The AB SCIEX Triple Quad™ 6500 and QTRAP® 6500 Systems for Bioanalysis – A New Level of Sensitivity

Unprecedented Sensitivity and Selectivity Achieves Sub pg/mL Limits of Quantitation in Bioanalysis

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The bioanalytical scientist is constantly challenged to achieve sufficient sensitivity and selectivity in method development, while maintaining robustness and throughput. Analytes with very low plasma levels such as inhalation, topical, and ophthalmic drugs are particularly challenging. Achieving the required LOQ's which can be lower than 1 pg/mL requires ultra-high sensitivity LC/MS/MS. In addition, high selectivity must be maintained.

The traditional solution to increase sensitivity of LC/MS/MS instruments has been to use wider sampling interfaces. However, this approach is nearing its limits due to the adverse effect on method robustness caused by the large number of neutrals and other contaminants entering the system.

A far more elegant approach is to improve the efficiency of ion production, sampling and transfer. In this technical note, we describe a new LC/MS/MS platform, the AB SCIEX Triple Quad™ 6500 and QTRAP® 6500 systems, with major improvements in sensitivity resulting from improved ionization efficiency, sampling, and transmission of ions. In addition, a new state of the art detector results in a major improvement in linear dynamic range.

The new AB SCIEX 6500 series provides an unprecedented level of sensitivity never before achieved in an LC/MS/MS system. Using new IonDrive™ technology, major new innovations have been implemented in every part of the system.

Key Benefits of IonDrive™ Technology for Bioanalysis

- Ultra-high sensitivity enables LOQ's well below 1 pg/mL
- Mass range of m/z 5 – 2,000 provides versatility for small molecule and peptide quantitation
- Increased dynamic range with new IonDrive high energy detector technology
- Increased ionization efficiency and heat transfer with the new IonDrive Turbo V source
- Increased ion sampling efficiency and ruggedness with the new IonDrive QJet ion guide

New 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 new 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 new curtain gas geometry which provides a more robust gas barrier, and better separation of ions from neutrals, and other particles.

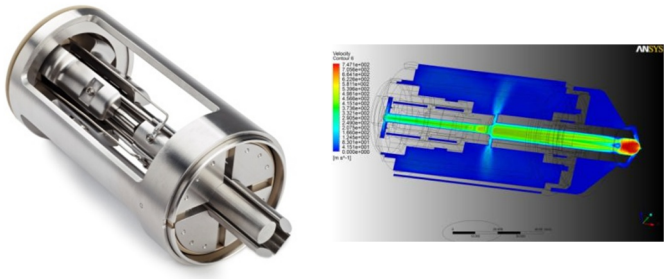


Figure 1. New IonDrive QJet ion guide. Dual RF stages maximize ion sampling from a large orifice while increasing ion transfer efficiency into the Q0 region – without increasing vacuum load in the analyzer region.

New IonDrive™ Turbo V Source

The new IonDrive Turbo V source builds on the efficiency and ruggedness of the original Turbo V source. The new design incorporates an optimized geometry and large diameter heaters.

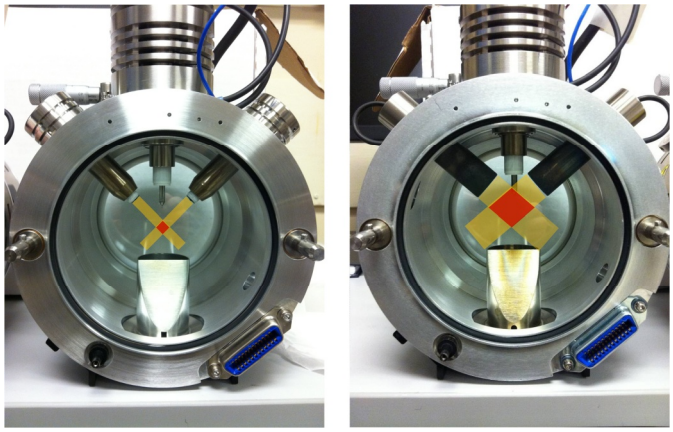


Figure 2. New IonDrive™ Turbo V source. Optimized geometry and larger diameter heaters result in higher ionization efficiency and robustness.

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).

New IonDrive™ High Energy Detector

The new high energy detector is a major step forward in improving both sensitivity and dynamic range. This advanced detector has 20x greater counting rate, 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 new 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.

Verapamil was evaluated in both neat solution and in plasma. In protein precipitated plasma, an 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.

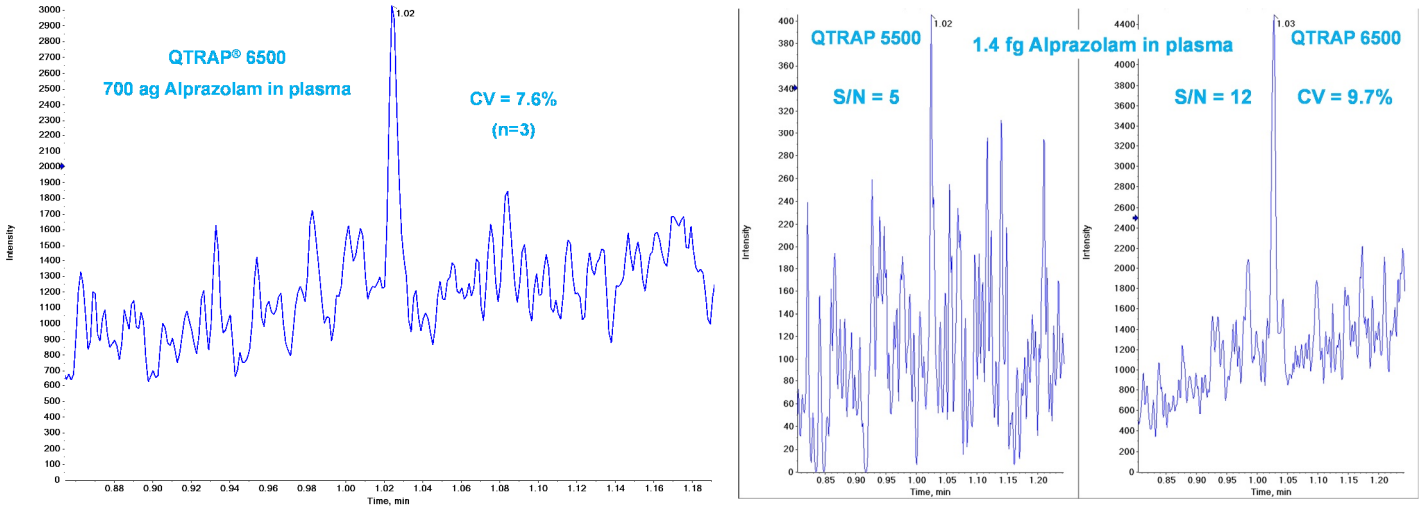


Figure 3. Ultra-High Sensitivity. IonDrive technology of the 6500 series achieves unprecedented levels of sensitivity in real bioanalytical matrices. 700 attograms of alprazolam in protein precipitated plasma can be quantified with CV of less than 10% (left). Major S/N gains are achieved in comparison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

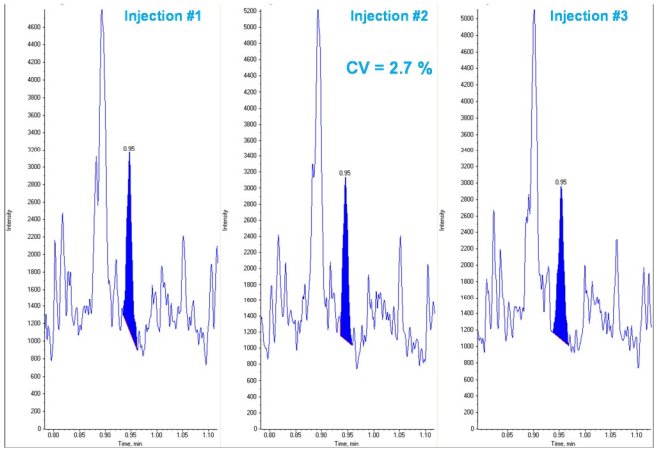


Figure 4 . Verapamil in Plasma at 120 attograms. Unprecedented sensitivity enabled an LOQ in protein precipitated plasma of 120 ag on-column with an outstanding CV of 2.7%**.**

Dynamic Range

With IonDrive™ detector technology, 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 new IonDrive™ detector achieves a very high pulse counting rate. As a result, intense peaks at 2e8 cps or greater no longer cause detector saturation.

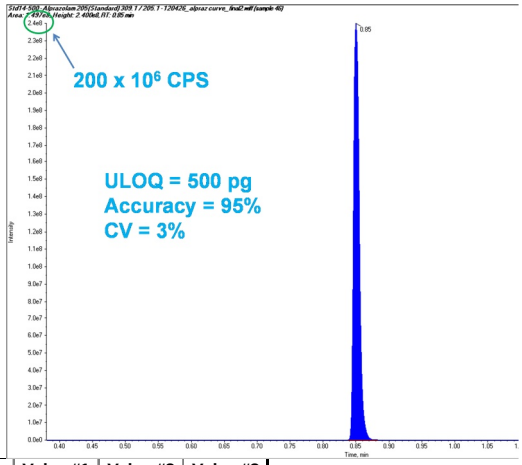
Robustness

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 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).

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).



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 new 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.

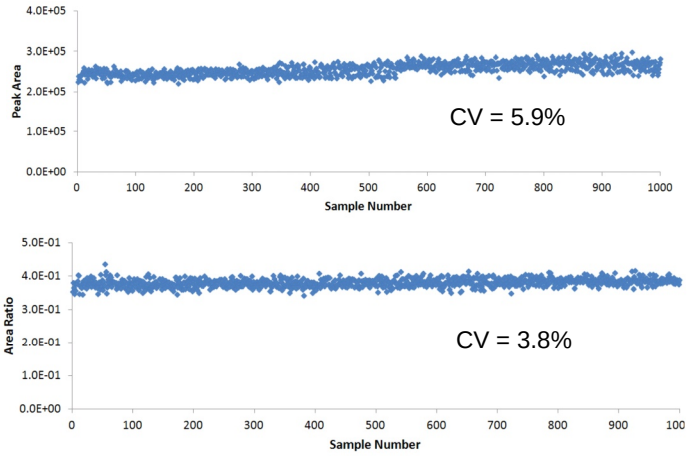


Figure 6. Robustness. 1000 consecutive injections of methamphetamine in protein precipitated rat plasma (no divert valve) continuously over 3 days.

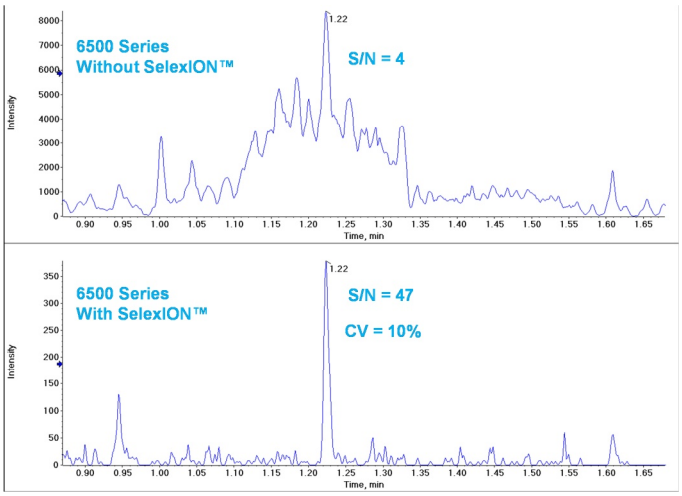


Figure 7. Salmeterol Analysis with SelexION. At a very low level of 1.4 fg on-column, matrix interference from the plasma poses a serious challenge (top). The 6500 system with SelexION solves this challenge, achieving a CV of 10% at the LOQ of 1.4 fg salmeterol (bottom).

Conclusions

- The AB SCIEX Triple Quad™ 6500 and QTRAP® 6500 systems with IonDrive™ technology have demonstrated unprecedented sensitivity gains, enabling sub pg/mL lower limits of quantitation in bioanalysis.
- The optimized geometry and new 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 remarkable sensitivity gains achieved are not at the expense of linear dynamic range. The new 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.

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