Significant sensitivity increases provides 30% more polar metabolites quantified in plasma

With the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

Robert Proos, Darren Dumlao, Kranthi Chebrolu, Christie Hunter
SCIEX, USA

Studying the impact of biological perturbations on the metabolome requires high sensitivity and specificity, as well as the ability to multiplex large numbers of known metabolites in a single assay. The utility of LC-MS/MS analysis for the quantification of metabolites from biological samples has become widely accepted as the key analytical solution due to the aforementioned requirements.[1]

In addition to very good sensitivity and specificity, targeted analysis using multiple reaction monitoring (MRM) on triple quadrupole MS systems has the advantage of throughput and simplified data processing. By focusing on known important metabolites that are key to biology, quantitative results provide key biological insights. These platforms are capable of fast polarity switching, allowing a broad range of analytes to be interrogated in a single injection. Finally, because of broad dynamic range typically required for biological samples, high sensitivity is a critical attribute of the LC-MS system for the analytes with needing lower detection limits that were previously undetectable and for smaller sample sizes.

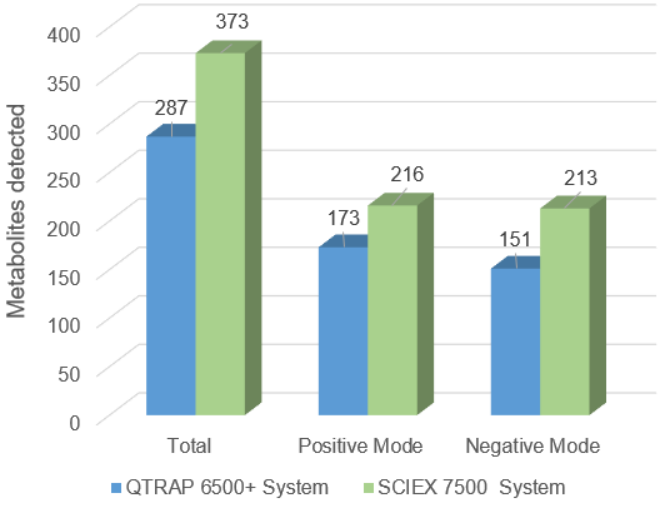

Figure 1. Increased sensitivity on the SCIEX 7500 System improved the number of metabolites detected. A similar analysis was run on both the QTRAP® 6500+ System and the SCIEX 7500 System was performed. The SCIEX 7500 System showed a 25% increase in metabolite detected was observed in positive ion mode, and a 41% increase was observed in negative mode. Note some metabolites were detected in both polarities and thus the overall gain in detected metabolites was 30%.

A targeted LC-MS/MS assay with MRM transitions for over 450 metabolites was used to assess the impact of added sensitivity on the detection and quantification of metabolites in plasma. Using a generic reversed-phase chromatography method and operating the MS systems in both positive and negative polarity modes to obtain broad coverage, a similar method was run on three separate QTRAP 6500+ systems and as well as three separate SCIEX 7500 systems under independent lab settings.

Key features of the SCIEX 7500 System for quantification of polar metabolites

- Provides significant gains in sensitivity though increased generation, capture, and transmission of ions with the:
  - OptiFlow® Pro Ion Source with E Lens™ Technology and the D Jet™ Ion Guide[2]
- NIST SRM 1950 plasma was used as reference material to compare platforms
- Higher sensitivity and S/N on the SCIEX 7500 System yielded large improvements in detected, quantifiable of metabolites- up to 30% more vs the QTRAP 6500+ System (Figure 1)
- Targeted assay is provided, complete with a standard operating protocol, an MRM list, and tools to facilitate method development (sMRM Builder template) can also integrate internal standard kits if required.[3]

Methods

Sample preparation: NIST SRM 1950 plasma was extracted using 8 volumes of methanol then spiked with internal standard QReSS kit (Cambridge Isotopes). After centrifugation to precipitate the proteins, the supernatant was directly analyzed.

Chromatography: Samples were analyzed using the ExionLC™ System with a Kinetex® F5 column (2.1 x 150 mm, 2.6 µm, Phenomenex). Simple linear gradient from 0 to 95% B was used with standard reverse phase mobile phases (A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile) with a flow rate of 200 µL/min. A 1 µL injection volume was used and the column temperature was maintained at 30 °C throughout the analysis.

Mass spectrometry: The same extracted sample was analyzed on both the QTRAP® 6500+ System and the SCIEX 7500 System. Data was collected using methods built with the Scheduled MRM™ Algorithm as well as unscheduled methods. Variations of the method were repeated on two additional instrument sets to confirm the observed sensitivity gains. Detailed method information is available in the supplementary method.[3] Finally, this high throughput method is used to analyze over 400 serum metabolites in a 20 min run period.

Data processing: All data was analyzed using the Analytics module in SCIEX OS Software.

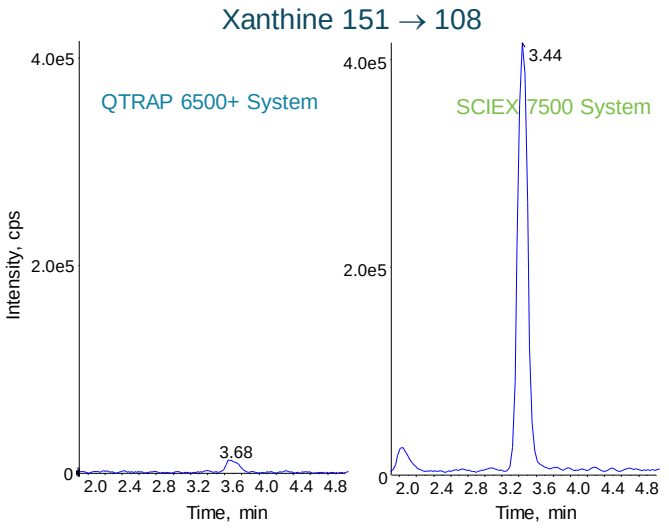

Figure 2. Signal to noise improvement for negative ion mode metabolites. Example shown here for Xanthine, created from guanine during purine degradation. Here the improved area and S/N observed when running the same assay on the SCIEX 7500 System as compared to the QTRAP 6500+ System is highlighted.

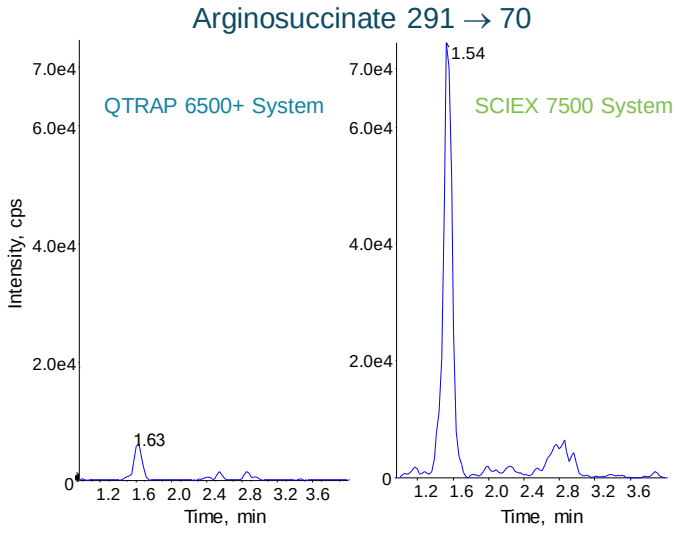

Figure 3. Signal to noise improvement for positive ion mode metabolites. Example shown here of argininosuccinate, an essential amino acid formed from citrulline in the uric acid biosynthetic pathway, highlights the improved area and S/N observed when running the same assay on the SCIEX 7500 System as compared to the QTRAP 6500+ System.

Sensitivity improvements on the SCIEX 7500 System

A number of innovations on the SCIEX 7500 System have resulted in increased sensitivity for MRM analysis. Here, the impact of these sensitivity gains on a targeted metabolomics assay was evaluated by comparing the signals observed after running a similar assay on six instruments (3 QTRAP 6500+ systems and 3 SCIEX 7500 systems). Example comparisons are shown in Figures 2 and 3 for positive and negative ion mode, highlighting the sensitivity gains on the raw signal. Xanthine analyzed in negative ion mode had a small detectable signal on the QTRAP 6500+ System but had a much improved signal on the SCIEX 7500 System (30x area and 8x S/N gains). An example for positive ion mode is argininosuccinate, showing a 13x gain in area and a 4x gain in S/N.

Perhaps more compelling was the ability to detect metabolites on the SCIEX 7500 System that were undetectable (below the noise) on the QTRAP 6500+ System. Two examples are showing in Figure 4, for both positive ion mode (retinal) and negative ion mode (pyridoxic acid). Retinal is a small molecule metabolized through oxidation of β-carotene in the human body and is essential, especially for night vision. Pyridoxic acid is a catabolic product of vitamin B6 metabolism.

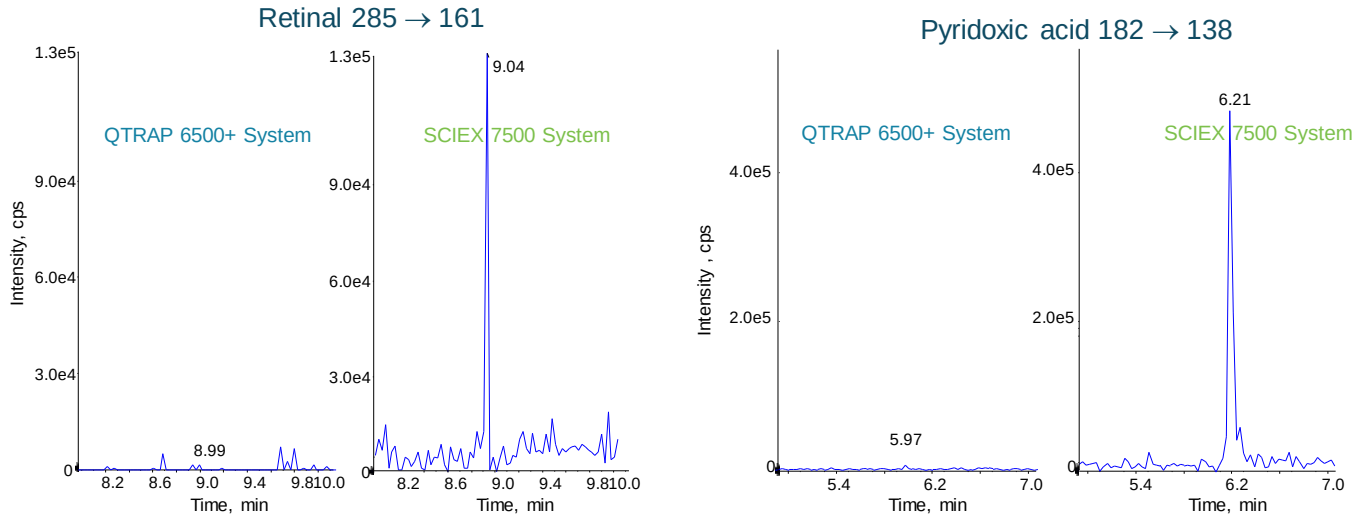

Figure 4. Improved S/N leads to quantification of additional metabolites. Compounds that were not detectable in the plasma sample on the QTRAP 6500+ System are now easily quantified on the SCIEX 7500 System. Retinal was easily quantified in positive ion mode (left) and pyridoxic acid in negative ion mode (right) also showed a large increase in signal and S/N on the SCIEX 7500 System.

Increased numbers of metabolites detected

Next, the results across the assay were compared looking at the peak areas, the peak heights and the S/N between the two instruments and by computing the fold increases for each metabolite. This allowed some general conclusions to be drawn between the two platforms (Table 1). In positive ion mode, the average peak area gain was 17x providing an overall signal / noise gain of 3x. In negative ion mode, the sensitivity increases were a bit more pronounced with a peak area gain of 40x and a S/N gain of 5x. The increased S/N and peak areas led to an increase in the number of metabolites that could be reliably detected and quantified in the assay. Peak detection used a minimum S/N value of 3.

Table 1. Average gains observed on the SCIEX 7500 System vs the QTRAP 6500+ System. The average peak area, peak height and S/N were compared between the two platforms to characterize the average observed gains.

	Average fold increase	
	Positive ion mode	Negative ion mode
Peak area	*17*	*40*
Peak height	*18*	*43*
Signal / noise	*3*	*5*

The resulting gains in metabolites detected and quantified is shown in Figure 1. In positive ion mode, 173 metabolites were detected on the QTRAP 6500+ System and 216 were detected on the SCIEX 7500 System, an increase in detected metabolites of 25%. The results were even more significant in negative mode, with 151 metabolites detected on the QTRAP 6500+ System and 213 detected on the SCIEX 7500 System - a gain of 41%. As some metabolites were measured and detected in both polarities, the overall gain in detected metabolites was 30%.

Confirmation of results on multiple instrument platforms

In order to ensure a robust conclusion, sample analysis was repeated across multiple instrument sets: a total of three QTRAP 6500+ systems and three SCIEX 7500 systems. The S/N for each metabolite detected was measured and then compared between both instruments to compute a fold increase in signal to noise. This was done across the dataset such that a normal distribution for each instrument set could be plotted (Figure 5). Due to the wide range of analytes covered in these global targeted methods, global source conditions were used on both instruments. This is likely the source of some of the larger, or smaller, than expected sensitivity differences between platforms. Nevertheless, the data demonstrated the general increase that can be expected upon translating this global metabolomics method from the QTRAP 6500+ System to the SCIEX 7500 System.

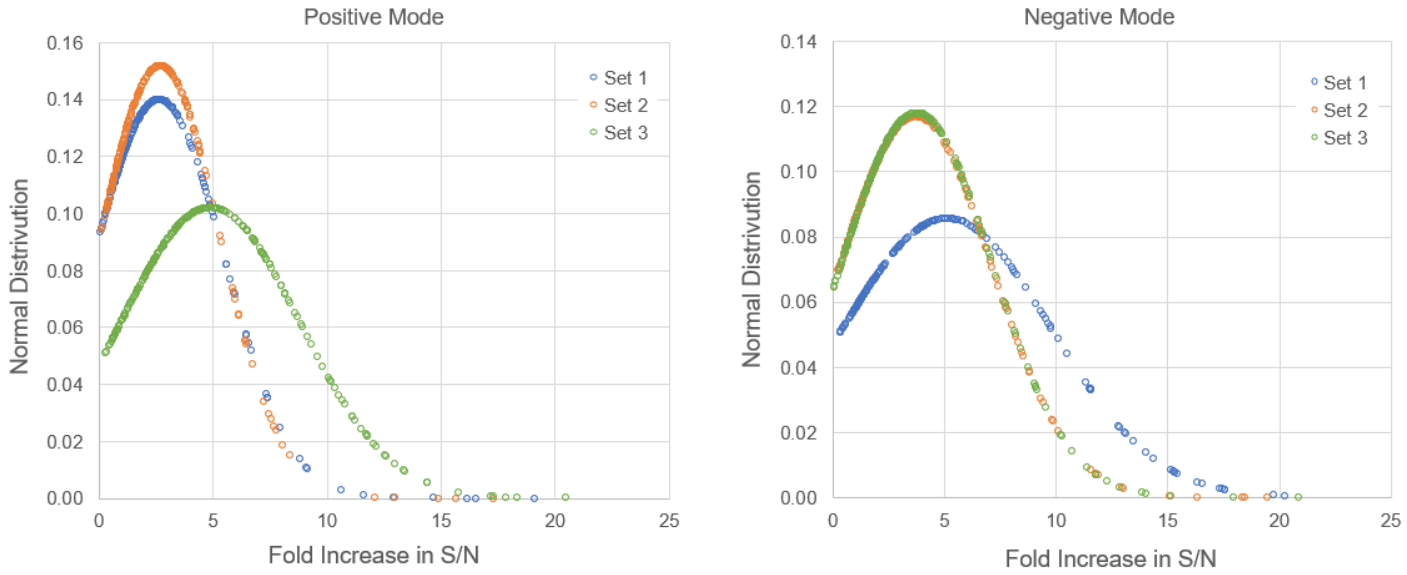

Figure 5. Repeating results on multiple instruments. To confirm the observed sensitivity gains from instrument set 1, similar methods were run on two more instrument sets. As shown in the distributions for the three instrument sets, similar sensitivity gains were observed in both polarities. On average, signal to noise gains of ~3.5 were observed for the SCIEX 7500 System for each instrument set tested. Note general source conditions must be used when monitoring such wide ranging analytes, likely resulting in the broader than expected distribution of S/N gains.

Conclusions

- A targeted MRM assay was developed and run on extracted NIST SRM 1950 plasma in order to compare the performance between the QTRAP 6500+ System and the SCIEX 7500 System
- The higher sensitivity of the SCIEX 7500 System allowed for the detection and quantification of ~30% more metabolites in plasma. Specifically, there was a 25% increase in positive mode, and a 41% increase in negative mode
- The assay was repeated on two other instrument sets to confirm the observation. Using the increase in S/N as the measure of improvement, all three instrument sets showed significant improvements in S/N for a large number of metabolites.

References

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3. Targeted profiling method for metabolomics.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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