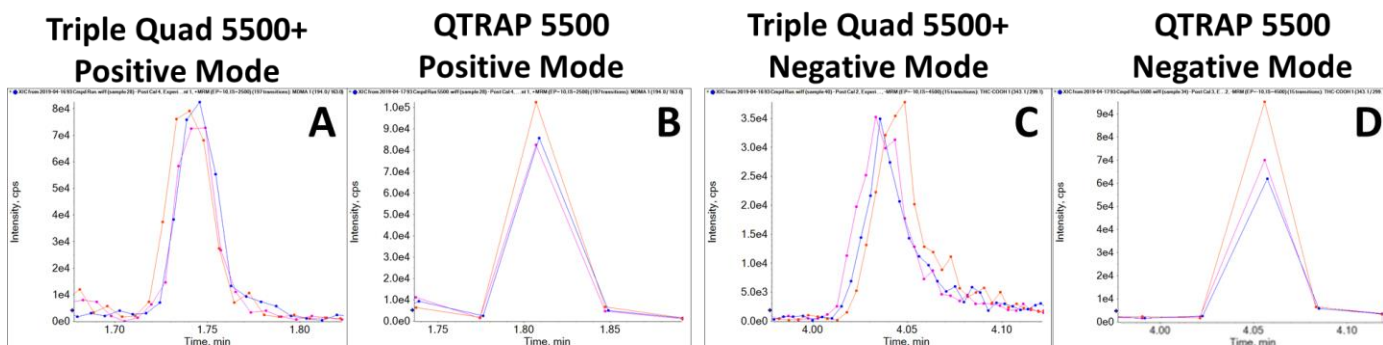| Analyte Concentration (ng/mL)	Mean Area (N=3)	Standard Deviation	CV (%)	Accuracy
0.01	68	15.63	13.53	86.83
0.1	151	55.12	6.50	104.74
1	952.7	83.66	8.78	96.08
10	10,251	862.55	8.41	102.51
100	111,866	3,700.45	3.30	111.85
1000	1,001,033	54,560.09	5.45	100.11

Figure 4: Improved Peak Sampling on the SCIEX Triple Quad 5500+ Using 5 msec Polarity Switching Time. A dramatic improvement in the data points across the LC peak is observed between the SCIEX Triple Quad 5500+ using 5 msec polarity switching time and the SCIEX QTRAP 500 using 50 msec. Plots show the improved sampling for a positive mode analyte (MDMA) and a negative mode analyte (THC-COOH).

Quantitative Accuracy of the SCIEX Triple Quad 5500+ – QTRAP Ready System

As detailed earlier in this technical note, the acquisition method makes use of the 5 msec polarity switching time to attain comprehensive coverage and data quality in both positive and negative modes. Fast polarity switching is especially critical in toxicology workflows with a very large number of target compounds to ensure that sampling is sufficient for accurate analyte quantification even at low concentration. This method has 196 transitions, so the 5 msec polarity switching time is critical to attaining comprehensive coverage and sufficient data sampling when switching from positive to negative mode.

Figure 4 details the number of data points acquired across the LC peak for a positive mode analyte (MDMA) and a negative mode analyte (THC-COOH), acquired on both the SCIEX Triple Quad 5500+ System and the SCIEX QTRAP 5500 System at concentrations of 1 ng/mL and 10 ng/mL, respectively. As seen in Figure 4, the number of points across the LC peak at baseline is only sufficient to enable accurate and confident quantification of the target analytes in this panel on the SCIEX Triple Quad 5500+ System. This is due to the reduction of the polarity switching time from 50 to 5 msec, which results in a gain of 90 msec per cycle. This time is efficiently used on the SCIEX Triple Quad 5500+ System to collect more data points across the peak. Table 2 shows the statistical result differences from the calibration curves for MDMA between the SCIEX Triple Quad 5500+ System and the SCIEX QTRAP 5500 System. These results demonstrate that the use of fast polarity switching times on the SCIEX Triple Quad 5500+ System enabled confident and accurate quantitation of the target analytes, even at low LOQ.

Table 2. Statistical Result Differences From the Calibration Curves for MDMA Between the SCIEX Triple Quad 5500+ System and the SCIEX QTRAP 5500 System.

Analyte Concentration (ng/mL)	5500+ CV (%)	5500+ Accuracy	5500 CV (%)	5500 Accuracy
0.1	18.72	78.89	N/A	N/A
1	12.84	82.74	8.78	86.08
10	7.30	92.77	8.41	86.93
100	7.36	112.38	3.30	111.18
1000	3.20	83.60	4.07	118.85
10000	4.02	95.31	3.10	80.63

Conclusions

A comprehensive and sensitive drug screening workflow for the analysis of a panel of 93 forensic compounds in urine is described using the SCIEX Triple Quad 5500+ System. The method combines both positive and negative polarity electrospray ionization modes into one comprehensive quantitation method which covers a large panel of target analytes. The results presented demonstrate how the HED detection system on the SCIEX Triple Quad 5500+ System extended the calibration range of the target analytes when compared to the SCIEX QTRAP 5500 System. Extending the high end of the calibration curve resulted in an additional order of linear dynamic range. This improvement allows toxicology laboratories to work with wider calibration ranges and reduces the risk of further sample preparation or re-analysis when analytes in some unknown samples fall above the linear range.

An additional improvement of the SCIEX Triple Quad 5500+ System is the faster polarity switching time which results in better analyte coverage and data quality without sacrificing workflow sensitivity or reproducibility. The results presented show that sufficient data quality and accurate target analyte quantitation can be achieved when using 5 msec polarity switching time even at low analyte concentration. Overall, these results demonstrate that the SCIEX Triple Quad 5500+ System provides substantial improvements for workflows requiring confident and accurate analyte quantification in biological matrices.

References

1. Rapid and Sensitive Analysis of a 93-Compound Forensic Panel in Urine Using the QTRAP®/Triple Quad™ 4500 LC-MS/MS System, SCIEX Technical Note. Document number: RUO-MKT-02-2870-A.

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