An Executive Summary

High-Sensitivity Mass Spectrometry: The Analytical Tool for Quantitative Analysis

Mark Roberts
Principal Product Manager
Mass Spectrometry
Waters Corporation

Dominic Foley
Senior Applications Scientist
Health Sciences
Waters Corporation

Kenneth J. Rosnack
Principal Business Development Manager
Food & Environmental Markets
Waters Corporation

The ability to quantify compounds at the molecular level is a critical step in the decision-making process for many scientists today. Whether you are analyzing small or large molecules, single analytes, or multiple panels of compounds, in most cases a tandem (triple) quadrupole mass spectrometer is the quantitative tool of choice. New developments in tandem (triple) quadrupole mass spectrometry demonstrate excellent quantitative performance, including a novel ionization technique that provides wider compound coverage and improved ionization efficiency.

A New Tandem Quadrupole Mass Spectrometer for Quantitative Applications

The Xevo® TQ-XS, a new tandem quadrupole mass spectrometer designed for high-sensitivity applications, was recently introduced into the Waters® family of Xevo products. The Xevo TQ-XS incorporates four key technological improvements:

- an updated ion guide, the StepWave XS™, for improved sensitivity with challenging compounds
- tool-free atmospheric pressure ionization probes for improved reproducibility among users
- a wider dynamic range detector for easier method transfer and more accessible sensitivity
- UniSpray™, a novel ionization technique that ionizes a wide range of compounds

The Xevo TQ-XS, based on the TQ-S platform, includes the StepWave XS ion guide, RADAR™ capabilities for obtaining qualitative full-scan mass spectrometry (MS) information simultaneous with the MRM experiment, ScanWave™ for enhanced product ion confirmation, and updated MassLynx® software.

StepWave XS ion guide. The new StepWave XS ion guide, similar to the original StepWave, ensures that neutrals and gas flow are removed from the first stage of the ion guide for enhanced transmission. Ions are transferred to the mass analyzer, resulting in a robust and sensitive ion guide. Improvements in the StepWave XS enhance its performance for challenging compounds:

1) The radio frequency (RF) voltage is now constant around the ion guide, which improves robustness while mass switching.
2) The profile of the ion guide is wider, allowing full encapsulation of the ion cloud that forms without ions interacting with the edges of the ion guide, which can compromise performance.
3) The first stage is modified with a series of horizontal plates for more controlled extraction of ions from the rapid gas flow because different voltages can be applied to each

SPONSORED BY
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®
LC GC north america

plate. Ions collide with gas molecules with less energy and are more likely to remain intact throughout the ion guide and through the MS analyzer. The segmented quadrupole second stage focuses the ion beam to ensure more efficient transfer of the ions from the source to the detector.

The Xevo TQ-XS demonstrates enhanced sensitivity over the Xevo TQ-S for challenging compounds in electrospray ionization (ESI) mode with standard multiple reaction monitoring (MRM) experiments. The range of improvement depends, of course, on the compound. **Figure 1** shows a range of positive and negative ionizing species, with peak area improvements from 2x to 50x and signal-to-noise (S/N) improved by 1x up to more than 10x.

An experiment **(Figure 2)** demonstrating robustness was run on the Xevo TQ-XS over 85 hours involving 2,000 consecutive injections analyzing sulphadimethoxine in protein-precipitated plasma (100 fg on column). Despite the mass switching and a short dwell time (5ms) used to simulate a real experiment, the resulting data showed little variation in peak area over the injection series and <3% relative standard deviation (RSD).

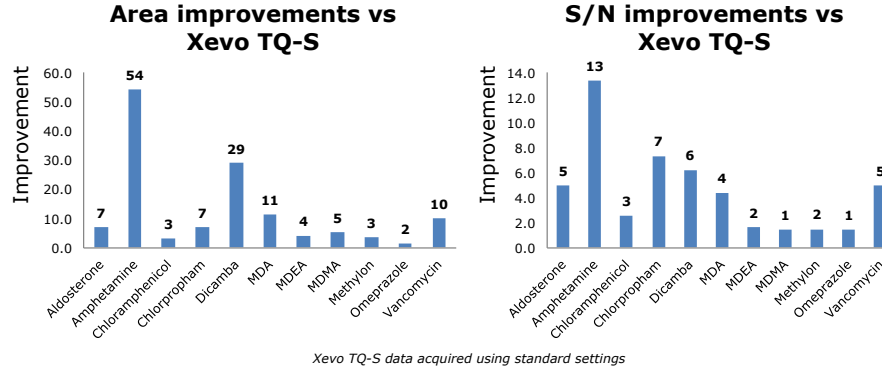
Wide dynamic range. The improved dynamic range allows easy transfer of a method run on a less sensitive instrument to a more sensitive instrument, as well as the analysis of a wide variety of concentrations in the same sample without the need to dilute and re-inject the sample. This capability is based on the long-life photomultiplier. Data for the peptide Val-Tyr-Val, for example, using UPLC/MRM with ESI showed 6 orders of linear dynamic range.

Tool-free ESI and APCI probes. Changing the probe assembly of the ESI and APCI probes, which include a capillary, is entirely tool-free and can be accomplished in less than 2 minutes versus the previous assembly time of 30-plus minutes. The 2-piece probe adaptor is taken apart to access the probe assembly. The probe assembly consists of a length of PEEK tubing that is connected to the capillary with a factory-fitted join, which is simply screwed into the probe adaptor. This simplified process improves reproducibility between operators and reduces dead volume.

UniSpray. UniSpray is a novel ion source option for the Xevo TQ-XS that improves the number and range of compounds that can be ionized in a single technique with robustness and linearity comparable to ESI. The analyst does not need to redevelop MRM methods and conduct different transitions when using UniSpray. The three diverse compounds shown in **Figure 3** were previously best ionized using other techniques, but now can be optimized in terms of S/N with

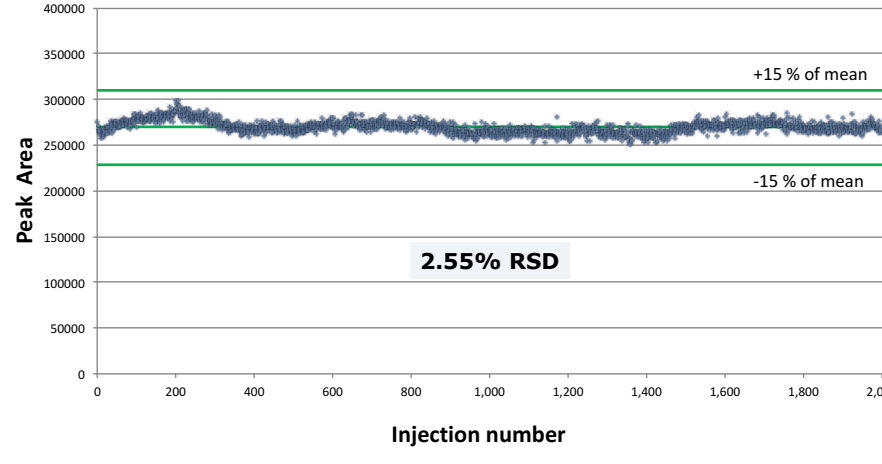
Figure 1: Peak area and signal-to-noise ratio improvements in Xevo TQ-XS versus Xevo TQ-S.

- Best sensitivity for challenging compounds - UPLC/MRM ESI mode



Xevo TQ-S data acquired using standard settings

Figure 2: Robustness improvements on the Xevo TQ-XS for sulphadimethoxine.



UniSpray. **Figure 4** illustrates the grounded probe of the UniSpray on the left. Beneath that probe is a target (a blunt pin) that has a high voltage applied to it. The probe emits a high velocity nebulized spray of the LC eluent, which hits the pin and breaks into smaller secondary droplets. Smaller droplets increase the ionization efficiency. Gas flow follows the curvature of the target pin and is directed toward the sample cone and into the mass spectrometer, resulting in a more sensitive sample analysis and enhanced desolvation.

Figure 3: Compounds previously best ionized using other techniques, but now optimized in S/N with UniSpray.

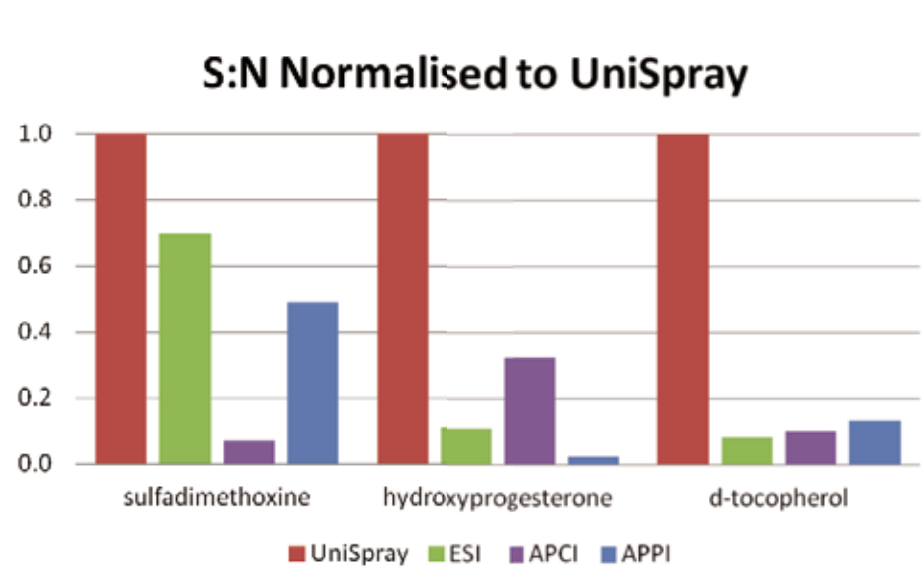
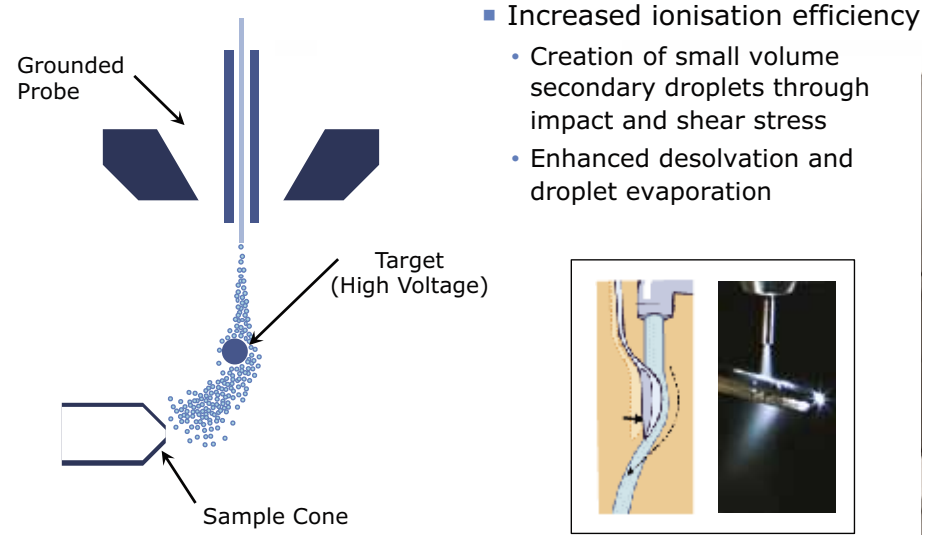


Figure 4: UniSpray mechanism.



Application: Mycotoxins and Pesticide Analysis in Cereals and Grain

Many labs run methods for mycotoxins and pesticides, which are regulated in cereals and grains around the world. Different methods are used for each pesticide and mycotoxin, so reducing the number of extractions and run times is desirable. As demands on labs grow, the demand for multiresidue analysis with multiclasses in a single method increases.

Experimental conditions for LC and MS of mycotoxins and pesticides in a variety of matrices including red corn, wheat, and barley are given in **Figure 5a** and **5b**. For each matrix, 0.5 g of homogenized sample was prepared using the CEN version of QuEChERS. A standard C18 BEH column was used with a water/methanol mobile phase and an injection volume of 1 µL. MRM methods were generated using QuanPedia, which is a database containing transitions and source parameters. No system tuning or modifications were carried out.

An example of the chromatography achieved for a selection of the 143 pesticides and mycotoxins in red corn is shown in **Figure 6**, acquired by LC-ESI-MS/MS. The inset table (upper left) provides an example of the spiking concentration, where all analytes were spiked at or below the maximum residue levels (MRLs) listed in the various relevant European Union regulations.

Figure 5a-5b: Experimental conditions for LC and MS of mycotoxins and pesticides in a variety of matrices including red corn, wheat, and barley.

Figure 5a

Sample Preparation

0.5 g homoginised sample

↓

QuEChERS CEN to 6 ml

↓

Centrifuge

↓

Collect extract and analyse

Analytical column:	2.1 x 100 mm BEH C18 1.7 µm
Column temp:	45 °C
Sample temp:	4 °C
Mobile phase A:	0.1 % formic acid in water
Mobile phase B:	0.1 % formic acid in methanol
Sample Man. wash:	50:50 Water/Methanol
Purge wash:	90:10 Water/Methanol
Seal wash:	90:10 Water/Methanol
Flow rate:	0.45 mL/min
Injection volume:	1 µL

Time (min)	A (%)	B (%)	Curve
0	98	2	6
0.25	98	2	6
12.25	1	99	6
13.0	1	99	6
13.01	98	2	6
17	98	2	6

Figure 5b

- MS: Xevo TQ-XS
- Capillary voltage: 1 kV
- Desolvation Temp: 550 °C
- Source Temp: 150 °C
- Desolvation gas: 1000 l/hr
- Cone gas: 150 l/hr

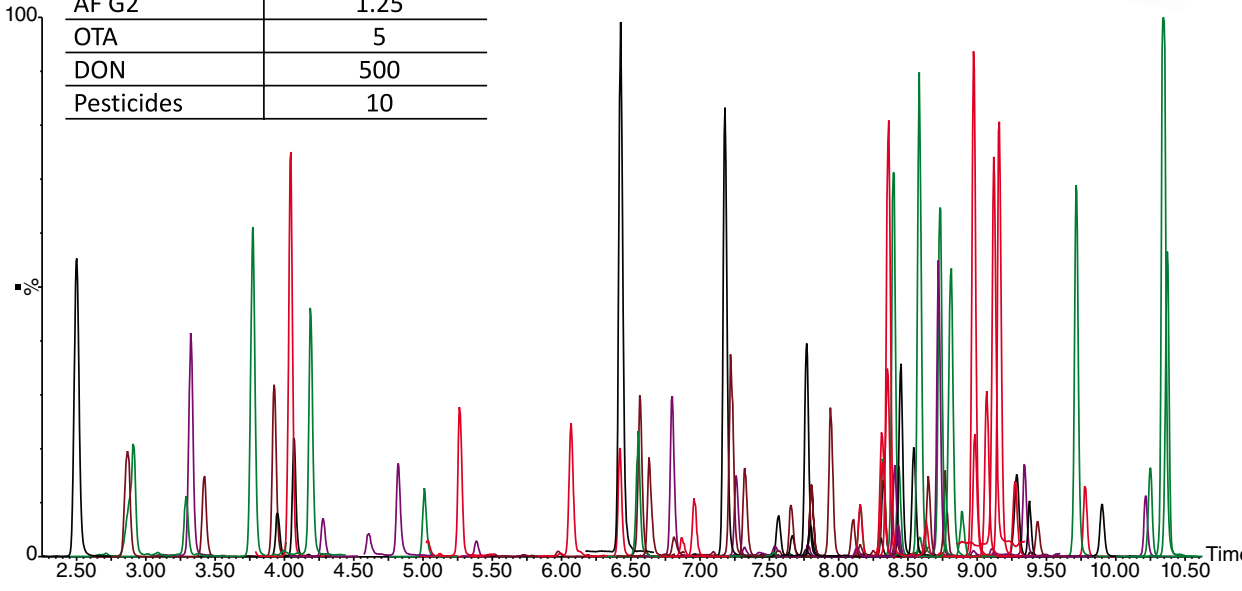
- MRM transitions: Method generated in QuanPedia, using Xevo TQ-S transitions and source parameters

Further to this analysis by ESI, UniSpray was investigated as an alternative, novel ionization source, to extend the range of multiclass residues with varying polarities in a single injection. An example of this is shown in **Figure 7**, where p,p'-DDE and captan (i.e., analytes traditionally analyzed by GC-MS) show excellent ionization by UniSpray and detection at or below the MRL in wheat and barley samples, respectively. This preliminary proof-of-concept work demonstrates excellent ionization efficiency for analytes that are traditionally challenging with GC, such as captan, which degrades in the injector and liner.

The RADAR scan capabilities of the Xevo TQ-XS assist in understanding sample complexity. With RADAR scan, full scan information can be acquired simultaneously with MRM

Figure 6: Chromatogram of single extraction and injection of multiresidue, multiclass analytes (mycotoxins and pesticides).

Compound	Concentration µg/kg
AF B1	1.25
AF B2	1.25
AF G1	3.75
AF G2	1.25
OTA	5
DON	500
Pesticides	10



transitions, which can reveal whether additional sample preparation or method changes are needed.

Application: Analysis of Steroid Hormones for Clinical Research

Analysis of steroid hormones is challenging due to the high number of structurally related analytes potentially causing interference with quantitation. Poor analytical method selectivity for these interfering analytes can result in high imprecision and inaccuracy for steroids such as aldosterone and testosterone, particularly at the lower concentrations. Greater analytical selectivity is achieved using LC–MS/MS, which provides chromatographic separation of isobaric steroid species. Mass spectrometry provides additional selectivity through MRM, which in turn optimizes the analytical sensitivity of these hormones.

In the following examples, methods previously developed on other Xevo tandem quadrupole platforms were transferred to the Xevo TQ-XS system to assess the quantitative performance for aldosterone and testosterone. Both methods used the UPLC conditions shown in **Figure 8**.

The analytical sensitivity improvement on the Xevo TQ-XS system compared to the Xevo TQ-S for an aldosterone solvent standard is shown in **Figure 9**. At 50 fg/mL on column, a >5x S/N improvement was observed using the Xevo TQ-XS system. Reproducibility also increased, as seen in the peak area for aldosterone on the Xevo TQ-XS (RSD =1.6%, n=3) compared to the Xevo TQ-S (RSD= 20.5%, n=3).

Aldosterone stripped serum samples were prepared at 2 pg/mL, extracted in triplicate over three occasions, and injected. Excellent reproducibility (RSD=12.3%) and analytical sensitivity (S/N:RMS >20) was demonstrated for this analysis over the three occasions. Quality Controls (QCs) over the calibration range for aldosterone were assessed at four different concentrations (21-669 pg/mL). These were analyzed in replicates of ten over three days. Total precision and repeatability in plasma was <7.9% on the Xevo TQ-XS.

Testosterone-stripped serum samples were prepared at 0.005 ng/mL, extracted in triplicate, and injected. Excellent reproducibility (RSD=7.1%) and analytical sensitivity (S/N:RMS >30) were demonstrated for this analysis over the three occasions. QC concentrations over the calibration range for testosterone were assessed at four different concentrations (0.02–14 ng/mL). These were analyzed in replicates of six over three days. Total precision and repeatability in serum was ≤4.4% on the Xevo TQ-XS.

Extracted calibration lines in stripped serum were linear on

Figure 7: p,p'-DDE and captan show excellent ionization by UniSpray and detection at or below the maximum residue limit.

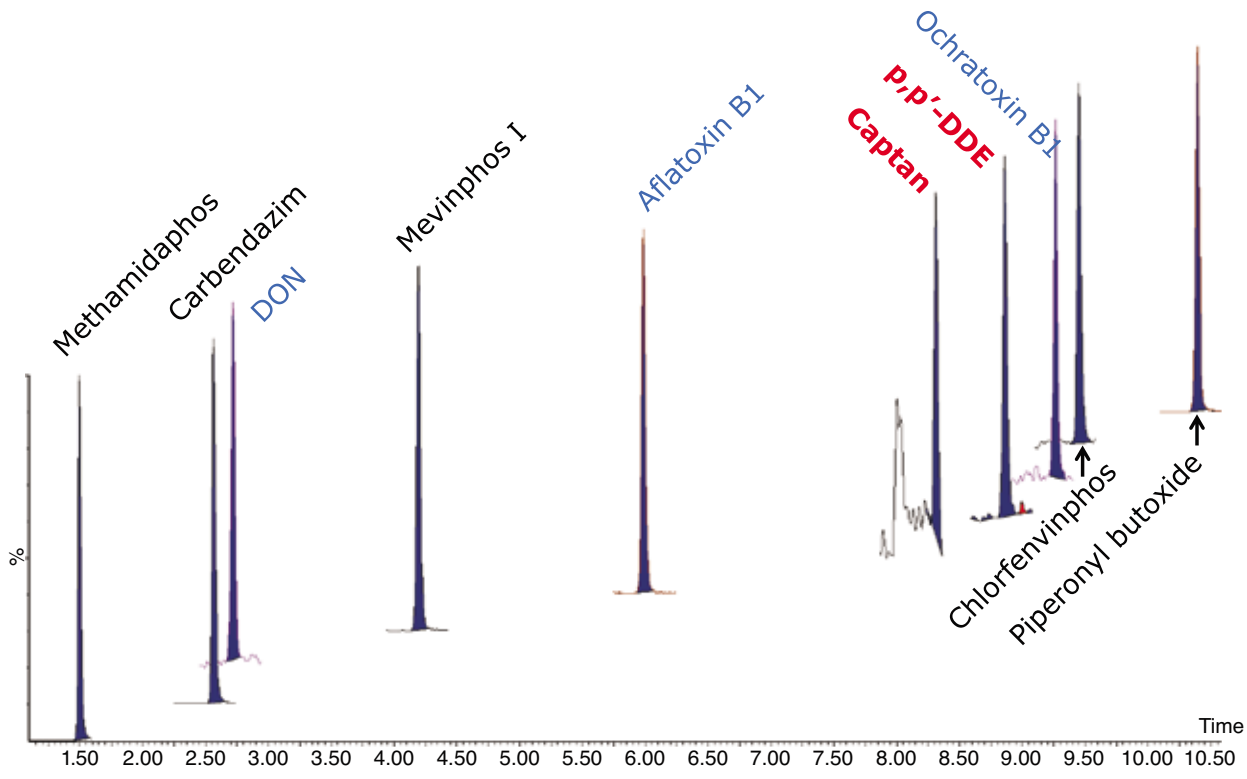


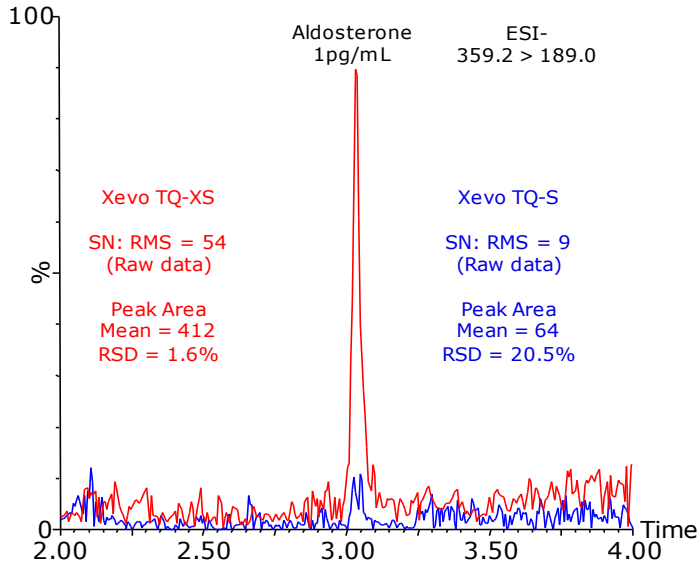
Figure 8: Experimental conditions for analysis of aldosterone and testosterone on Xevo TQ-XS.

- Methods for the analysis of aldosterone and testosterone were transferred to the Xevo TQ-XS to assess quantitative performance

	Aldosterone	Testosterone + others
Column	CORTECS C18, 1.6µm, 100mm x 2.1mm	ACQUITY HSS T3, 1.8µm, 50mm x 2.1mm + VanGuard T3
Mobile Phase	MP A: Water MP B: MeOH	MP A: Water + 2mM NH4Ac + 0.1% Formic Acid MP B: MeOH + 2mM NH4Ac + 0.1% Formic Acid
Sample Volume	200 µL	100 µL
Sample Preparation	Protein Precipitation + Oasis MAX SPE µElution	Protein Precipitation + Oasis PRiME HLB SPE µElution
MS ESI mode	Negative	Positive
Technical Brief	720005728EN	TBC

Figure 9: Sensitivity improvements on the Xevo TQ-XS system versus the Xevo TQ-S for aldosterone.

- Aldosterone solvent standard was prepared at 1 pg/mL, injected in triplicate and analyzed using the Xevo TQ-S and Xevo TQ-XS systems
- Observed >5x improvement in calculated S/N using the Xevo TQ-XS



Injection of a 1 pg/mL solution (n=3, 50fg on column) of aldosterone on the Xevo TQ-XS and Xevo TQ-S

S/N performed using RMS on raw data

Figure 10: Performance of the low calibrator serum samples for cortisol, 21-deoxycortisol, 11-deoxycortisol, androstenedione and 17-OHP, analyzed over three days.

- S/N (RMS) assessment of low concentration serum calibrators over 3 separate runs
- The table and chromatograms show concentrations that meet or are well within this criteria

Analyte	Concentration (ng/mL)	Peak Area		S/N	
		Mean	%RSD	Mean	%RSD
Cortisol	0.125	19628	0.7%	157	19.8%
21-Deoxycortisol	0.075	3407	15.7%	29	7.1%
11-Deoxycortisol	0.0125	6741	9.0%	31	11.2%
Androstenedione	0.025	35449	4.7%	105	9.5%
17-OHP	0.0625	25008	3.6%	118	12.7%

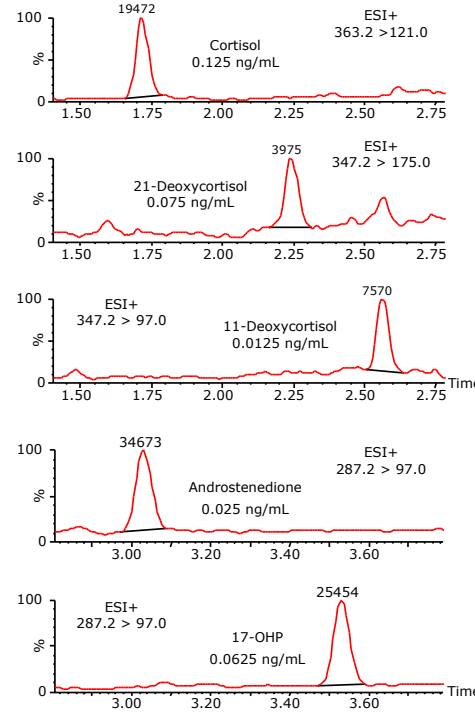
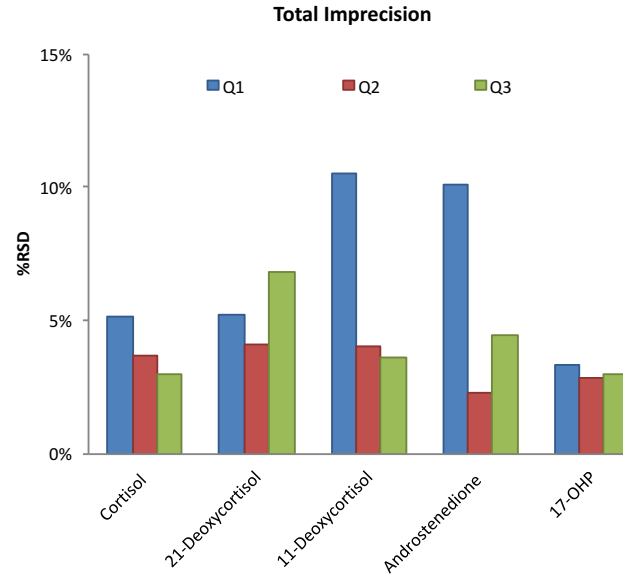


Figure 11: Total imprecision for cortisol, 21-deoxycortisol, 11-deoxycortisol, androstenedione and 17-OHP QC samples, analyzed over three days.

- Steroid hormone serum QCs were analysed in replicates of 6 over 3 days (n=18)
- Total imprecision across all analytes was ≤10.5% on the Xevo TQ-XS

Analyte	Concentration (ng/mL)		
	Q1	Q2	Q3
Cortisol	0.30	25	350
21-Deoxycortisol	0.15	2.5	35
11-Deoxycortisol	0.10	2.5	35
Androstenedione	0.030	5.0	70
17-OHP	0.25	12.5	175



the Xevo TQ-XS system for both aldosterone and testosterone, with correlation coefficients >0.998 over three occasions.

Using the testosterone LC-MS/MS methodology, steroids including; cortisol, 11-deoxycortisol, 21-deoxycortisol, androstenedione and 17-OHP were evaluated using the Xevo TQ-XS system. As seen in **Figure 10**, excellent analytical sensitivity and reproducibility of the low calibrator serum samples was achieved, where S/N was >20 for all analytes (<20% RSD) over three days. As illustrated in **Figure 11**, total imprecision across all analytes was ≤10.5% on the Xevo TQ-XS system.

The Xevo TQ-XS provides excellent quantitative performance for the steroid hormones for clinical research purposes. The analytical sensitivity for aldosterone improved up to fivefold using the new Xevo TQ-XS compared to the TQ-S system.

Excellent analytical sensitivity for aldosterone and testosterone was achieved using small sample volumes, and the calibration lines from these extractions were linear across the range for both analytes. Other steroid hormones showed excellent analytical sensitivity and precision performance on the Xevo TQ-XS.

Research Use Only, Not for Use in Diagnostic Procedures

Conclusion

The technology incorporated into the Xevo TQ-XS tandem (triple) quadrupole mass spectrometer demonstrates improved sensitivity that is reproducible for multiple injections despite complex sample matrices. The novel ion source, UniSpray, provides results for a wider range of compounds in a single analysis without the need to change the ion source or probe. This analytical tool allows analysts to carry out reproducible quantitative analyses and reach lower limits of quantitation.