Leveraging sensitivity improvements for low-level detection of drugs, metabolites, and endogenous hormones in complex biological matrices

Using the SCIEX 7500 system, powered by SCIEX OS software

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The ability to accurately quantify low levels of analytes in a wide variety of complex biological matrices is a critical requirement for many bioanalytical workflows. In the forensic laboratory, expanding the number of analytes per method—while maintaining the required level of laboratory throughput—is becoming increasingly challenging given the complexity and diversity of the relevant matrices in which the analytes need to be detected. These requirements highlight the need for analytical instrumentation capable of delivering the quantitative performance and sensitivity necessary to meet these analytical challenges.

The SCIEX 7500 system has demonstrated potential for the quantification of low-level analytes in multiple biological matrices. The introduction of hardware features, including the OptiFlow Pro ion source with E Lens probe and the D Jet ion guide, has resulted in improvements in the generation, capture and transmission of ions into the mass spectrometer. These technological improvements have enabled scientist to go beyond the current limits of sensitivity for their most challenging and demanding workflows.[1]

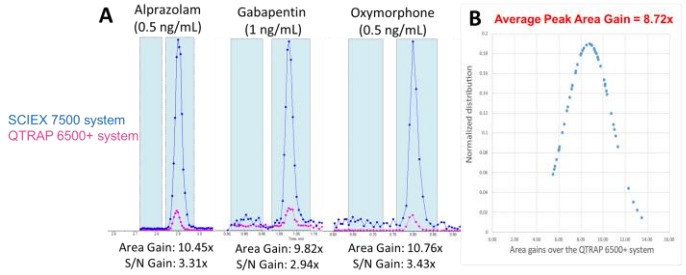

Figure 1. Improved sensitivity on the SCIEX 7500 system for the detection of drugs of abuse. A) Extracted ion chromatogram (XIC) comparisons between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for three drugs of abuse extracted from human whole blood show a significant improvement in area gain and signal to noise. B) Bell distribution plot showing the normalized distribution area gains for the 98 MRM transitions monitored in this workflow. The average peak area gains were 8.72-fold.

In this technical note, the technological innovations on the SCIEX 7500 system are leveraged to improve the sensitivity and overall quantification performance of a number of challenging forensic workflows. The impact of these sensitivity gains was investigated by comparing the signals observed on the SCIEX 7500 system and a previous generation instrument, the QTRAP 6500+ system. The results showcase improvements in both lower limits of detection (LLOD) and quantification (LLOQ), which provide the ability to routinely and robustly detect ultra-low levels of analytes extracted from challenging biological matrices. This increased sensitivity also enables smaller sample and injection volumes to be used, significantly minimizing matrix interferences and, therefore, providing more consistent ionization. The results also demonstrate that the added sensitivity can be leveraged to simplify sample preparation procedures, which improves productivity for routine analysis. The ability to analyze these compounds without the need for laborious and time-consuming sample preparation procedures improves the overall operational efficiency and throughput, enabling forensic testing laboratories to exceed their current productivity levels.

Advantages of the SCIEX 7500 system for forensic workflows

- Lower limits of detection and quantification, improving the overall quantification performance when compared to data acquired on the previous generation instrument, the QTRAP 6500+ system
- Average peak area gains ranging from 7.25 to 8.72x for compounds with a wide range of chemical and physical properties analyzed in several complex biological matrices (Figure 1)
- Low-level, previously undetectable drugs and metabolites can be detected and accurately quantified
- Higher precision and accuracy achieved at the LLOQ, leading to more confidence in the quantification of those low-level analytes
- Simplified sample preparation procedure, negating the need for laborious and time-consuming sample cleanup techniques such as solid-phase extraction (SPE) or liquid-liquid extraction (LLE)
- Lower sample volume and smaller injection volumes can be used for the analysis, minimizing matrix effects, which improves overall ionization efficiency and allows multiple analyses from precious low-volume samples
- Overall sensitivity improvements enable the development of rapid, simplified, and robust workflows in complex biological matrices while consistently delivering high-quality data time and time again.

Methods

Sample preparation: Details on each sample preparation procedure are provided in the corresponding technical notes and referenced in next sections.

Liquid chromatography: Separations were performed on an ExionLC system using a variety of Phenomenex columns and mobile phase compositions. Details on LC conditions are provided in the corresponding technical notes.

Mass spectrometry: A SCIEX 7500 system equipped with an OptiFlow Pro ion source with an analytical probe, an E Lens probe and an electrospray ionization (ESI) probe was used. For comparison purpose, a QTRAP 6500+ system equipped with an IonDrive Turbo V ion source was used. Both instruments were optimized for maximum sensitivity. The same LC stack was used and moved between the instruments to reduce variability and allow direct instrument performance comparison. Additional (and in the case of THC-COOH in oral fluid, tertiary) multiple reaction monitoring (MRM) transitions were monitored for the suite of analytes targeted in the different methods.

Data acquisition and processing: Data were acquired using SCIEX OS software on the SCIEX 7500 system. For the QTRAP 6500+ system, data was acquired using Analyst software 1.7. All data were processed using SCIEX OS software where detection and integration of the peaks from the background were accomplished within the viewing window using the MQ4 algorithm. The Peak to Peak algorithm was used for signal to noise calculations in the Analytics module of SCIEX OS software.

Drugs of abuse and metabolites in human whole blood

In this experiment, an optimized and sensitive method for the detection of a panel of 49 drugs of abuse and metabolites extracted from human whole blood was developed using the SCIEX 7500 system. The sensitivity improvements provided by the SCIEX 7500 system enabled sensitive quantification of the analytes in the sub-ng/mL range, with some down to pg/mL levels, while maintaining linearity, precision, and accuracy of measurement.[2] Figure 1A shows example XIC comparisons between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for three drugs of abuse. As seen in Figure 1A, the raw signal gain and the improved signal to noise are clearly visible for A) alprazolam at the 0.5 ng/mL concentration level, B) gabapentin at the 1 ng/mL concentration level and C) oxymorphone at the 0.5 ng/mL concentration level. A significant increase in both the integrated peak area and the signal-to-noise was observed across all the analytes targeted in this study. For example, the SCIEX 7500 system achieved peak area gains ranging from 9.82 to 10.76 and signal-to-noise gains ranging from 2.94 to 3.43 over the QTRAP 6500+ system for the three analytes highlighted in Figure 1A.

To further demonstrate the drastic improvement in sensitivity, the normalized distribution of the peak areas (N=3) for each of the two MRM transitions monitored for the 49 drugs and metabolites was plotted, to compare the gain of the SCIEX 7500 system over the QTRAP 6500+ system. The resulting bell distribution curve is shown in Figure 1B. The peak area gains for the analytes included in this panel ranged from 5.48 to 13.09-fold when using the SCIEX 7500 system. This averaged 8.72-fold increase in peak areas ultimately resulted in higher signals, greater S/N and lower limits of quantification (LLOQ) for all compounds, transitions, and replicates included in this workflow.

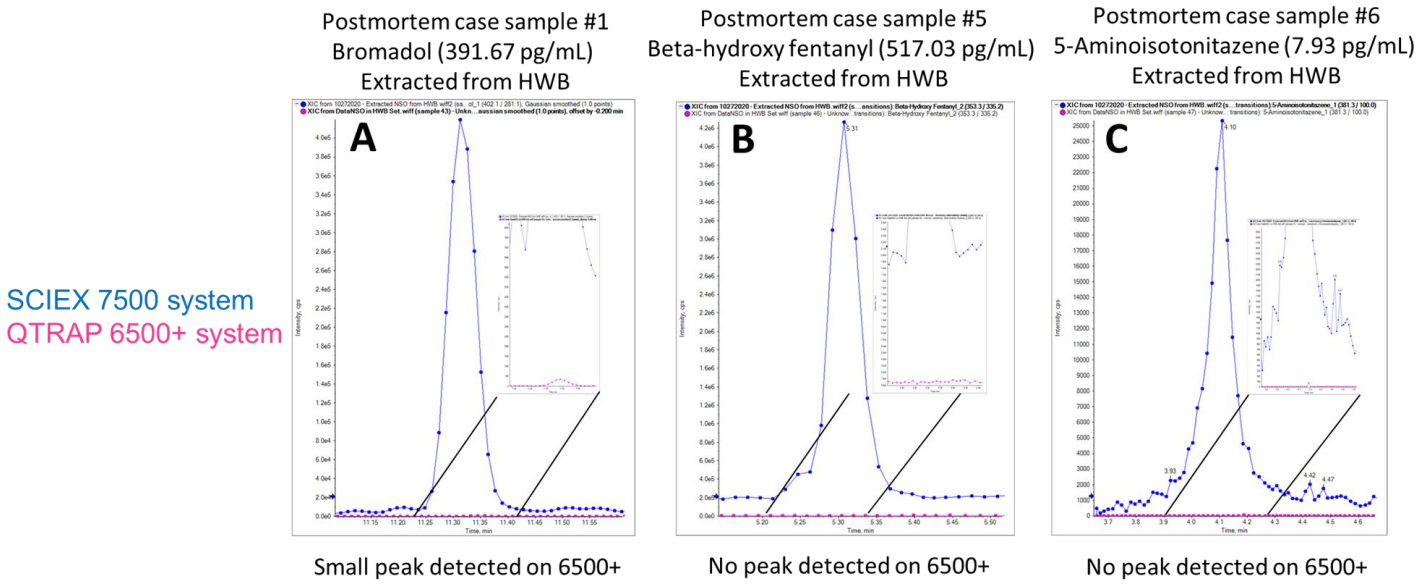

Figure 2. High sensitivity for the detection of low-level NSO and metabolites in human whole blood. Extracted ion chromatogram (XIC) comparisons between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for three authentic postmortem case samples. Measurable signals enabled quantification of low-level analytes using the SCIEX 7500 system. Small peaks or no measurable signals were detected on the QTRAP 6500+ system.

Fentanyl analog isomers and novel synthetic opioids (NSO) in human whole blood

The sensitivity of the SCIEX 7500 system was further investigated for the detection of 32 fentanyl analog isomers and NSO in human whole blood. The quantification performance of the system provided the ability to accurately quantify low levels of NSO in postmortem case samples that would normally go undetected, providing the evidence of combined opioid drug toxicity leading to death.[3] Figure 2 compares the sensitivity levels for the same postmortem case samples analyzed with both the SCIEX 7500 system and the QTRAP 6500+ system in which NSO and metabolites were present at low concentrations. As seen in Figure 2, the SCIEX 7500 system showed measurable signals that enabled detection and quantification of three low-level analytes: bromadol at 391.67 pg/mL in authentic postmortem case sample #1, beta-hydroxy fentanyl at 517.03 pg/mL in authentic postmortem case sample # 5, and 5-aminoisotonitazene at 7.93 pg/mL in authentic postmortem case sample #6. Small peaks (Figure 2A) or no measurable signals (Figure 2, B and C) were detected when analyzed on the QTRAP 6500+ system. This demonstrates that the SCIEX 7500 system provides the necessary sensitivity for the detection and accurate quantification of low levels of drugs and metabolites that were not previously detectable (below the noise) on the QTRAP 6500+ system, providing a means to monitor ultra-potent NSO in overdose scenarios.

To further investigate the sensitivity improvement across the 32 analytes targeted in this study, the normalized distribution of the peak areas (N=3) for each of the two MRM transitions monitored for each analyte was plotted to compare the gain of the SCIEX 7500 system over the QTRAP 6500+ system. Figure 3 shows the resulting bell distribution curve where the peak area gains observed for the SCIEX 7500 system over the QTRAP 6500+ system range from 3.57 to 20.70-fold. Sensitivity gains for the detection of novel synthetic opioids in human whole blood were significant on the SCIEX 7500 system, with average peak area increases of 8.37-fold for the 64 transitions monitored. This increase in sensitivity lead to the detection and accurate

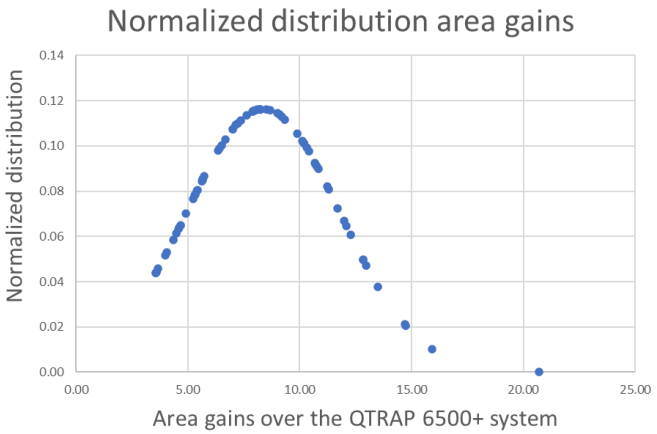

Figure 3. Peak area gains for a panel of 32 analytes on the SCIEX 7500 system. Averaged (N=3) peak areas for each of the 64 MRM transitions monitored in this workflow were normalized and the gains observed for the SCIEX 7500 system over the QTRAP 6500+ system were plotted. The average peak area gains were 8.37-fold.

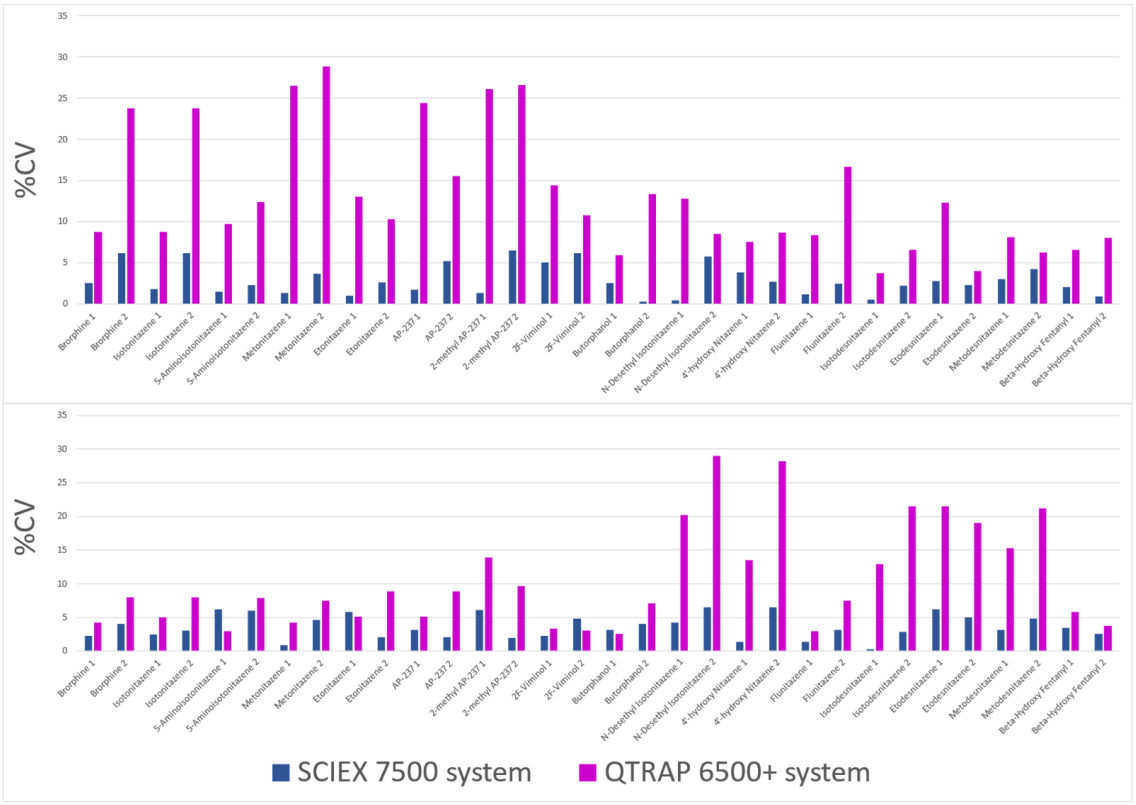

Figure 4. Increased sensitivity on the SCIEX 7500 system leads to significantly improved precision. Excellent precision (%CV of less than 10%) was observed on the SCIEX 7500 system (blue bars) for three replicate injections of a low-level (1 ng/mL) human whole blood calibrator. The %CV values for the QTRAP 6500+ system were generally less than 20%, with a number of outliers nearing 30% (pink bars). Increased precision provides more confidence in quantification levels of low-level drugs and metabolites present in postmortem case samples.

quantification of low-level drugs and metabolites which provides the necessary evidence to support medicolegal death investigation following intake of high-potency drugs.

In addition to enabling detection of low levels of highly potent substances in postmortem case samples, the added sensitivity provided by the SCIEX 7500 system improved the reproducibility of the assay. Three replicates of a low-level (1 ng/mL) human whole blood calibrator were injected and %CV were calculated. As shown in the histograms in Figure 4, excellent precision was observed on the SCIEX 7500 system with %CVs of less than 10% for the low-level calibrator. CVs for the same calibrator solution analyzed on the QTRAP 6500+ system were generally less than 20%, with a number of outliers nearing 30%. The lower %CV observed on the SCIEX 7500 system is a clear measure of improved assay precision on the platform, which in turn contributes to an overall improvement of quantitative performance at or around the LLOQ. Overall, this improvement leads to more confidence in the quantified amounts of drugs and metabolites detected in postmortem case samples, which is critical when determining the cause of death following an accidental overdose.

Drug analysis using DBS

Here, an optimized sample extraction procedure was combined with a robust detection method for the analysis of 24 drugs and metabolites extracted from dried blood spots (DBS). The performance of the SCIEX 7500 system was leveraged to provide the required level of sensitivity for drug analysis, with quantification of all the analytes in the subng/mL range.[4] Figure 5A shows signal comparisons between the SCIEX 7500 system and the QTRAP 6500+ system for three analytes targeted in this assay: buprenorphine at the 5 ng/mL concentration level, EDPP at the 5 ng/mL concentration level and imipramine at the 10 ng/mL concentration level. As seen in Figure 5A, the analytes had a small detectable signal on the QTRAP 6500+ system but had a much-improved signal on the SCIEX 7500 system. Higher levels of sensitivity for drug analysis using DBS were observed in both integrated peak area and signal-to-noise across the 24 analytes targeted in this study. For example, the SCIEX 7500 system achieved peak area gains ranging from 7.5 to 7.9 and signal-to-noise gains ranging from 2.7 to 3.1 over the QTRAP 6500+ system for the three analytes showcased. This higher sensitivity enabled detection and accurate quantification of low levels of drugs and metabolites in 30 µL of human whole blood extracted from DBS.

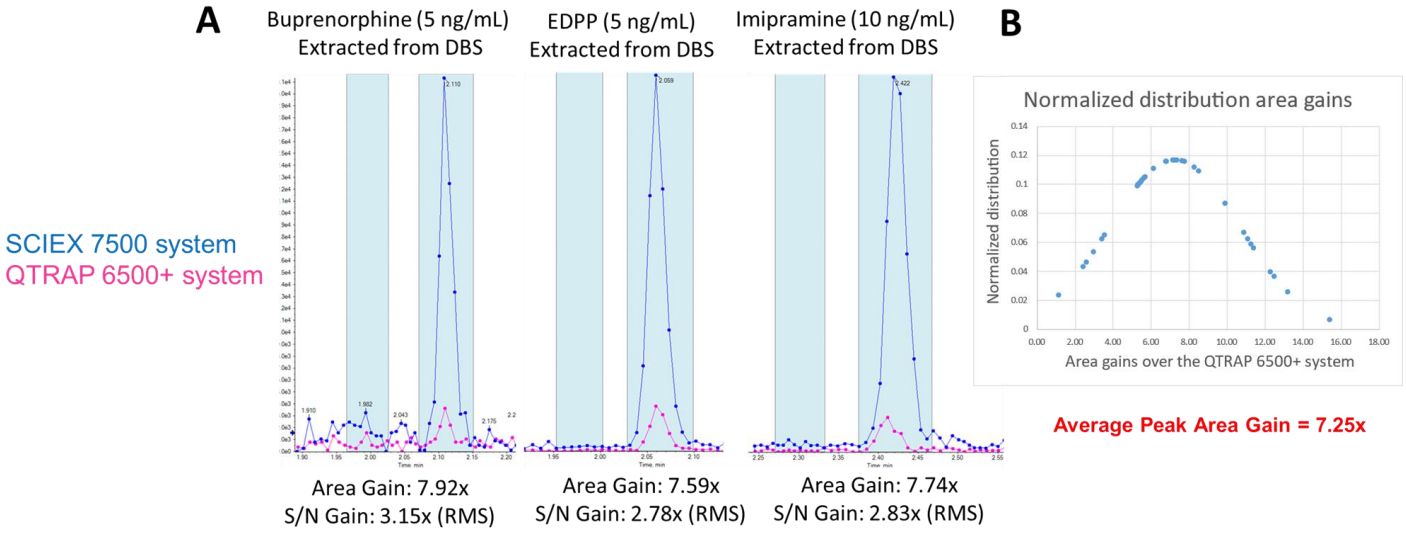

Figure 5. Increased sensitivity for the detection of drugs extracted from DBS. A) Extracted ion chromatogram (XIC) comparisons between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for three drugs extracted DBS. The signals show a considerable increase in area gain and signal to noise across the drugs included in the panel. B) Peak area gains showing the normalized distribution area gains for the 48 MRM transitions monitored in this workflow. The average peak area gains were 7.25-fold.

The normalized distribution of the peak areas (N=3) for each of the two MRM transitions monitored for the 24 drugs and metabolites was plotted to compare the gain of the SCIEX 7500 system over the QTRAP 6500+ system. Figure 5B shows the sensitivity improvements for the 48 MRM transitions monitored using the SCIEX 7500 system and the QTRAP 6500+ system. Peak areas for the 24 analytes targeted in this assay were 7.25x higher, on average, when using the SCIEX 7500 system. The gains observed ranged from 1.13 to 15.38, demonstrating the improvement in sensitivity for drug analysis using DBS on the SCIEX 7500 system.

THC-COOH in oral fluid

In this experiment, the sensitivity of the SCIEX 7500 system was leveraged for the accurate quantification of pg/mL levels of THC-COOH in oral fluid using a simple dilute and shoot approach, which negates the need for time-consuming procedures such as solid-phase extraction (SPE), evaporation and reconstitution. The use of the MRM workflow enabled quantification of THC-COOH down to 50 pg/mL, while the MRM^3 workflow successfully eliminated background interferences, further improving the LLOQ of the workflow down to 10 pg/mL. [5] The same samples were also acquired on the QTRAP 6500+ system to compare the sensitivities of the two workflows between the two platforms. Figure 6 shows overlaid data for both the MRM (A) and MRM^3 (B) workflows from the two platforms. The two plots highlight the signal difference at 25 ng/mL (left) and 5 ng/mL (right) using the MRM and MRM^3 workflows, respectively. As seen in the XIC trace comparisons, the SCIEX 7500 system showed significant improvements in both measured peak area and signal-to-noise over the QTRAP 6500+ system. The area gains were 7.9x (MRM) and 7.3x (MRM^3) while the signal-to-noise gains were 3.1x (MRM) and 3.2x (MRM^3). The results demonstrate that the sensitivity of the SCIEX 7500 system can be leveraged by simplifying the sample preparation procedure, enabling a dilute-and-shoot approach as opposed to tedious and time-consuming extraction techniques such as SPE or LLE. Effectively employing a dilution rather than a concentration in the sample preparation leads to significantly reduced introduction of matrix components into the system, minimizing matrix effects that can affect assay performance in many ways. In addition, the results demonstrate that the ion trap functionality of the SCIEX 7500 system can be leveraged to further minimize matrix effects and improve the

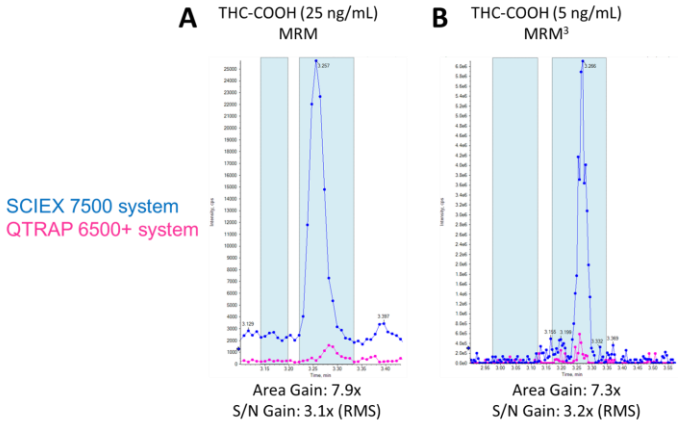

Figure 6. Sensitivity improvements for the detection of THC-COOH in oral fluid using the MRM and MRM^3 workflows on the SCIEX 7500 system. XICs were compared between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) using the MRM (A) and the MRM^3 (B) workflows. The signals show a considerable increase in area gain (7.9x for the MRM workflow and 7.3x for the MRM^3 workflow) and signal to noise (3.1x for the MRM workflow and 3.2x for the MRM^3 workflow).

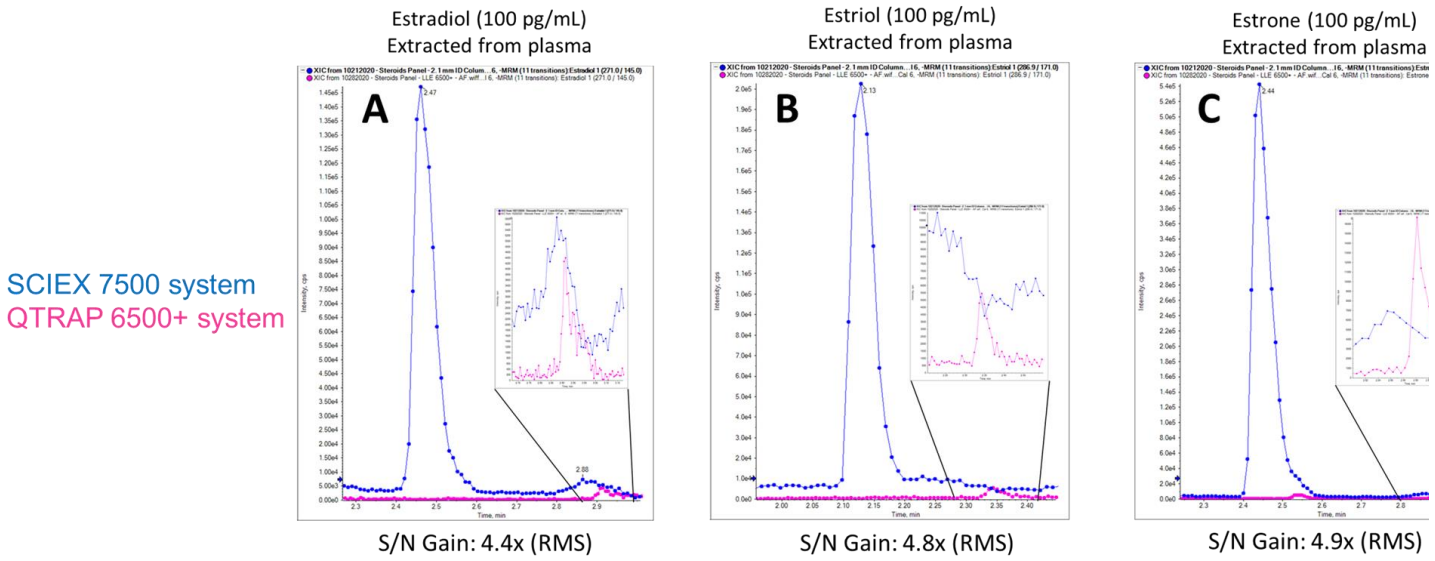

Figure 7. Sensitivity improvements for the analysis of endogenous species. Signal comparison between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for the analysis of estradiol, estriol and estrone at the 100 pg/mL concentration level. The average S/N gains were 4.7-fold.

LLOQ. These attributes are shown to be advantageous for routine analysis as they improve overall assay sensitivity as well as laboratory throughput and efficiency.

Steroids in plasma

The SCIEX 7500 system was used for the analysis of estrogens spiked in human plasma. The improved sensitivity enabled robust and accurate quantification of low-levels (1 pg/mL, 0.05 pg on column) of endogenous hormones using a reduced sample volume and faster workflow.[6] Figure 7 shows signal comparisons between the SCIEX 7500 system and the QTRAP 6500+ system for the three estrogens targeted in this assay: A) estradiol, B) estriol and C) estrone, spiked in human plasma at the 100 pg/mL concentration level. Despite the higher background level observed on the SCIEX 7500 system, the XICs show an average improvement in signal-to-noise of 4.7 across the three estrogens. This higher sensitivity enabled detection and accurate quantification of low levels of estrogen species from only 200 µL of sample with excellent reproducibility and robustness.

To further investigate the sensitivity of the SCIEX 7500 system over the QTRAP 6500+ system, two sets of calibrators were prepared. The first set of calibrators were extracted out of a 500 µL starting sample volume and 50 µL was injected into the QTRAP 6500+ system. The second set of calibrators were extracted out of a 200 µL starting sample volume and 25 µL was injected into the SCIEX 7500 system. Figure 8 displays the example XIC comparisons between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for A) estradiol at 0.1 ng/mL and B) testosterone at 0.05 ng/mL. The XICs show similar signal-to-noise to the QTRAP 6500+ system with a five times lower amount injected on column. This work suggests that the SCIEX 7500 system can deliver the same results as the QTRAP 6500+ system with: 1) reduced sample volume, 2) lower injection volume, and 3) a larger dilution factor during the sample preparation procedure. These advantages have additional benefits in matrix interference reduction, which provides more consistent ionization efficiencies for the analysis of endogenous species. Added benefits also include less sample injected onto the analytical column and mass spectrometer which improves column lifetime and reduces mass spectrometry maintenance, respectively.

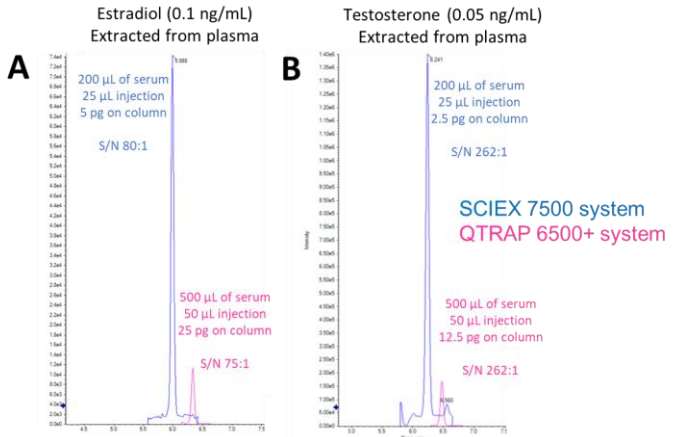

Figure 8. The SCIEX 7500 system achieves similar S/N results than the QTRAP 6500+ system with 5x less injected on column. The XIC traces show the signal comparison between the SCIEX 7500 system (blue) and the QTRAP 6500+ system (pink) for the analysis of estradiol at the 0.1 ng/mL concentration level and testosterone at the 0.05 ng/mL concentration level.

Conclusions

The sensitivity improvements afforded by the SCIEX 7500 system to accurately detect and quantify low levels of drugs, metabolites, and endogenous hormones were demonstrated for a number of forensic workflows. The examples showed that the hardware features on the SCIEX 7500 system enable low-level quantification of analytes with a wide range of chemical and physical properties. The resulting increase in sensitivity can be leveraged to:

- Achieve robust and accurate quantification of previously undetectable compounds
- Simplify sample preparation procedures, perform larger dilutions, and use lower sample and injection volumes, which have the added benefits of minimizing matrix effects and maintaining ionization efficiencies
- Increase confidence in quantification, improving both accuracy and precision at the lower concentration levels and resulting in increased confidence in the quantification of low-level analytes
- Reduce the amount of materials loaded on the head of the column and into the mass spectrometer, which should improve column lifetime
- Improve laboratory efficiency through potentially reduced complexity of sample preparation procedures, resulting in improved laboratory productivity and overall performance

References

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