The SCIEX Triple Quad™ LC-MS/MS 6500 and the QTRAP® 6500 LC-MS/MS Systems for Targeted Quantitation

For High Sensitivity and Selectivity in Quantitative Analysis

Researchers are constantly challenged to achieve sufficient sensitivity and selectivity in method development, while maintaining robustness and throughput. Detection of analytes at very low levels requires ultra-high sensitivity and good selectivity from the LC-MS/MS system. Whether it is detecting drugs at low levels in protein precipitated plasma, pesticides in food matrices or protein biomarkers in biological fluids, high sensitivity of the platform for detection of the target compounds combined with lowered background noise to get the best signal/noise for the assay is key.

Enhancing the sensitivity of LC-MS/MS instruments requires efficiency along every part of the instrument be optimized to obtain the highest possible transmission. The efficiency of ion production, ion sampling and ion transfer along the full ion path needs to be evaluated.

The SCIEX Triple Quad 6500 and QTRAP 6500 systems have major improvements in sensitivity resulting from improved ionization efficiency, sampling, and linear dynamic range. Using IonDrive™ system technology, major new innovations have been implemented in every part of the system.

Key Benefits of IonDrive™ System Technology for Quantitation

- The SCIEX Triple Quad 6500 and QTRAP 6500 systems have improvements across the ion rail that enhance quantitative experiments
- Ultra-high sensitivity enables very low LOQ's in complex matrices
- Mass range of m/z 5 – 2,000 provides versatility for small molecule and peptide quantitation
- Increased dynamic range with IonDrive high energy detector technology
- Increased ionization efficiency and heat transfer with the IonDrive Turbo V source
- Increased ion sampling efficiency and ruggedness with the IonDrive QJet ion guide

IonDrive™ QJet Ion Guide

Increasing the efficiency of ion transfer into the vacuum region is the key to further gains in sensitivity without compromising robustness. The IonDrive QJet ion guide represents a ground breaking design, significantly increasing ion transfer efficiency. It consists of a dual stage RF guide which improves ion capture from a larger orifice while increasing transmission efficiency into the Q0 region (Figure 1). This is combined with the curtain gas geometry which provides a more robust gas barrier, and better separation of ions from neutrals, and other particles.

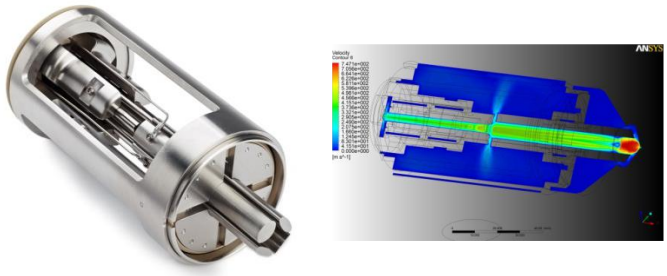


Figure 1. IonDrive QJet Ion Guide. Dual RF stages maximize ion sampling from a large orifice while increasing ion transfer efficiency into the Q0 region – without increasing vacuum load in the analyzer region.

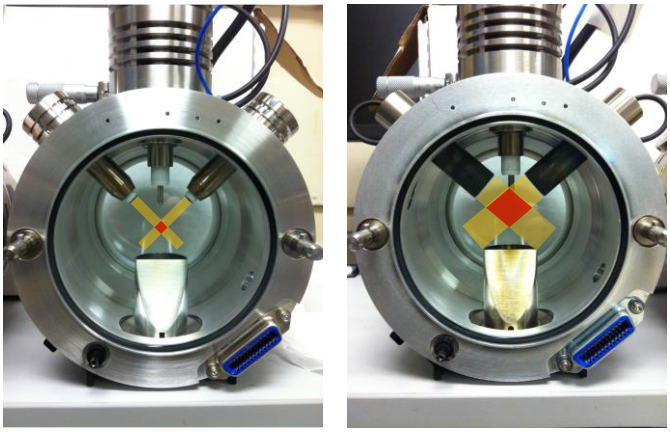


Figure 2. IonDrive™ Turbo V source. Optimized geometry and larger diameter heaters result in higher ionization efficiency and robustness.

IonDrive™ Turbo V Source

The IonDrive Turbo V source builds on the efficiency and ruggedness of the original Turbo V source. The design incorporates an optimized geometry and large diameter heaters. By covering a larger cross-section of the spray cone, two important gains are achieved. The first is improvement in ionization efficiency, especially at higher flow rates and high aqueous mobile phase. The second advantage is a wider region with optimum heating. This makes source optimization faster and simpler, and results in a more robust method (Figure 2).

IonDrive™ High Energy Detector

The high energy detector improves both sensitivity and dynamic range. This advanced detector has a 20x greater counting rate over previous designs, resulting in linear dynamic range gains up to 1 order of magnitude. At the same time, very high sensitivity at the low end is achieved by the pulse counting technology. This detector represents the best of both worlds, with technology that does not sacrifice sensitivity for the sake of dynamic range, or vice versa.

Experimental

To evaluate the bioanalytical performance of the 6500 series system, standard curves of a series of commonly studied analytes were prepared in protein precipitated plasma and in neat solution. UHPLC conditions were employed for all experiments by using a Shimadzu UFLC-XR or Nexera series HPLC system, and a Phenomenex Kinetix C-18 column 2.1 x 50 mm, 2.6 um column. Flow optimization of the IonDrive Turbo V source was performed using flow injections or T-infusion. A QTRAP 5500 system was also used as a benchmark. All comparisons were performed under the same conditions by performing back to back runs on the same sample set, using the same HPLC stack, column, mobile phase bottles, and samples.

Sensitivity

A lower limit of quantitation of 700 attograms (ag) was achieved for alprazolam in protein precipitated plasma (Figure 3). Excellent precision was observed with a CV of 7.6% for triplicate injections at the LOQ. This represents a 4-5x gain in signal to noise compared to the 5500 series.

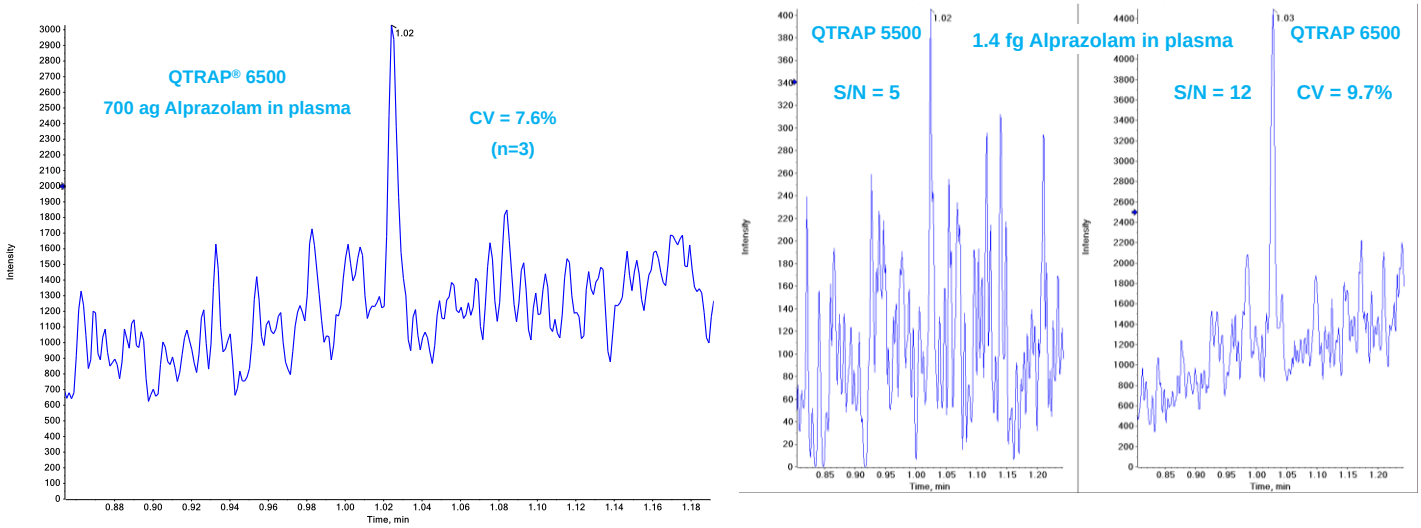


Figure 3. Ultra-High Sensitivity. IonDrive system technology of the 6500 series achieves high levels of sensitivity in real bioanalytical matrices. 700 attograms of alprazolam in protein precipitated plasma can be quantified with CV of less than 10% (left). Major S/N gains are achieved in comparison to the QTRAP 5500 system (right).

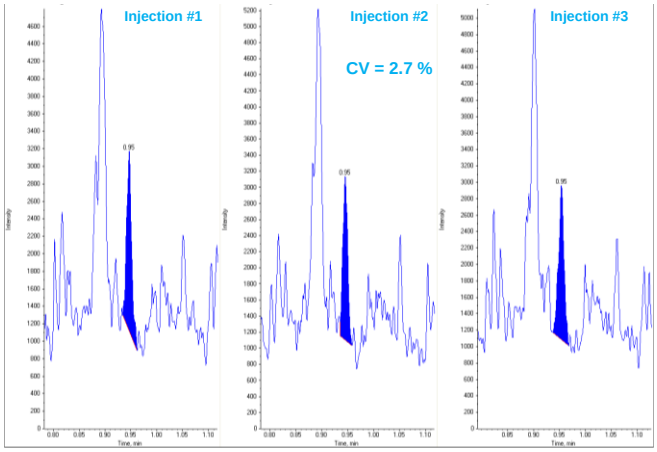


Figure 4 . Verapamil in Plasma at 120 attograms. Unprecedented sensitivity enabled an LOQ in protein precipitated plasma of 120 ag on-column with an outstanding CV of 2.7%**.**

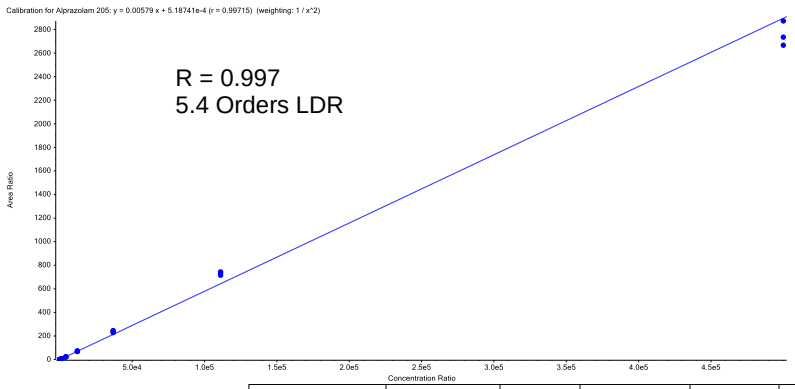
Verapamil was evaluated in both neat solution and in plasma. In protein precipitated plasma, a LOQ of 120 ag on-column was achieved with a CV of 2.7% (Figure 4). This allows lower limits of quantitation in the range of 100 fg/mL in plasma. Bioanalysis for topical and ophthalmic drug candidates below 1 pg/mL is now a reality.

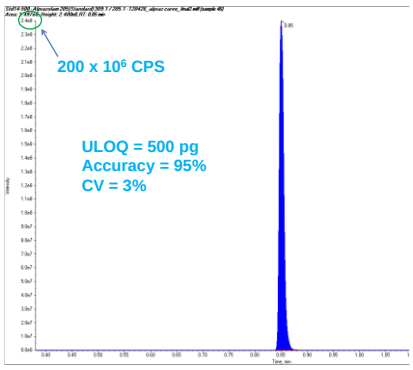
Dynamic Range

With IonDrive™ detector, sensitivity does not come at the expense of dynamic range. A linear range of 5.4 orders of magnitude was achieved allowing the upper limit of quantitation to reach 500 pg on-column with 95% accuracy and CV of 3.8% for triplicate injections (Figure 5). The IonDrive™ detector achieves a very high pulse counting rate. As a result, intense peaks of up to 2e8 cps no longer cause detector saturation.

Robustness

Multi-day robustness was evaluated by using 1,000 consecutive injections of methamphetamine in plasma in a continuously running batch lasting 3 days. The optimized curtain gas geometry and the IonDrive QJet Ion Guide result in rugged performance in biological matrix. No divert valve was used. The relative standard deviation for all 1000 injections was 5.9% for raw peak areas, and 3.2% for analyte to internal standard ratio (Figure 6).





Actual Conc (fg on column)	Num. Values	Mean	Standard Deviation	%CV	Accuracy	Value #1	Value #2	Value #3
1.88	3 of 3	1.86	0.12	6.2	99.0	1.99	1.76	1.84
5.65	3 of 3	5.89	0.23	3.9	104.3	6.08	5.64	5.96
16.9	3 of 3	16.7	0.60	3.6	98.4	17.1	16.0	16.9
50.8	3 of 3	49.0	1.07	2.2	96.3	48.4	48.2	50.2
152	3 of 3	145	4.65	3.2	95.3	140	149	147
457	3 of 3	423	20.0	4.7	92.5	422	403	443
1372	3 of 3	1319	37.7	2.9	96.2	1294	1363	1301
4115	3 of 3	4004	80.1	2.0	97.3	4080	3920	4011
12346	3 of 3	12391	191.6	1.5	100.4	12283	12278	12612
37037	3 of 3	41317	1311	3.2	111.6	39896	42479	41576
111111	3 of 3	126138	2684	2.1	113.5	123526	125999	128888
500000	3 of 3	476169	18206	3.8	95.2	495981	460175	472349

Figure 5. Extended Linear Range. The IonDrive high energy detector expands ion counting capacity by 20x, resulting in a wider dynamic range *and* high sensitivity for alprazolam in rat plasma with 5.4 orders of linear dynamic range achieved. High count rates in excess of 2e8 cps do not result in detector saturation.

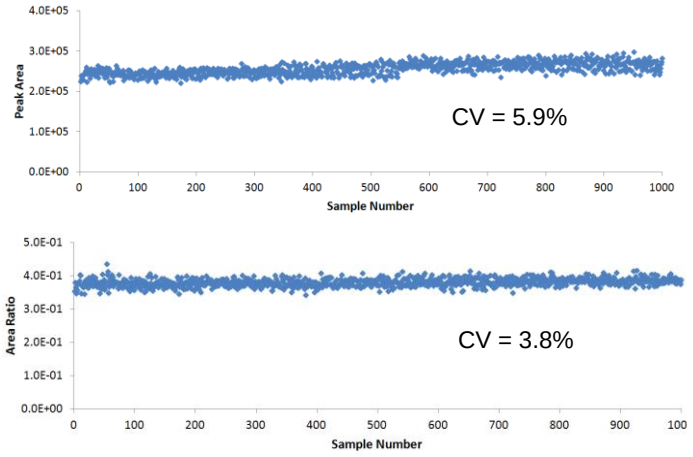


Figure 6. Robustness. 1000 consecutive injections of methamphetamine in protein precipitated rat plasma (no divert valve) continuously over 3 days.

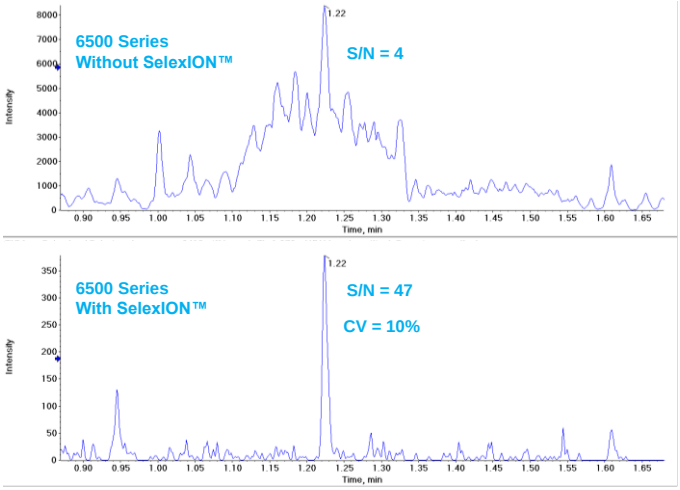


Figure 7. Salmeterol Analysis with SelexION Differential Mobility Separation Technology. At a very low level of 1.4 fg on-column, matrix interference from the plasma poses a serious challenge (top). The QTRAP 6500 system with SelexION Technology solves this challenge, achieving a CV of 10% at the LOQ of 1.4 fg salmeterol (bottom).

Selectivity

With this dramatically increased sensitivity and lower LOQ's, matrix interference can be the limiting factor in achieving the desired LOQ. Even with the high selectivity of MRM, interfering peaks and high baseline can pose serious challenges.

For the analysis of Salmeterol in rat plasma, both sensitivity and selectivity are required to reach sub pg/mL LOQ's. The matrix was found to interfere with the analyte peak, severely limiting the LOQ in spite of achieving the required analyte sensitivity. By taking advantage of SelexION® technology, differential ion mobility was used for additional orthogonal selectivity. This lowered the baseline substantially, enabling 1.4 fg on-column to be easily detected with a 10x improvement in signal to noise (Figure 7).

Conclusions

- The SCIEX Triple Quad 6500 and QTRAP 6500 systems with IonDrive system technology have demonstrated significant gains in sensitivity enabling low-level detection of analytes in complex matrices.
- The optimized geometry and large diameter heaters in the IonDrive Turbo V source result in improved ionization efficiency at high flows and more robust source conditions
- Efficiency gains in ion sampling with the IonDrive QJet ion guide increase sensitivity without compromising robustness
- The high sensitivity gains achieved are not at the expense of linear dynamic range. The IonDrive High Energy Detector increases ion counting capacity up to 20-fold, resulting in up to 6 orders of magnitude linear dynamic range
- The increased mass range of m/z 5 – 2,000 provides versatility and high sensitivity for both small molecules and peptides

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices