



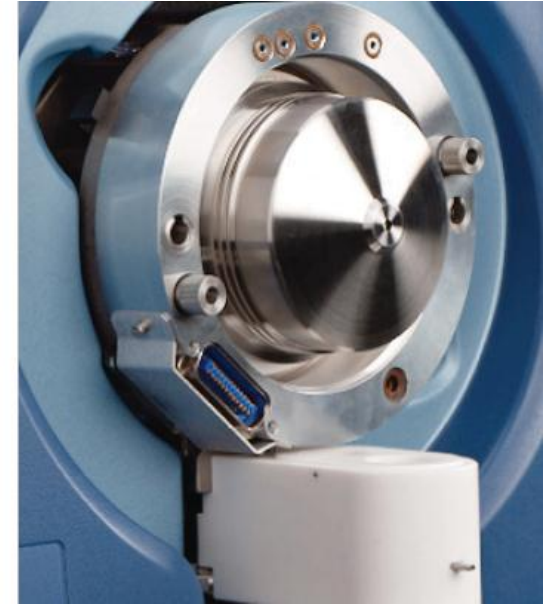
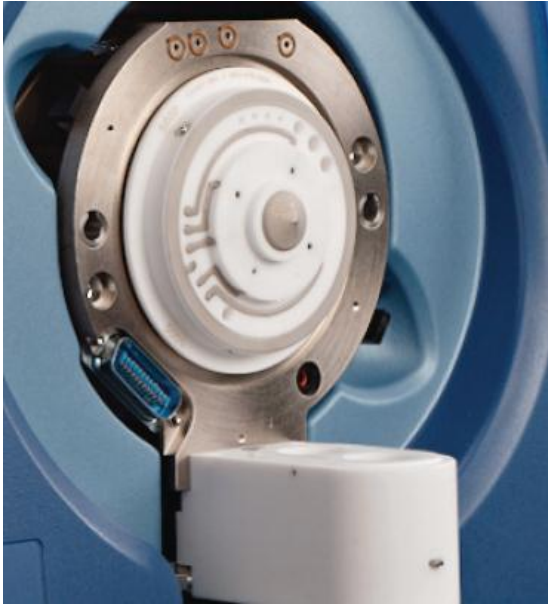
Differential Mobility Spectrometry with the AB SCIEX SelexION™ Technology for 5500 Systems A New Dimension of Selectivity

Mauro Aiello, Senior Product Manager



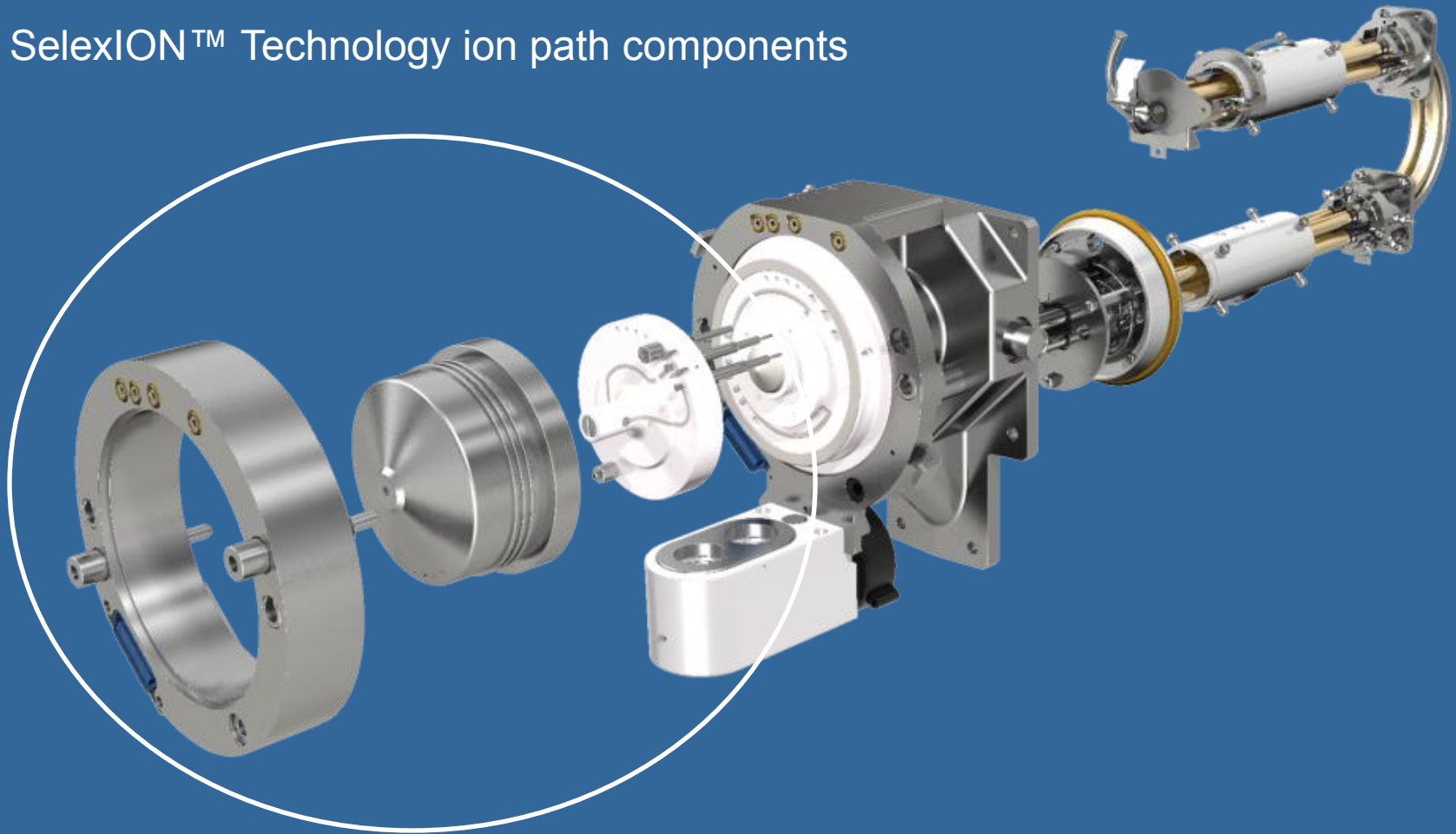
Differential Mobility Spectrometry with the SelexION™ Technology for 5500 Systems delivers a **new dimension of selectivity** and performance for any application requiring the separation of isobaric species, isolation of challenging co-eluting contaminants and reduction of high background noise.





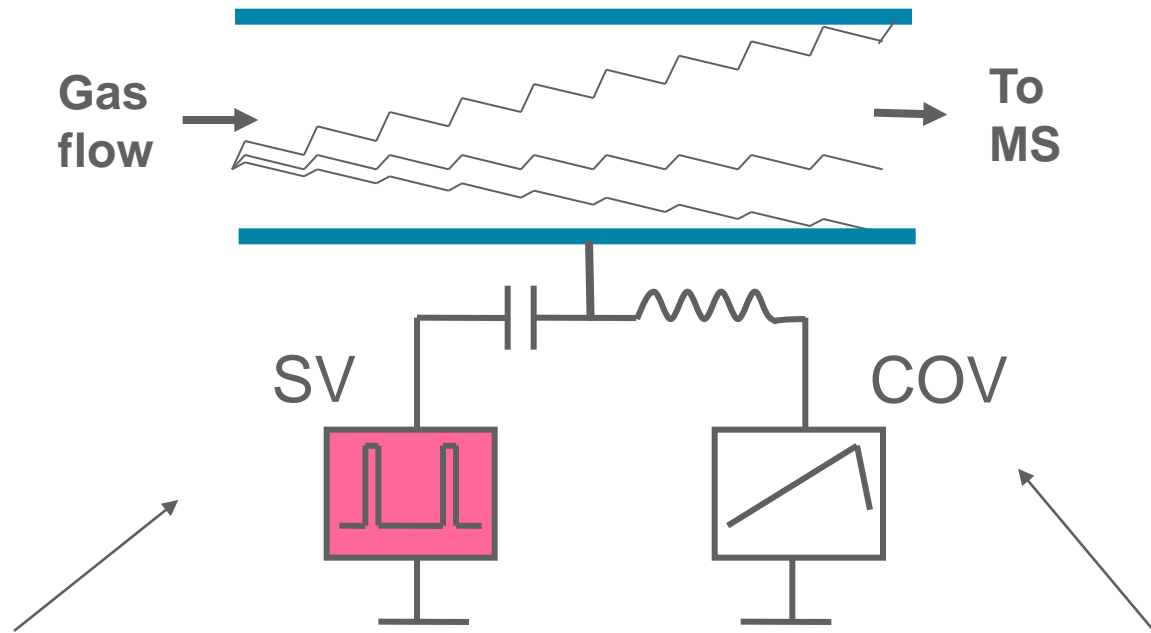
- **Robust hardware designed for ease of use**
 - Few minutes to install/remove
 - No tools required
 - No need to break vacuum

SelexION™ Technology ion path components



How does SelexION™ Technology separate ions?

- Differential Mobility Spectrometry (DMS) is the term used for planar geometry



Separation waveform (SV):

Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

Compensation voltage (COV):

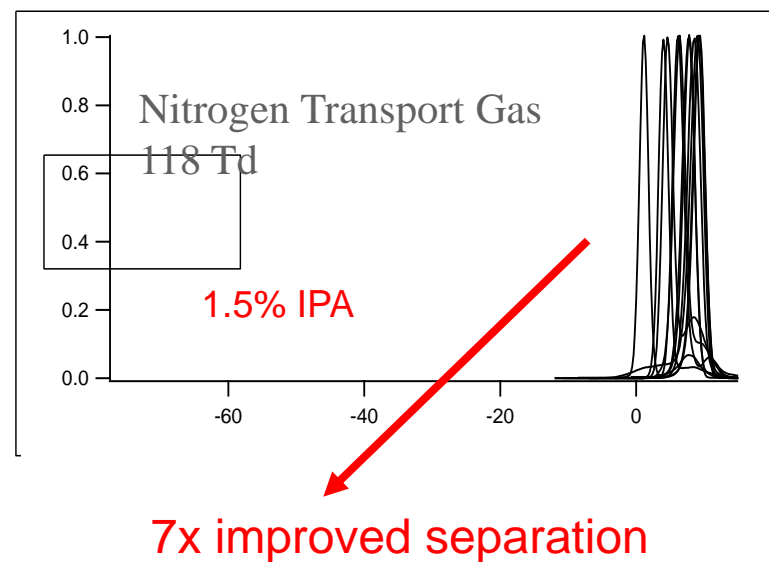
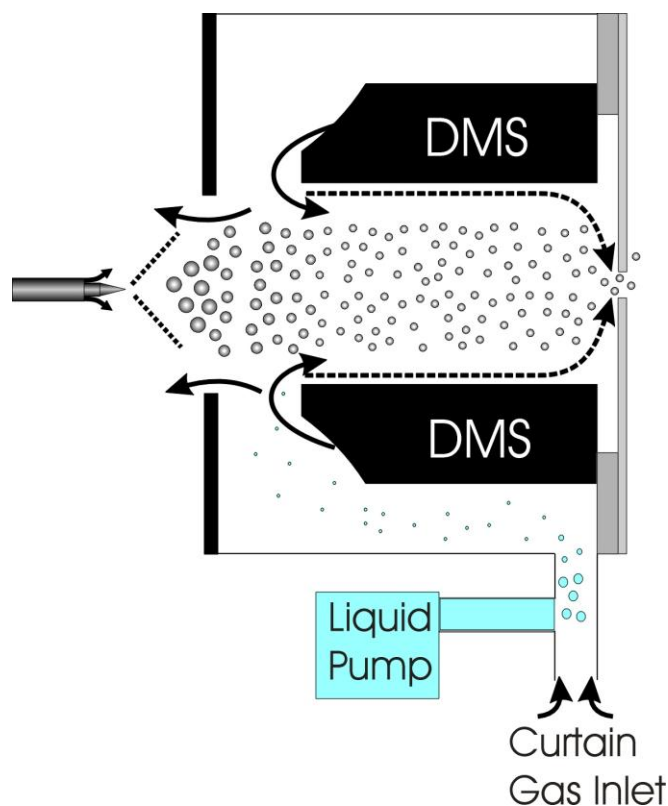
Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

- **Short residence times**
- **Rapid voltage changes for MRM operation**
 - **MRM cycle times of 25 msec, (20 msec pause time)**
 - **Fast LC support**
- **Transparent Mode**
 - **Allows all ions to be transmitted by turning off voltages**
- **Minimal diffusion losses**
- **Homogeneous electric fields within the DMS analyzer.**
 - **Improved resolution at high voltages**
- **Uniform conditions for the addition of chemical modifiers**
 - **More on this to come...**

Also:

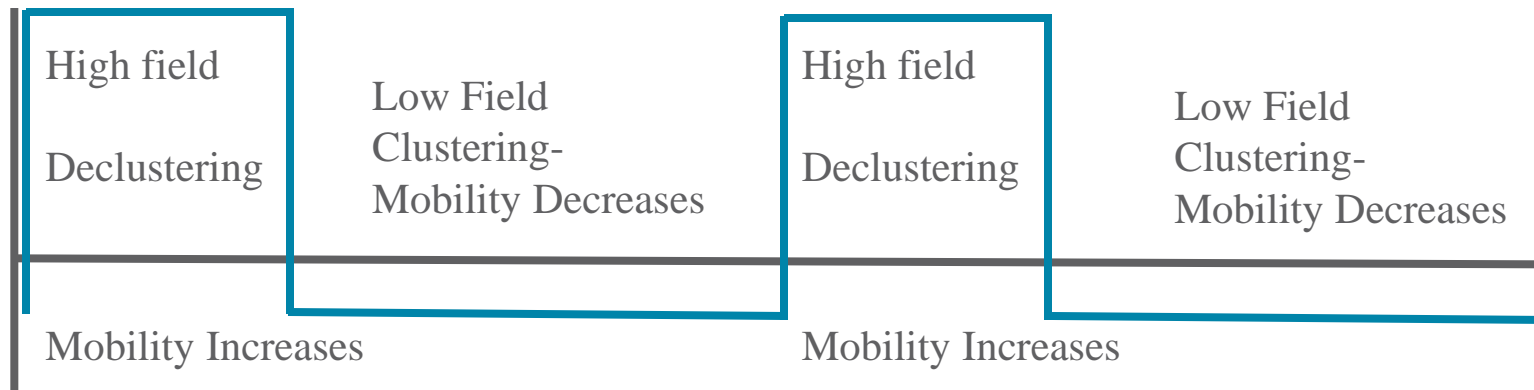
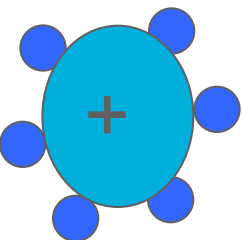
- *Scheduled MRM™* support

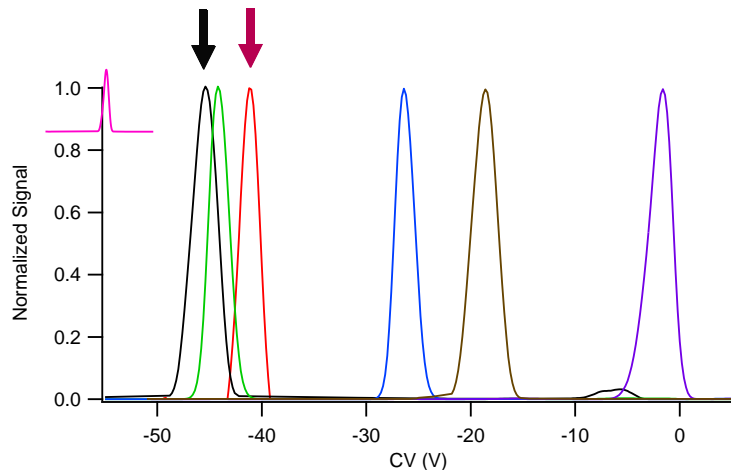
- Liquid modifiers can be added to the curtain gas flow
- Improves separations
- More options for separation in difficult cases



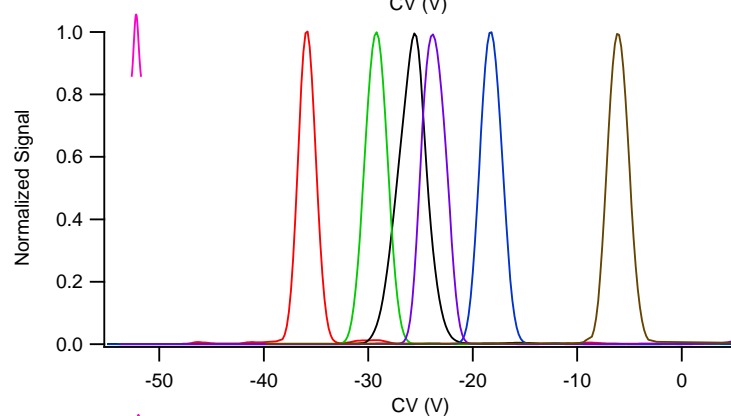
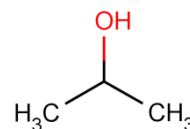
11 compounds: methylhistamine, minoxidil, ephedrine, norfentanyl, acyclovir, clenbuterol, tramadol, quinoxifen, pamaquin, fendiline, buscopan.

Separations are Chemical in Nature

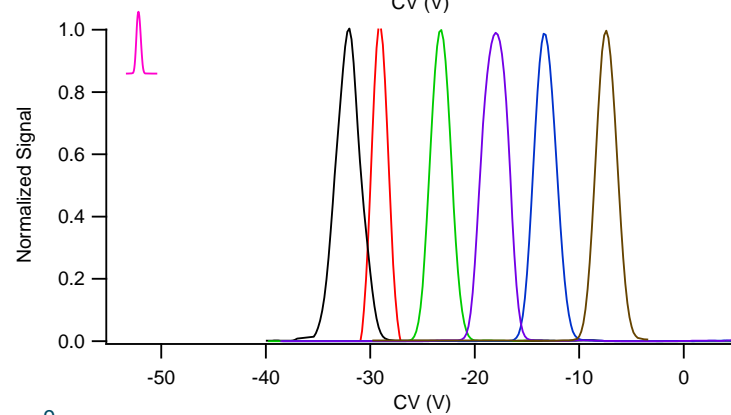
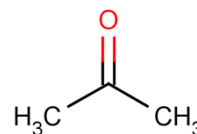




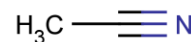
Isopropanol Separation



Acetone Separation



Acetonitrile Separation



Red: Ephedrine

Black: Acyclovir

Green: Norfentanyl

Blue: Clenbuterol

Purple: Imipramine

Brown: Diazepam

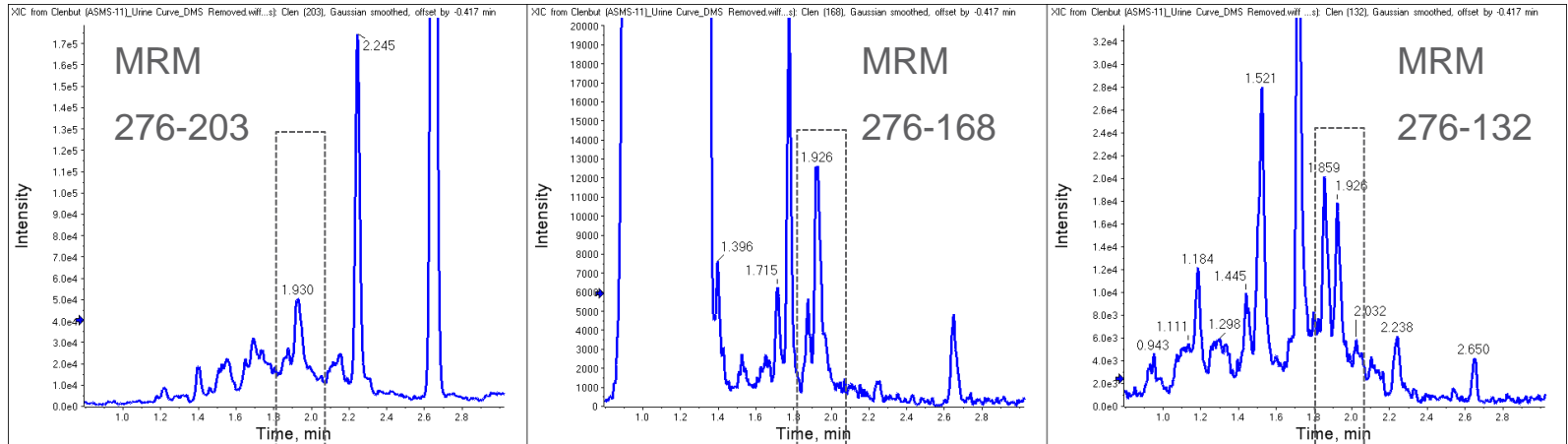
Pink: Quinoxifen

Clenbuterol analysis from urine samples

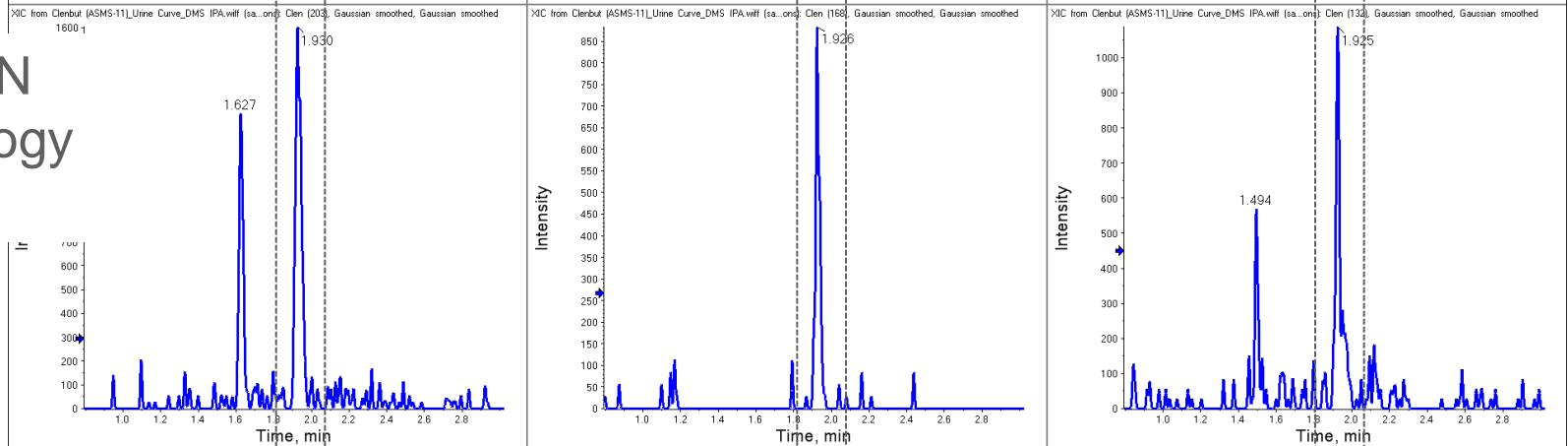
- LC-MSMS analysis of clenbuterol from urine samples is known to exhibit high degree of interferences in all major MRM transitions monitored.
- These interferences also vary greatly in terms of complexity and intensity levels between subject samples.
- Here we investigated the use of DMS to increase the selectivity of the LC-MSMS analysis

Clenbuterol Spiked in Human Urine (dil. 1:1 prior to analysis) QTRAP® 5500 vs 5500 with SelexION™ Technology

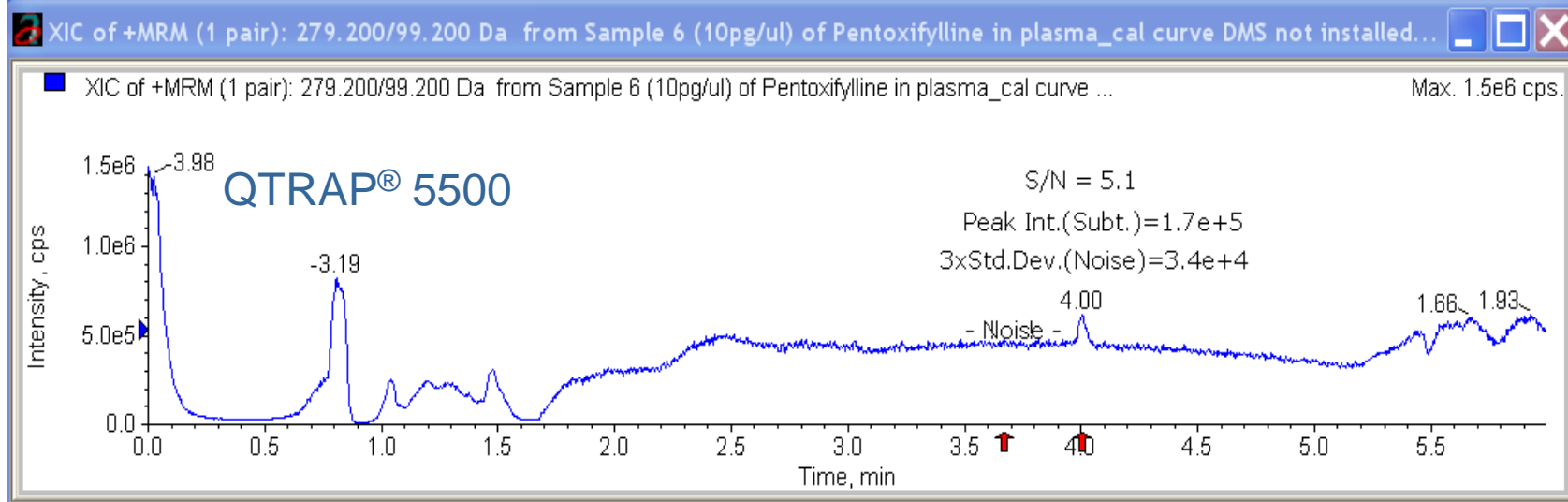
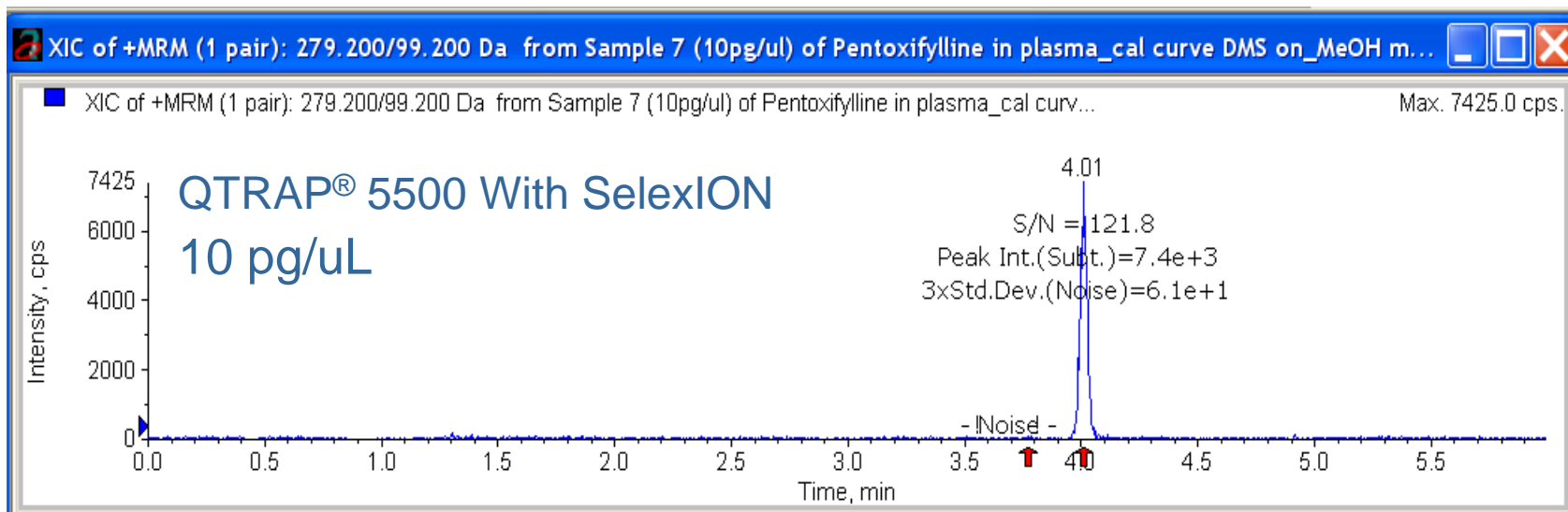
QTRAP
5500



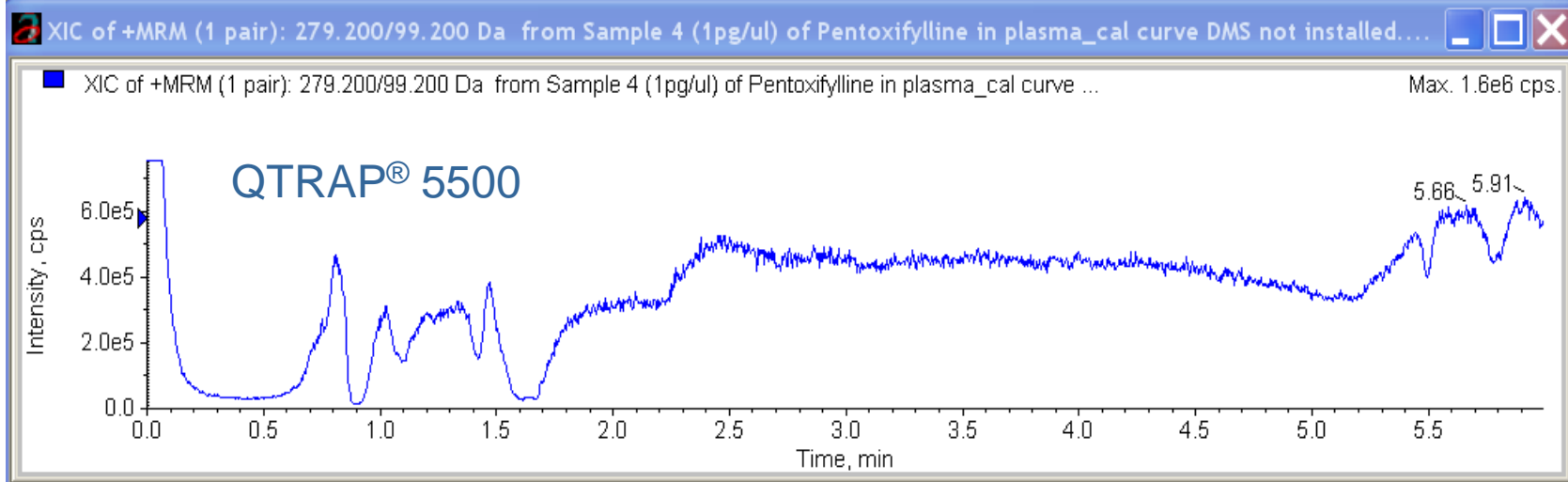
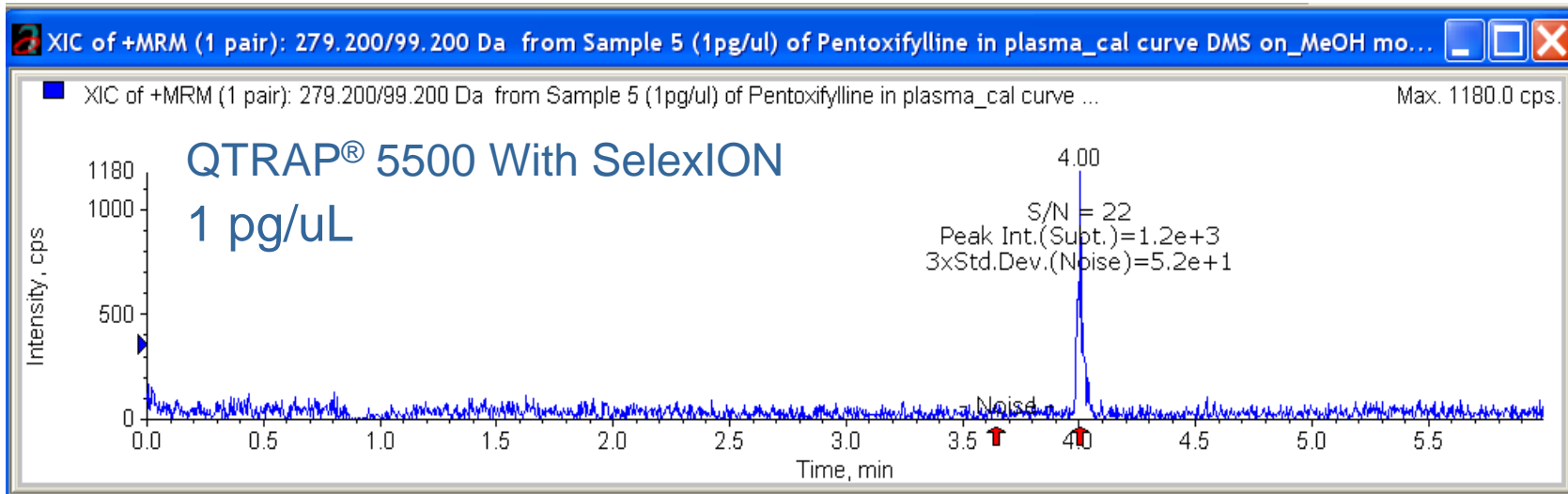
SelexION
Technology
on 5500



Pentoxifylline ~20 times LOQ Improvement



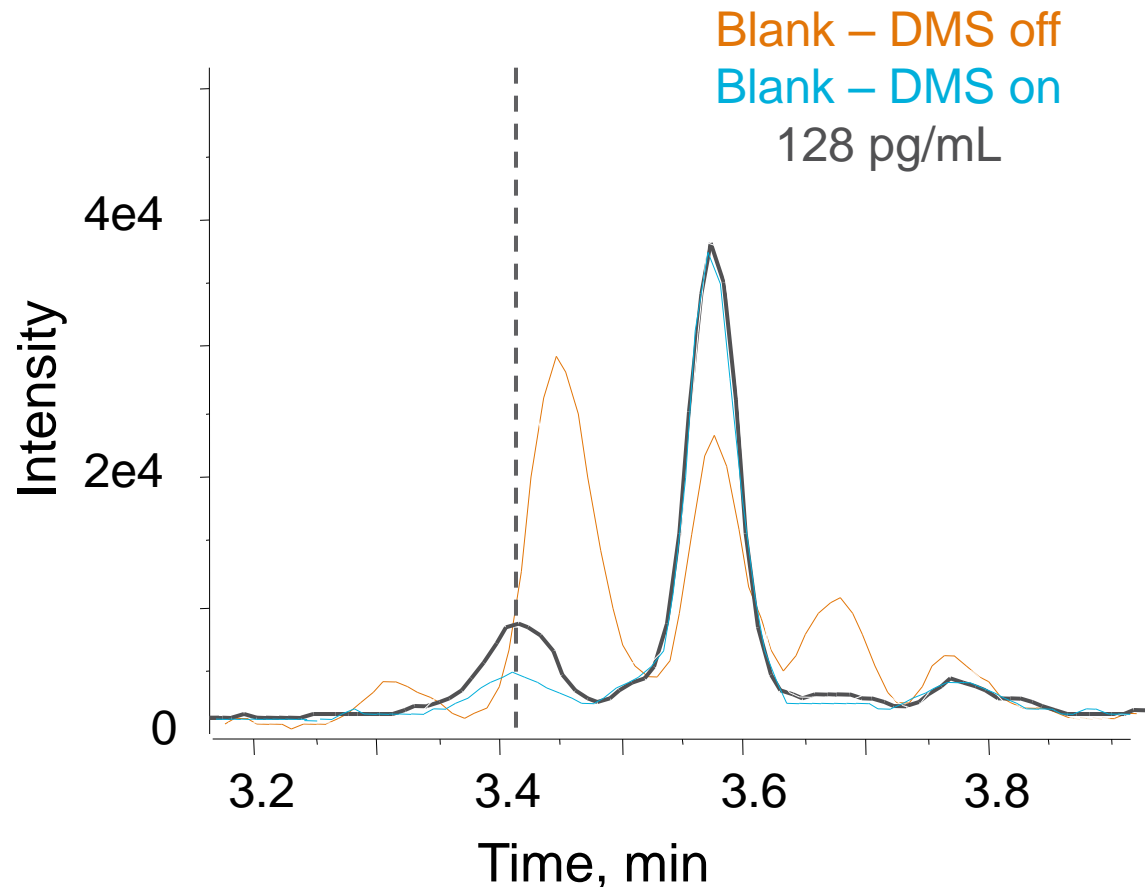
Pentoxifylline ~20 times LOQ Improvement



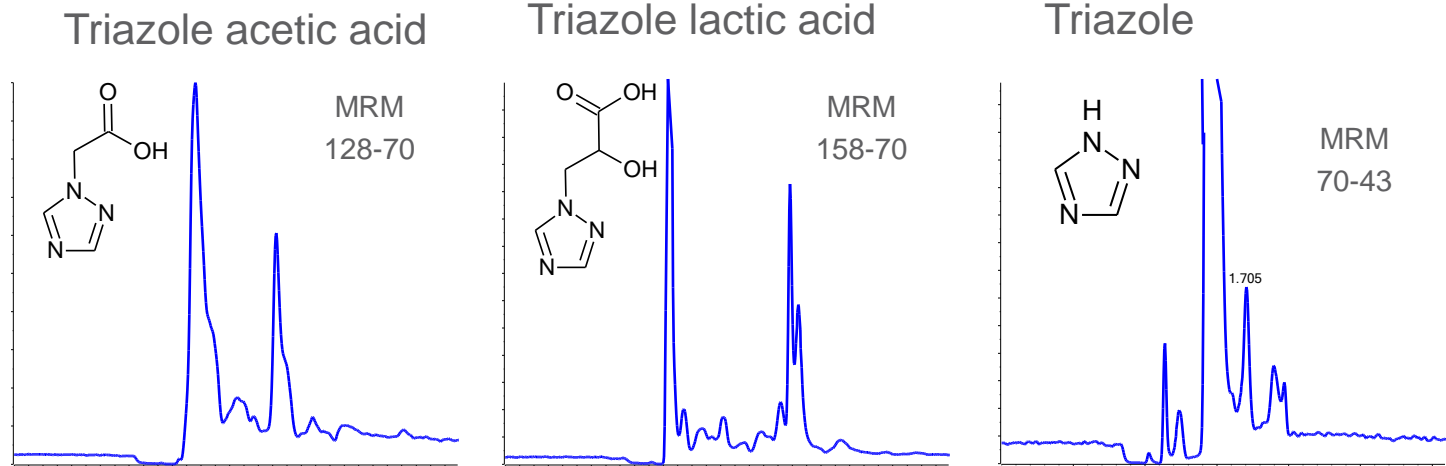
Measuring Large Peptides

BNP +6 Charge State

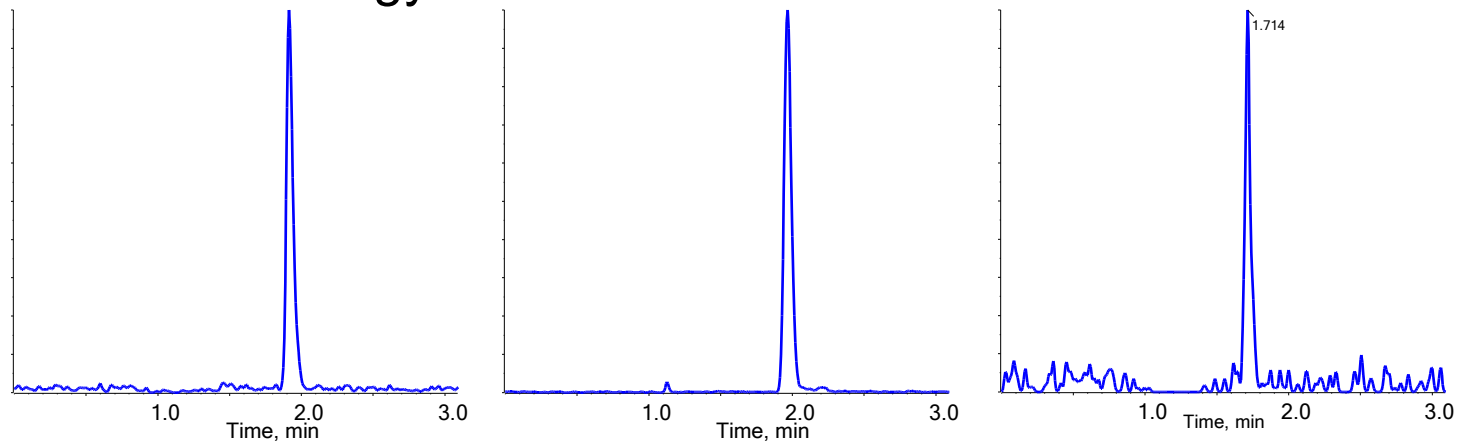
- For some large endogenous peptides that don't fragment well, monitoring intact by SIM can provide good sensitivity but is prone to interferences
- DMS can significantly reduce the presence of a co-eluting nearly isobaric matrix peak, to improve detection limits



Regular QTRAP[®] 5500



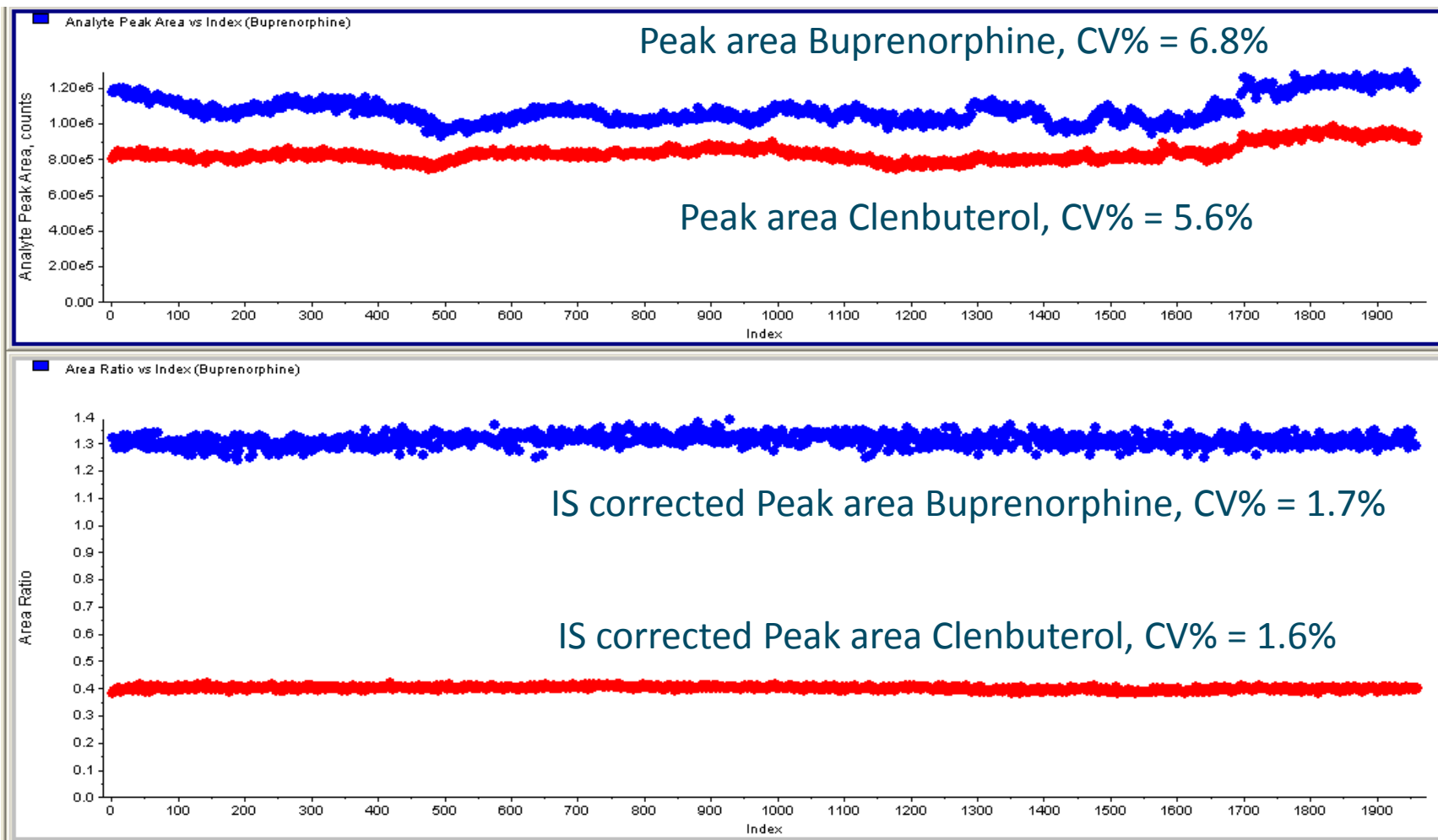
With SelexION[™] technology



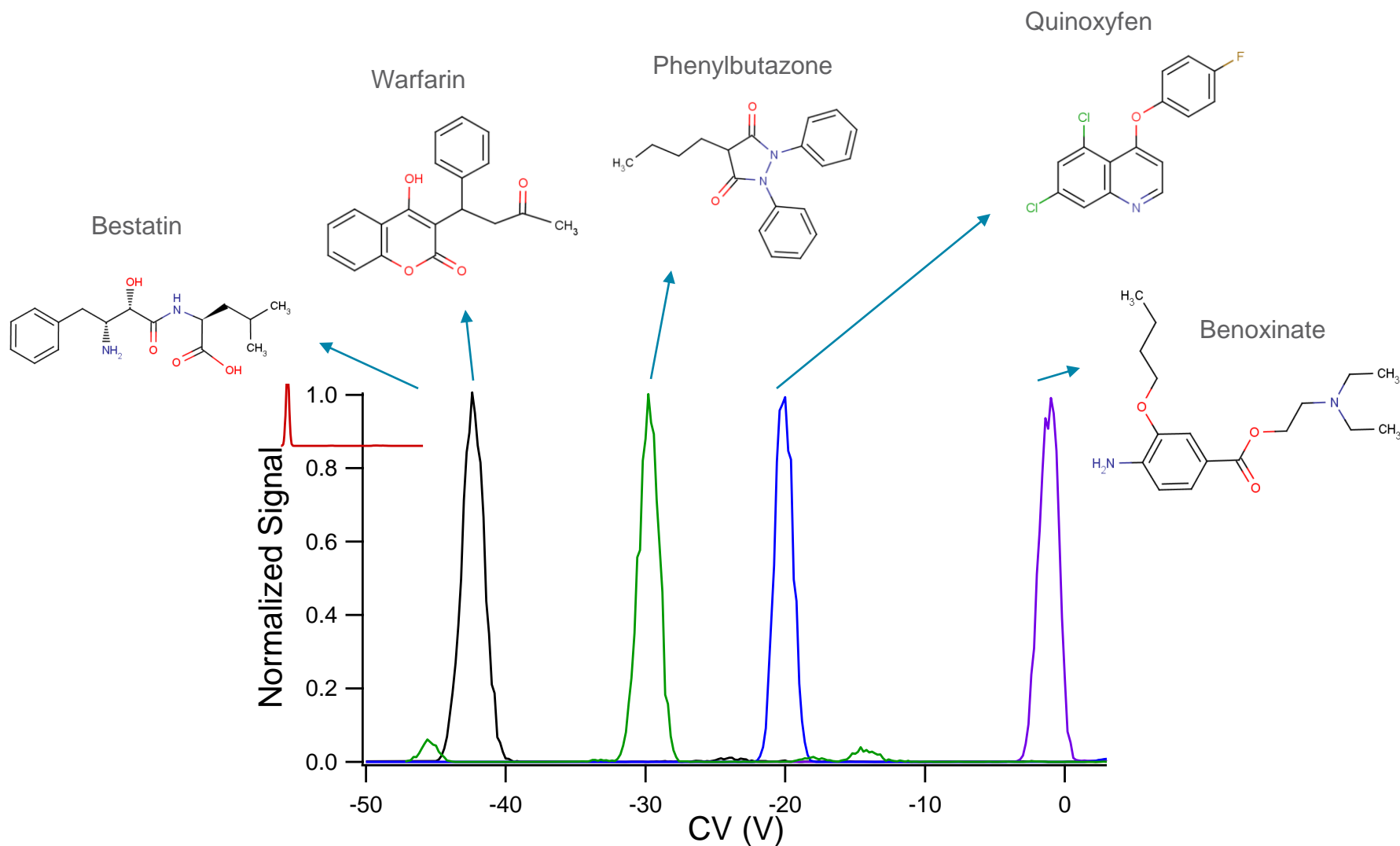
Grape fruit extract fortified with conazole degradant products at 0.01mg/kg. Samples kindly supplied by R. Schoening, Bayer CropScience, Monheim, Germany, & J. Jasak, Technical University, Institute of Food Chemistry, Dresden, Germany

Robustness for Demanding Applications in regulated labs

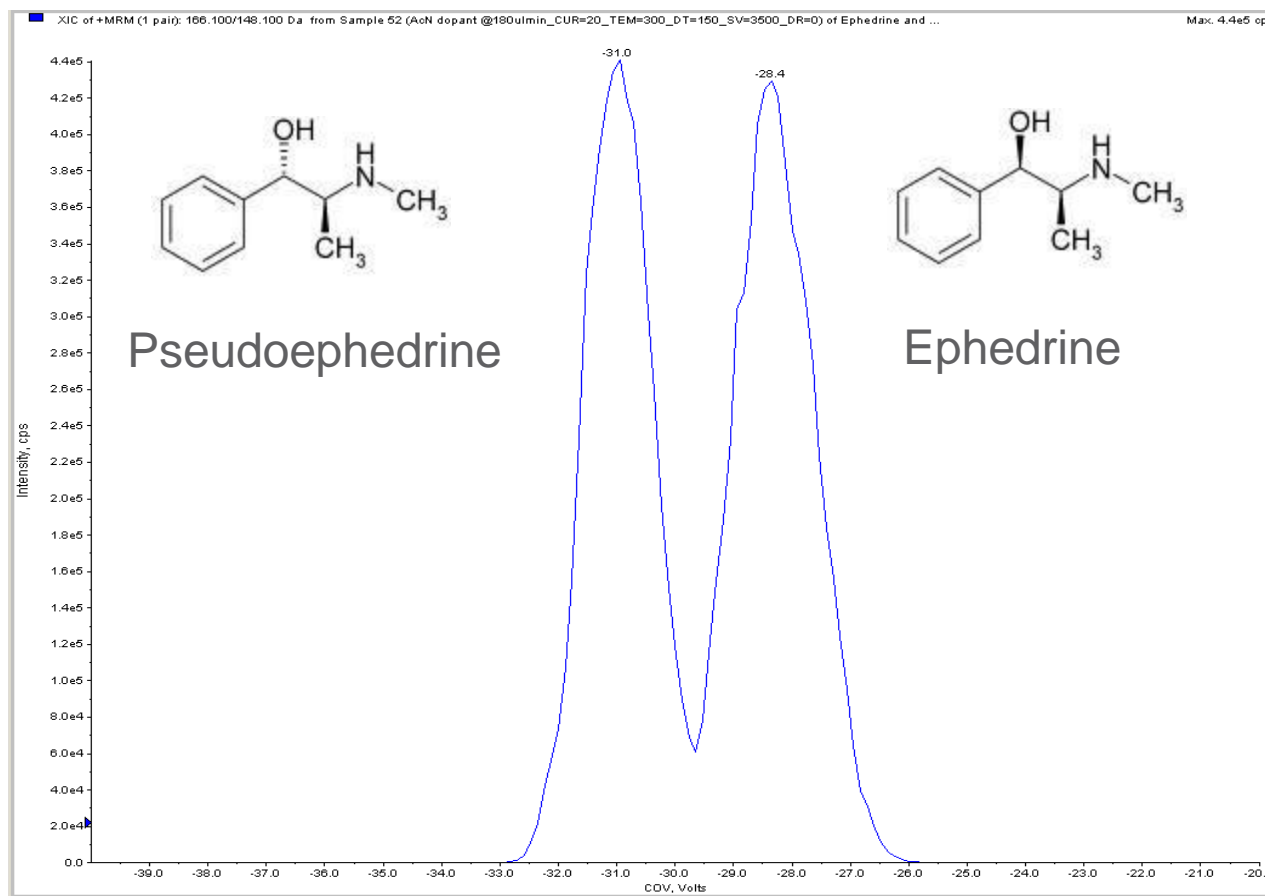
1000 *plasma* samples injected over 66 hours



Separation of Isobaric Compounds (m/z 309)



Separation of Isomers - Pseudoephedrine/Ephedrine



- Separation of pseudoephedrine and ephedrine with SelexION™
- Indistinguishable by MS or MS/MS

Steroid Interference Removal with SelexION™ Technology

Experiment Conditions

- 5 subjects each of male and female serum samples obtained from a customer laboratory
- STD was prepared in pooled children under age 7 serum samples.
- Two sample preparation methods:
 - LLE with 90/10 hexane/ ethyl acetate (200 uL serum; 1 mL solvent; recon 150 uL with 50/50 MeOH/H2O)
 - PPT with ACN (200 uL serum; 600 ACN; dry down and recon 150 uL with 50/50 MeOH/H2O)

Pumps Autosampler Oven Controller Time Program				
	Time	Module	Event	Parameter
1	0.50	Pumps	Pump B Conc.	10
2	1.50	Pumps	Pump B Conc.	70
3	2.50	Pumps	Pump B Conc.	75
4	4.30	Pumps	Pump B Conc.	75
5	4.50	Pumps	Pump B Conc.	95
6	6.20	Pumps	Pump B Conc.	95
7	6.40	Pumps	Pump B Conc.	10
8	7.50	Controller	Stop	
9				

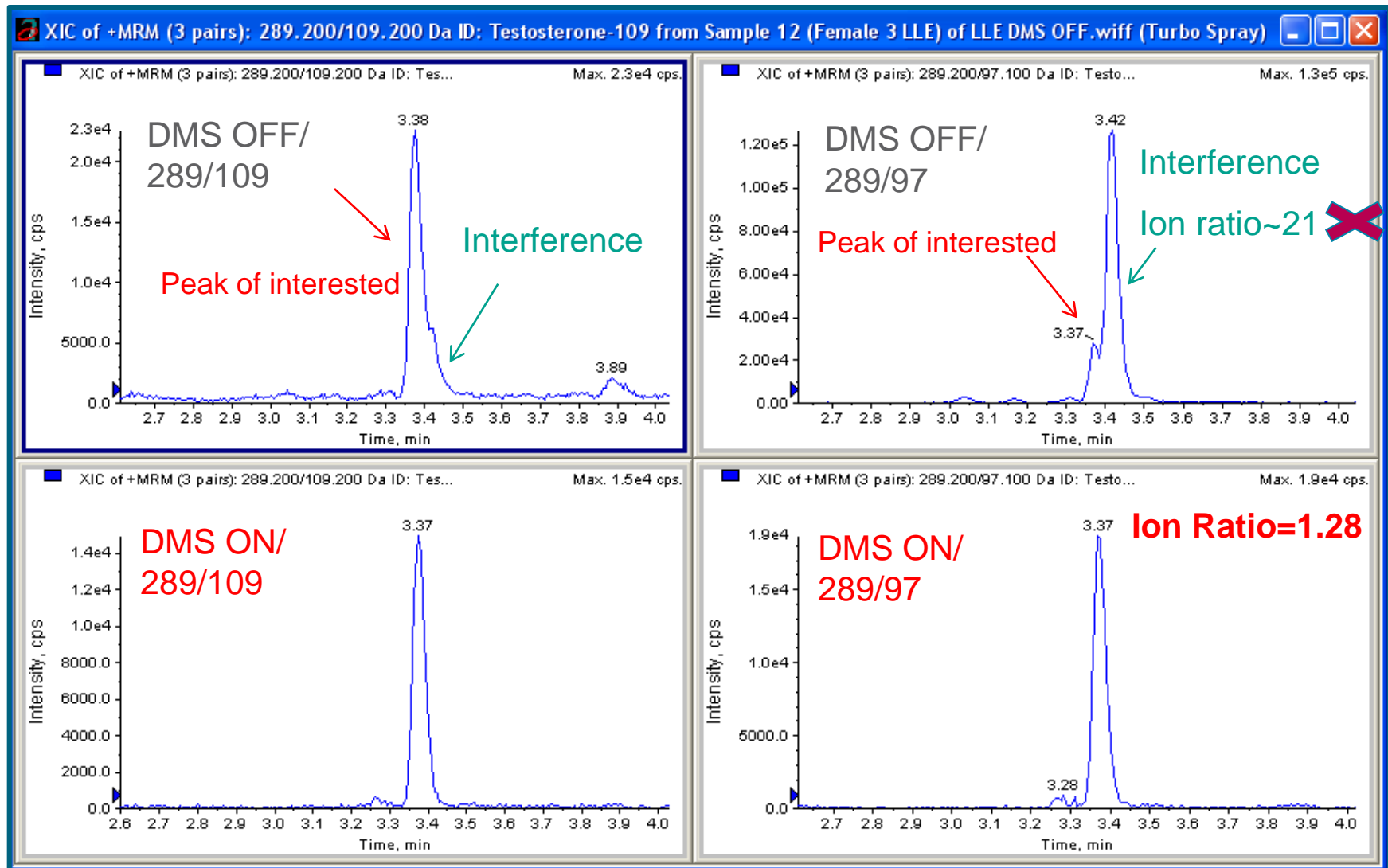
Column: Kinetex 2.6 u 50x2.1 mm; run time: 7 min; flow rate 0.3 mL/min

MPA: Water+0.1% FA;

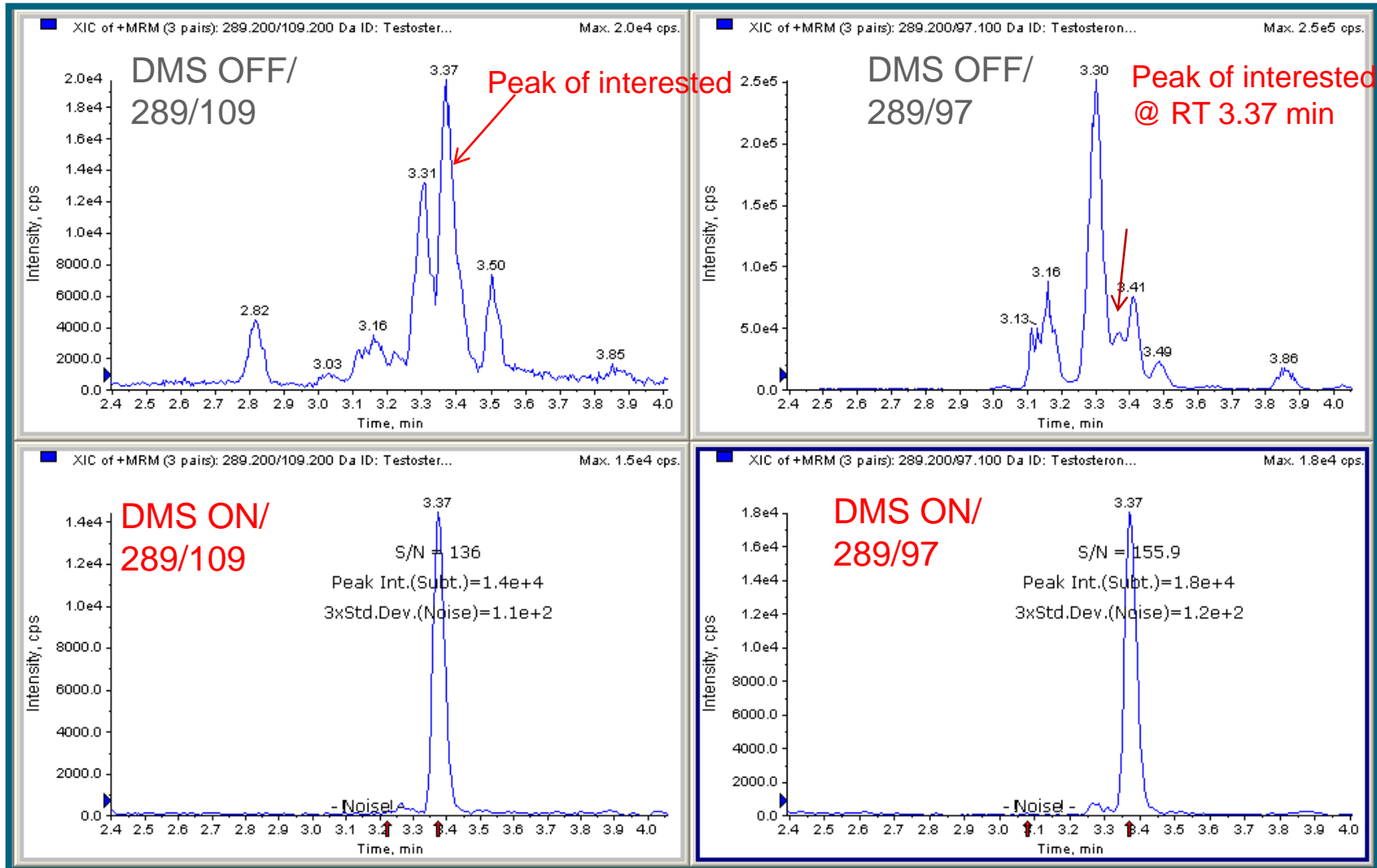
MPB: MeOH+0.1% FA

ESI: positive

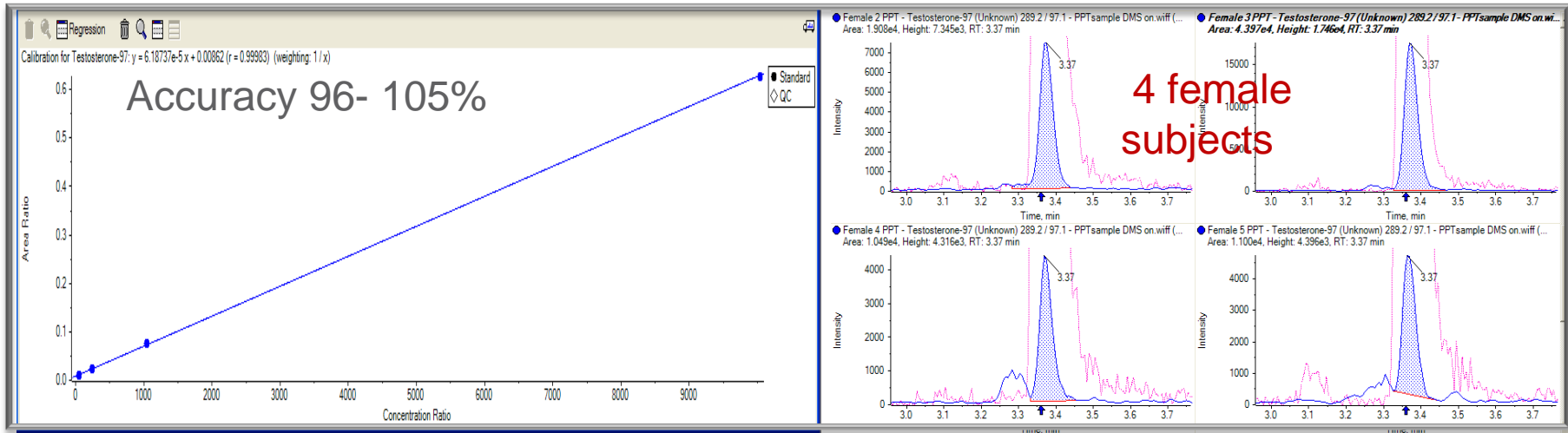
Testosterone Female #3 Serum Sample: LLE



Testosterone Female #3 Serum Sample: PPT

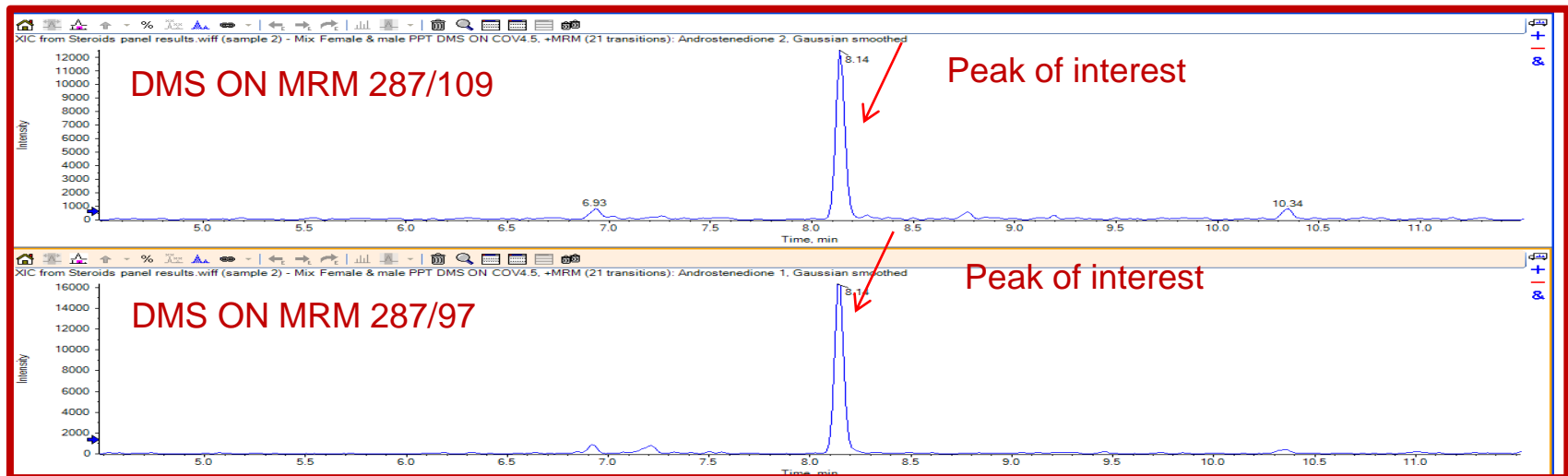
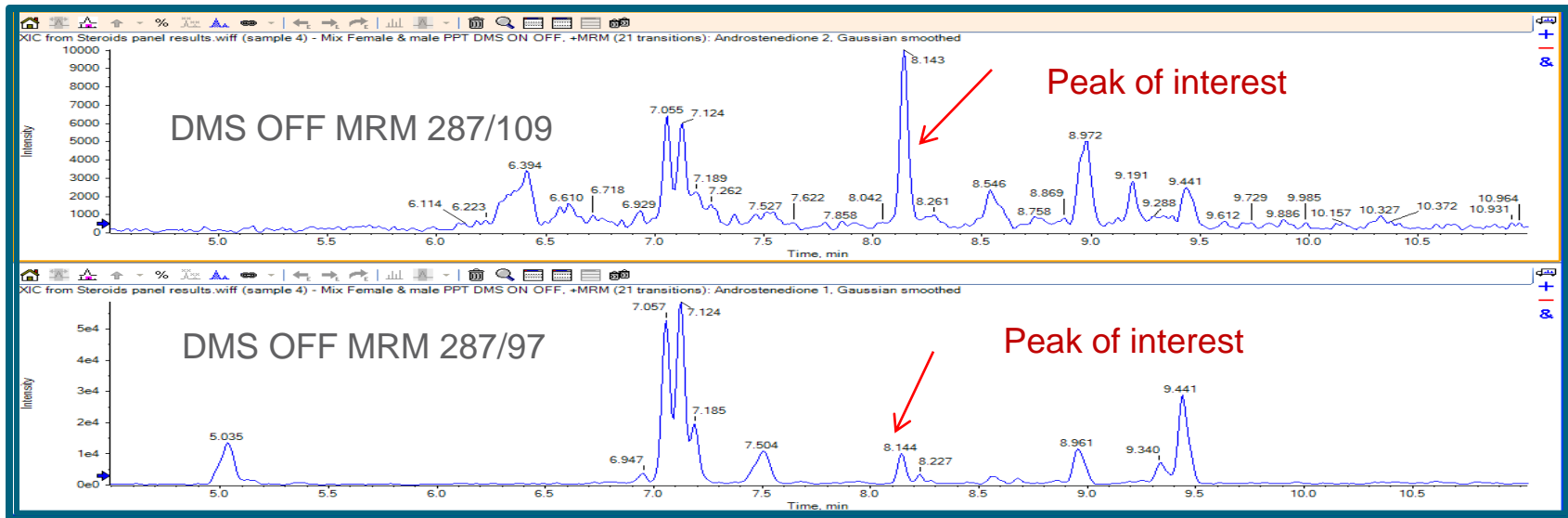


STD Curve in Serum Sample with DMS on (PPT)



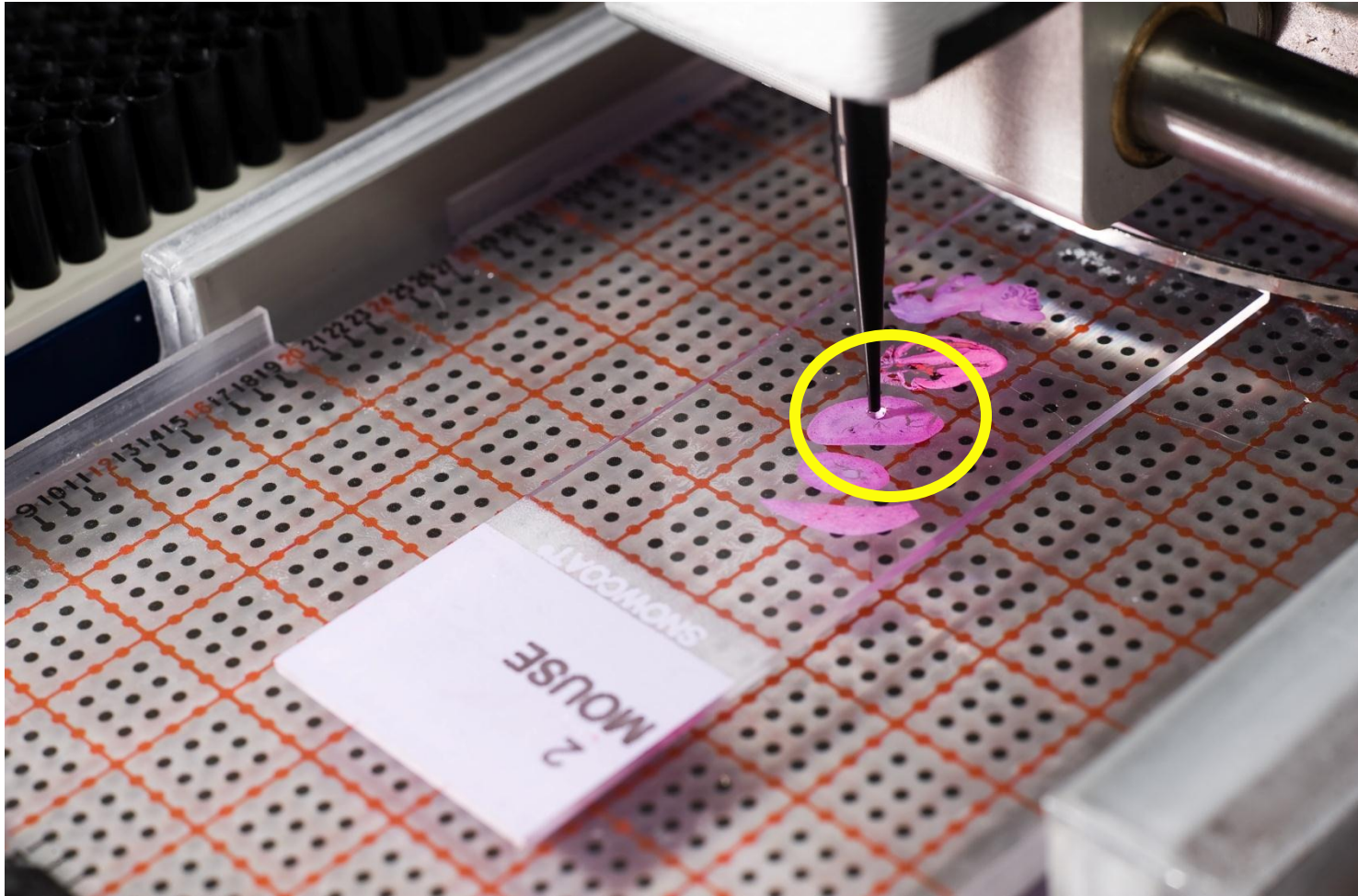
Sample	Neat (n=28)	Serum Sample PPT (n=25)	Serum Sample LLE (n=25)
Average Ion Ratio (97/109)	1.23	1.28	1.24
STDEV	0.04	0.07	0.04
%CV	3.44	5.47	3.12

Steroids panel: androstenedione at RT=8.14 min / protein precipitation sample preparation

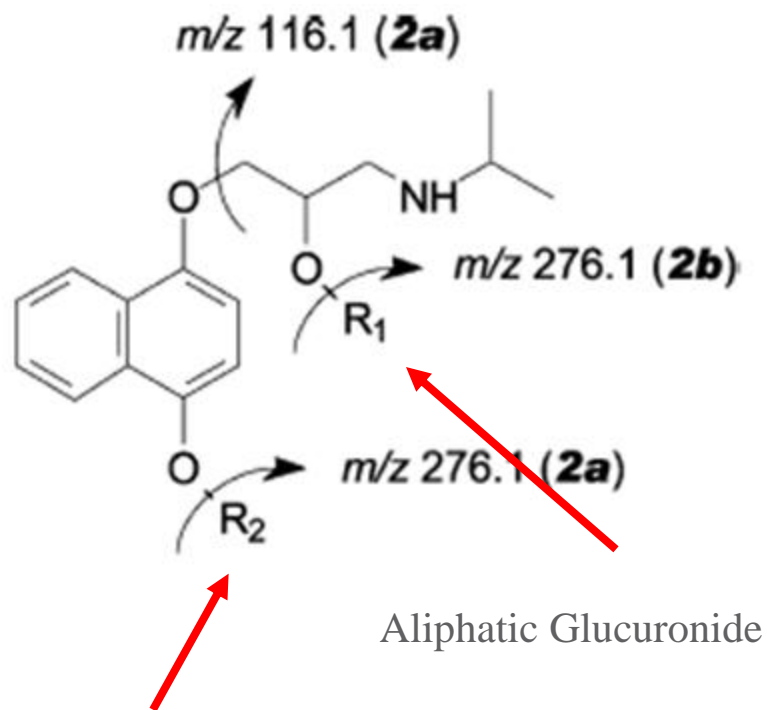


DMS as separation technique when LC is not an option...

- Infusion based analysis
- Surface Sampling
 - LESA
- Direct desorption technique
 - DESI
 - LDTD



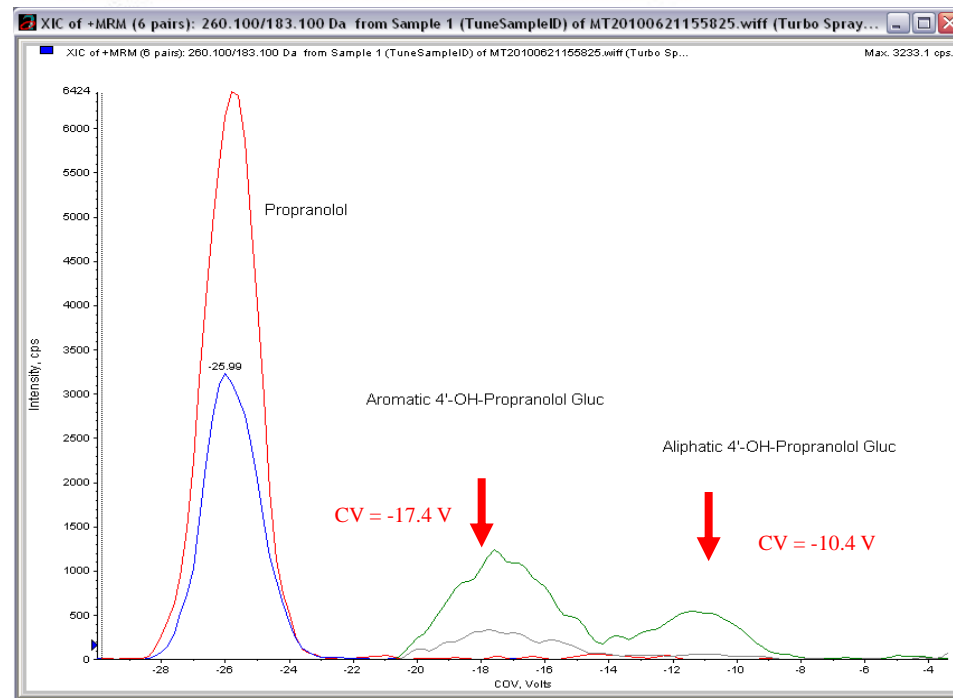
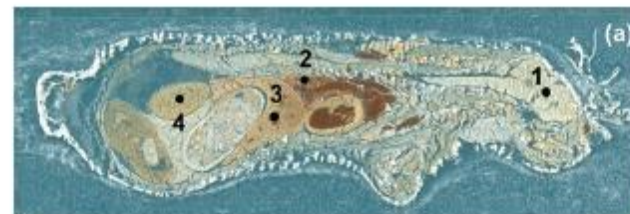
Propranolol Glucuronide Structural Isomers (acyl glucuronide)



Aromatic Glucuronide

Isobaric metabolites

Same fragments and dominant MRM transition!



DMS separation with 1.5% ACN modifier
 DMS resolves propranolol and the 2
 glucuronide structural isomers!

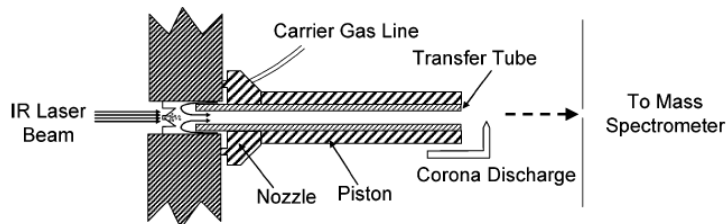
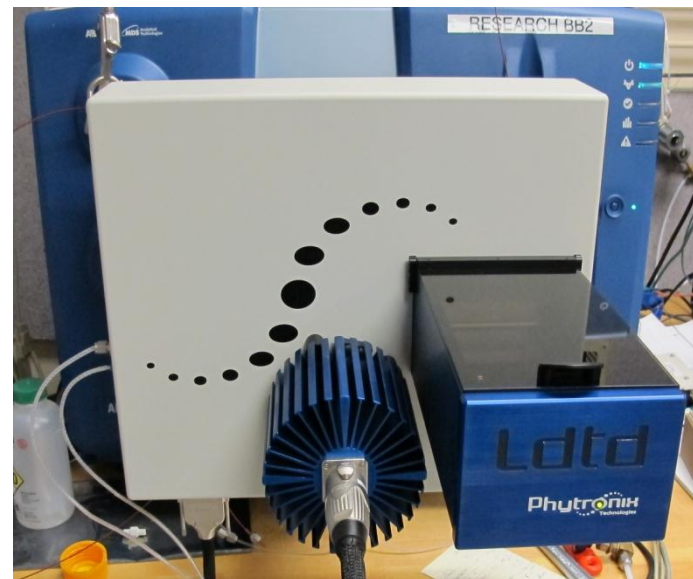


Figure 2. Schematic of the LDTD source. J Wu et al., *Anal. Chem.*, 2007, 79, 4657-4665.

Thermal desorption/APCI

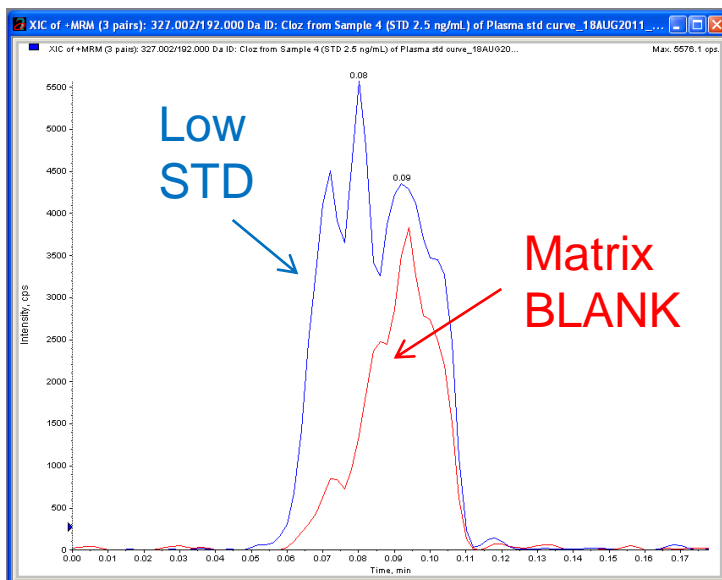
2.5 pg Clozapine in protein precipitated plasma

LDTD on 5500

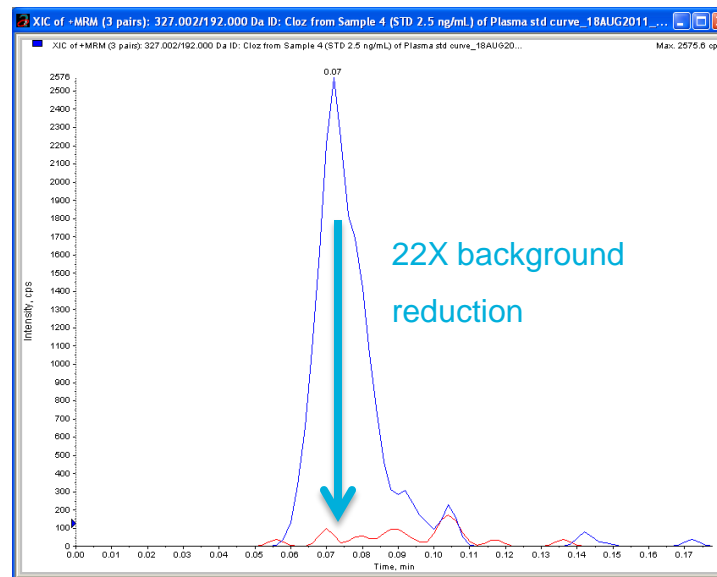


LDTD on 5500 with DMS

5576 cps



2576 cps



Summary

- An added dimension of selectivity through differential mobility spectrometry with the AB SCIEX SelexION™ Technology on the QTRAP® 5500 system and the Triple Quad™ 5500 system
- Improvement in data quality and LOQs for assays with interferences
- Robust and reliable for use in a regulated environment
- Improved throughput
 - Less need for chromatographic separations and sample prep
 - Can be coupled to non-LC sample introduction
- Combined with MRM³ on the QTRAP 5500 system, SelexION Technology gives several options
 - High sensitivity in MRM mode
 - High *selectivity* for analytes that fragment well with MRM³
 - High *selectivity* for analytes that fragment poorly with DMS-MS or DMS-MS/MS with the SelexION Technology
 - Separation for isobaric analytes with common fragments



Thank You



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