

## **μSPEed® | Application Note 2017**

### μSPEed recovery of Drug Mix from Serum and Blood

Pub No. 96-10006 Rev 01

#### SUMMARY

Developed of μSPEed trap and elute of 0.002ppm-2ppm 8 component drug standard from neat and samples – analysed using UHPLC -MS detection.

Development of an extraction clean-up method for 8 specific drug compounds from aqueous, serum and using μSPEed cartridges on ePrep Automated Sample Preparation platform.



#### EXTRACTS



250μL raw blood tapped on μSPEed cartridge



Cartridge after elution of trapped drugs from raw blood



Serum Extract from μSPEed cartridge

#### PROCEDURE

##### Stock Solutions

##### Individual Stock Solutions

1000ppm of each component into separate vials made up with ultra pure water:

- Verapamil HCl
- Caffeine
- Acebutolol HCl
- Propranolol HCl
- Dextromethorphan HBr
- Ketoconazole
- Atenolol
- Tolbutamide

### **Mixed Stock Standard (100ppm)**

Aliquot each of the 8-components made up to 100ppm with ultra pure water

### **Working Standard #1 (10ppm)**

1:10 of Mixed Stock made up with 50:50 Methanol:Water

### **Working Standard #2 (20ppm)**

2:10 of Mixed Stock made up with Ultra Pure Water

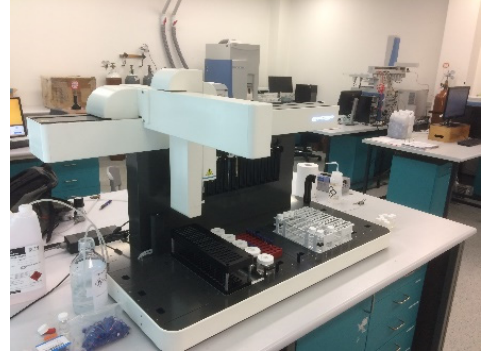
## **Instrumentation and $\mu$ SPE**

### **ePrep**

Sample Preparation Workstation used for the preparation of standards and automated  $\mu$ SPE.

Workflow programs developed for:

- a) Individual Stock Solutions
- b) Working Mixed Stocks
- c) Calibration Standards
- d) Point Standards
- e)  $\mu$ SPEed Processing



### **$\mu$ SPEed Cartridges**

$\mu$ SPEed, C18RPS-3 $\mu$ m/120Å

3 $\mu$ m/ 120Å ODS spherical silica packing. Part Number: 01-10110

All cartridges activated with Methanol and conditioned with 100% Ultra Pure Water before use.

New cartridge used for each sample.

### **HPLC (UV)**

Thermo Scientific Vanquish UHPLC systems and Chromeleon Chromatography Data System

### Column

Accucore Vanquich C18+

100mm x 2.1mm

1.5 $\mu$ m Particle

P/N 27101-102130

### **MSQ (Mass Spectrometer)**

Thermo Scientific single quadrupole mass spectrometer

## **Sample Preparation**

### **Method A: Calibration Standards – SIMS/MS**

Mixed calibration standards made up at 2, 1, 0.2, 0.1, 0.02 and 0.002 ppm

### **Method B: Extraction of Calibration Standards using $\mu$ SPEed**

200 $\mu$ l of each calibration standard loaded onto SPE cartridges. Washed with 100 $\mu$ l of water and eluted with 100 $\mu$ l of MeOH. Each sample was then diluted with 100 $\mu$ l of water to eliminate any viscous fingering.

### **Method C: Concentration Extraction of Spiked Serum Sample using $\mu$ SPEed**

100 $\mu$ l of Serum spiked with 0.02ppm of drug mix

100 $\mu$ l of each Serum loaded onto SPE cartridges. Washed with 100 $\mu$ l of water and eluted with 20 $\mu$ l of methanol. Each sample was then diluted with 20 $\mu$ l of water to eliminate any viscous fingering.

## Chromatography

Mobile Phase A = Ultra Pure Water with 1% Formic Acid

Mobile Phase B = Acetonitrile with 1% Formic Acid

0 min = 95% A and 5% B

Linear Gradient @ 0.3mL/min

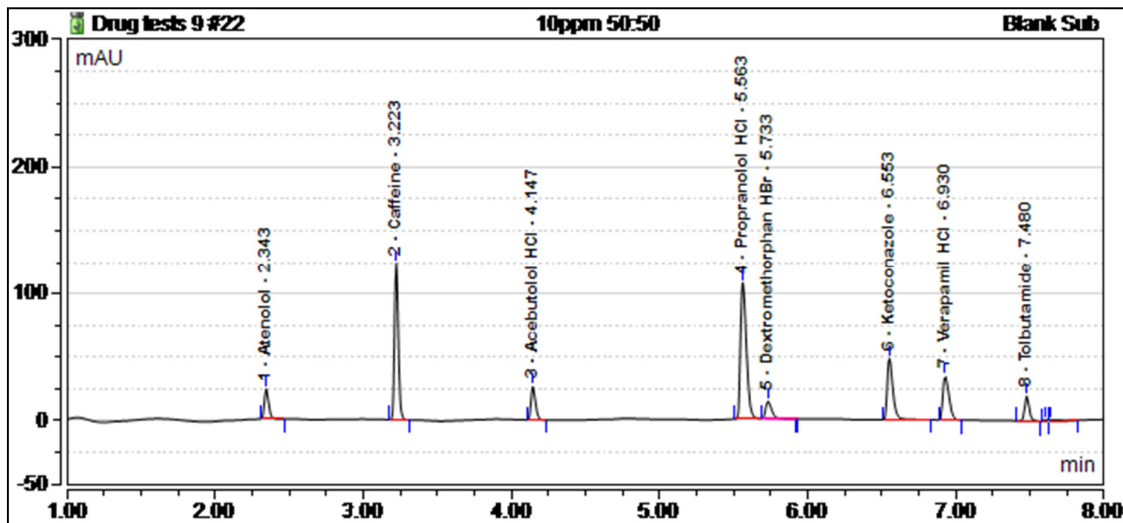
8 min = 40% A and 60% B

Column Temperature = 25°C

Autosampler Temperature = 8°C

1uL Injection

## UV Detection



Initial method development on UV detector at 254nm. Elution profile optimised. Limit of detection found to be 2ppm

## Mass Spectrometry

Probe Temperature (°C) = 349

Needle (kV) = 3.00

Scan Time (Sec) = 0.5

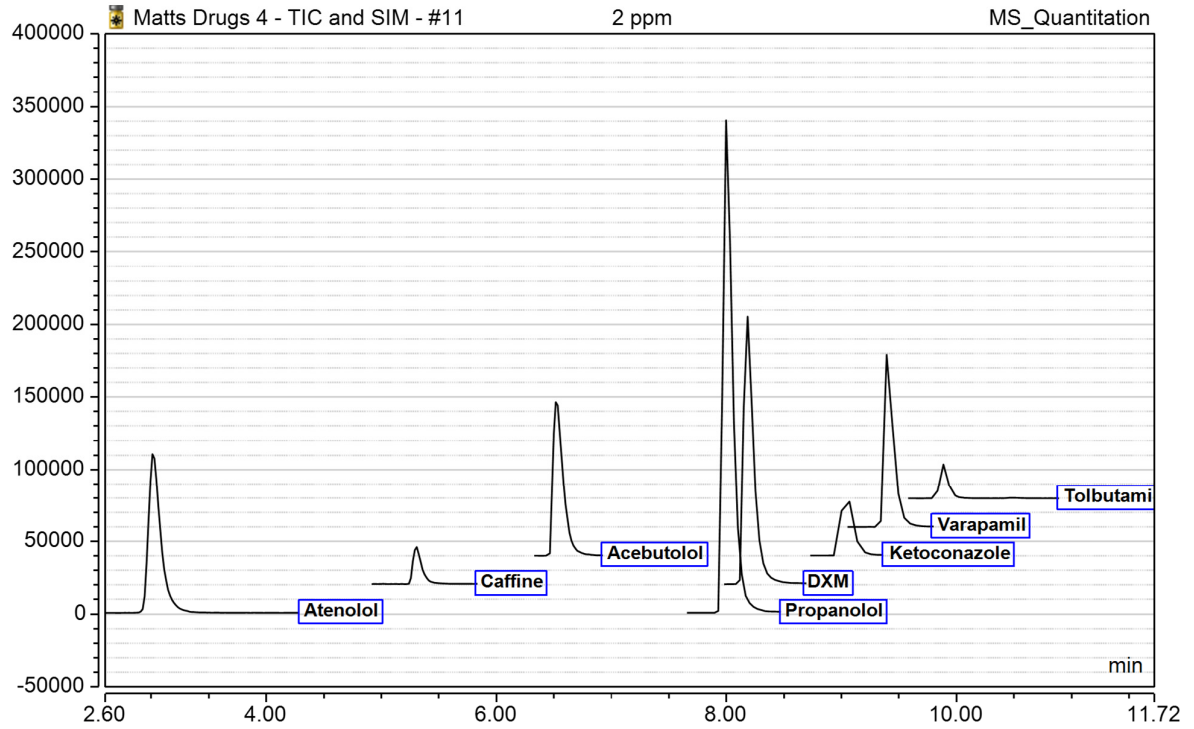
Selective Ion Monitoring at the following ions for each compound:

Compound	m/z	Window
Atenolol	267.2	2.5 - 3.2
Caffeine	195.1	5 - 5.5
Acebutolol	337.2	7.7 - 8.4
Propanolol	260.2	7.7 - 8.6
DXM	272.2	7.7 - 8.6
Ketoconazole	531.2	8.8 - 9.3
Varapamil	455.3	9.2 - 9.7
Tolbutamide	271.1	9.7 - 10.5

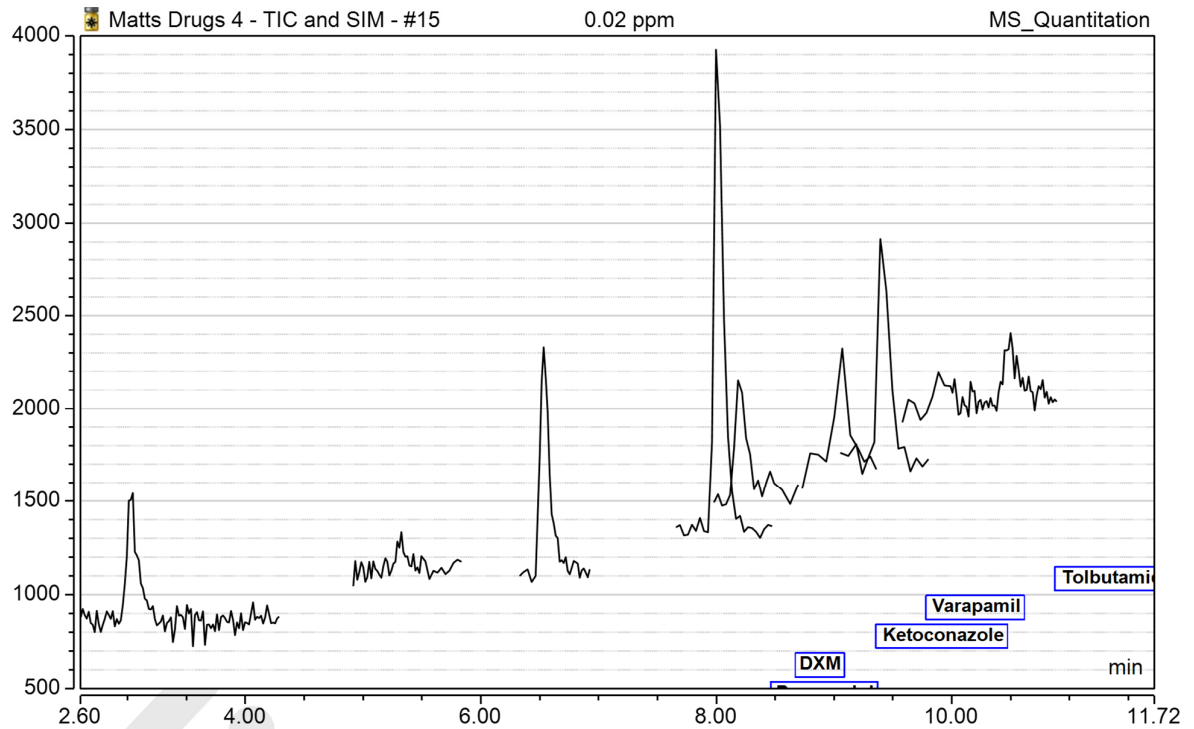
## RESULTS AND DISCUSSION

### Calibration Standard – SIMS/MS

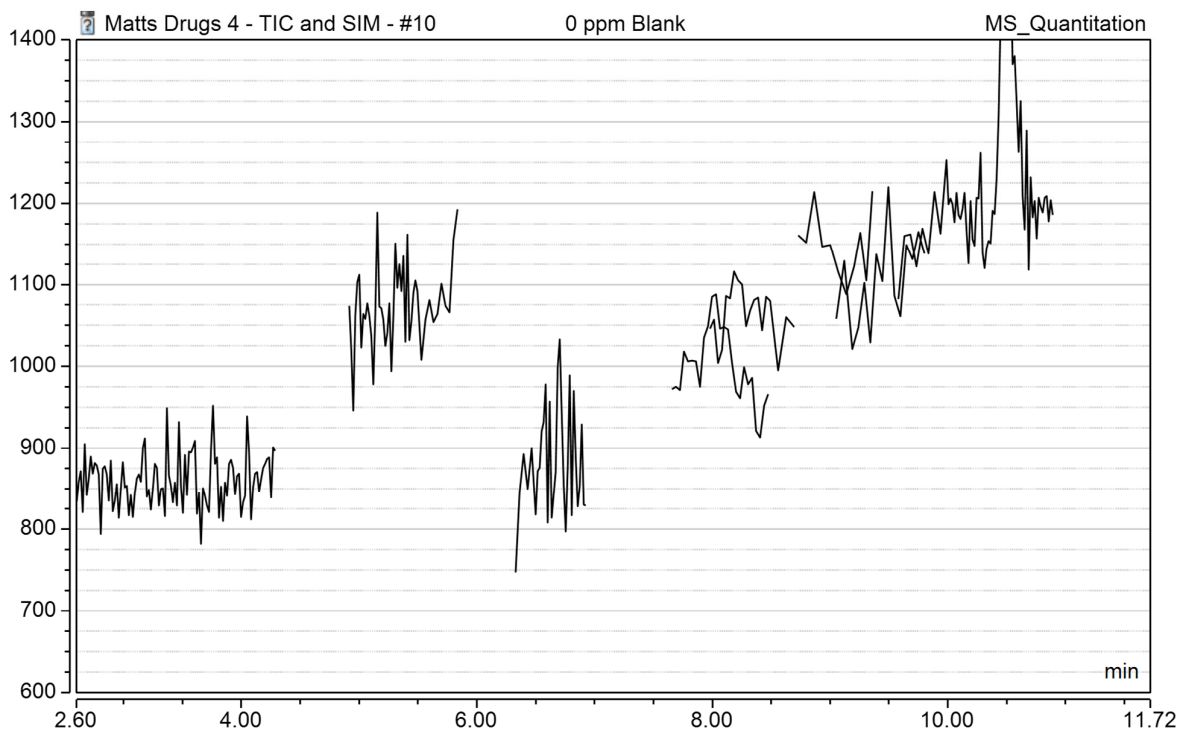
Selective Ion Monitoring at 2ppm sample



Selective Ion Monitoring at 0.02ppm sample



Selective Ion Monitoring in blank sample



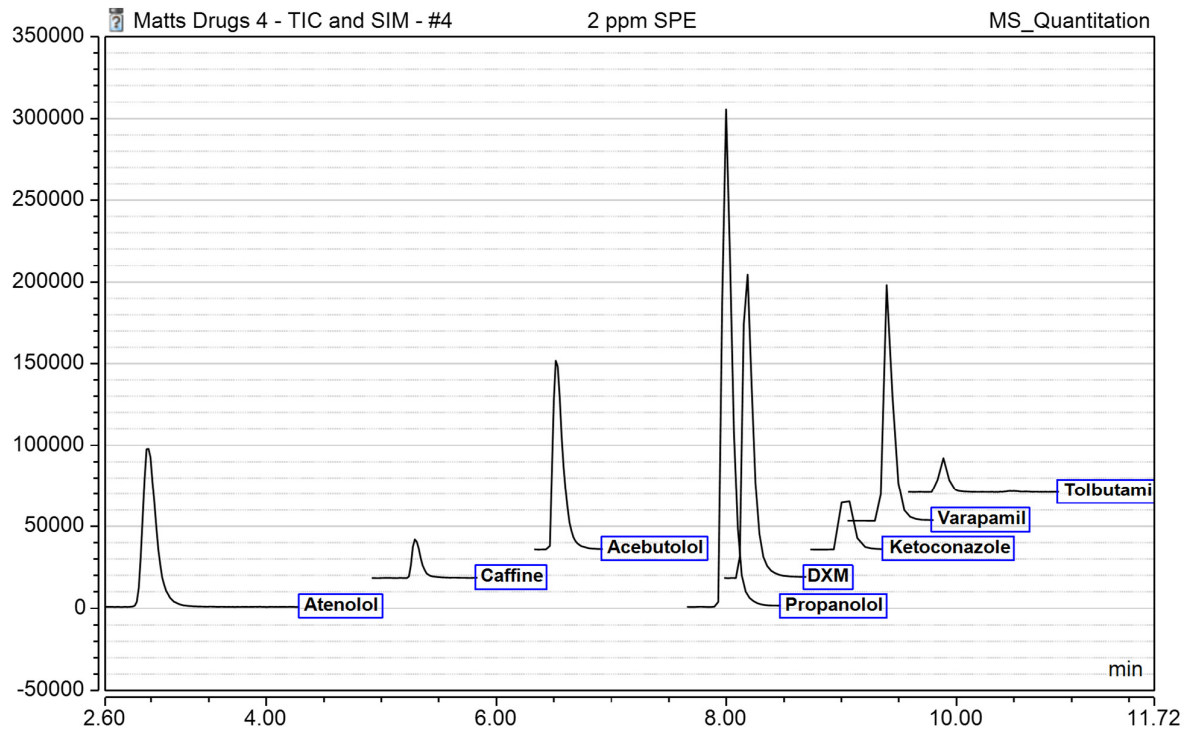
Calibrations for Neat Standards – 2 ppm to 0.002 ppm in elution order

<b>Compound</b>	<b>Offset (C0)</b>	<b>Slope (C1)</b>	<b>R-Square</b>
Atenolol	-279.0525	9923.5475	0.9974
Caffeine	-137.9212	1087.5172	0.9966
Acebutolol	2196.0562	8656.7169	0.9990
Propranolol	588.7025	25146.1453	0.9994
DXM	-376.4213	17721.4423	0.9967
Ketoconazole	106.0764	2371.3935	0.9847
Varapamil	-278.7006	11147.8838	0.9763
Tolbutamide	32.6215	1593.5637	0.9957

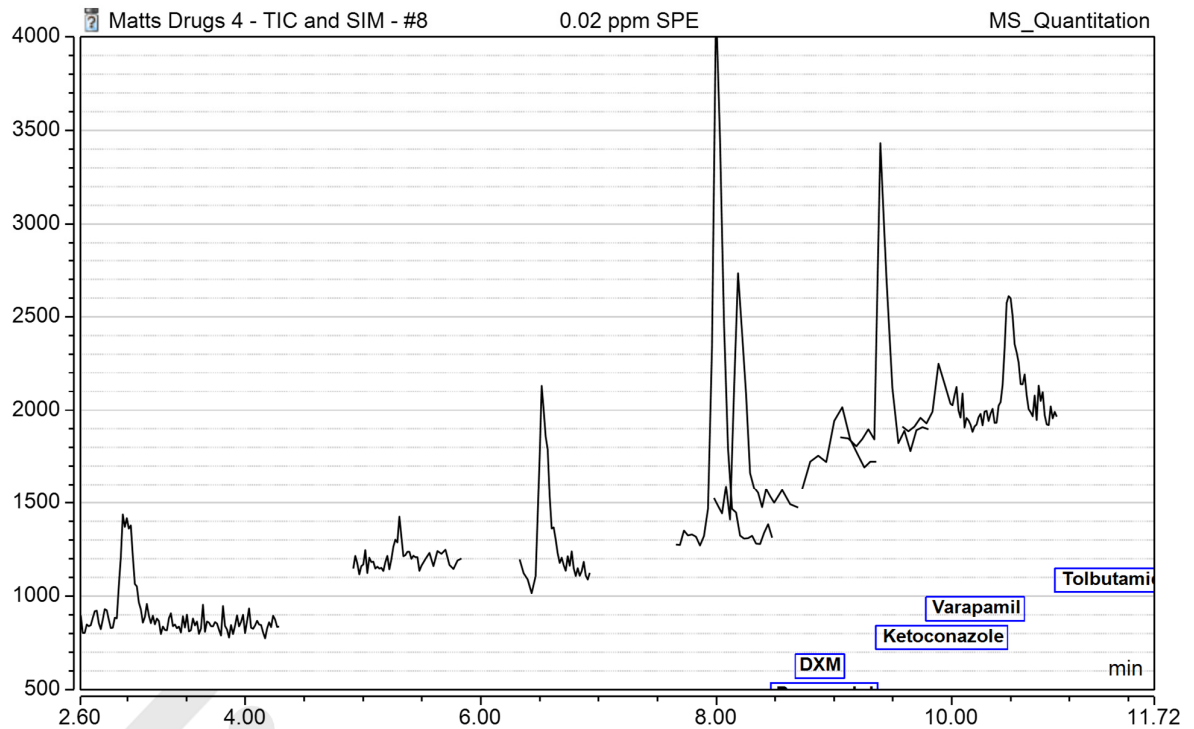


## Extraction of Calibration Standards using $\mu$ SPEd

### Selective Ion Monitoring at 2ppm $\mu$ SPE sample



### Selective Ion Monitoring at 0.02ppm $\mu$ SPE sample

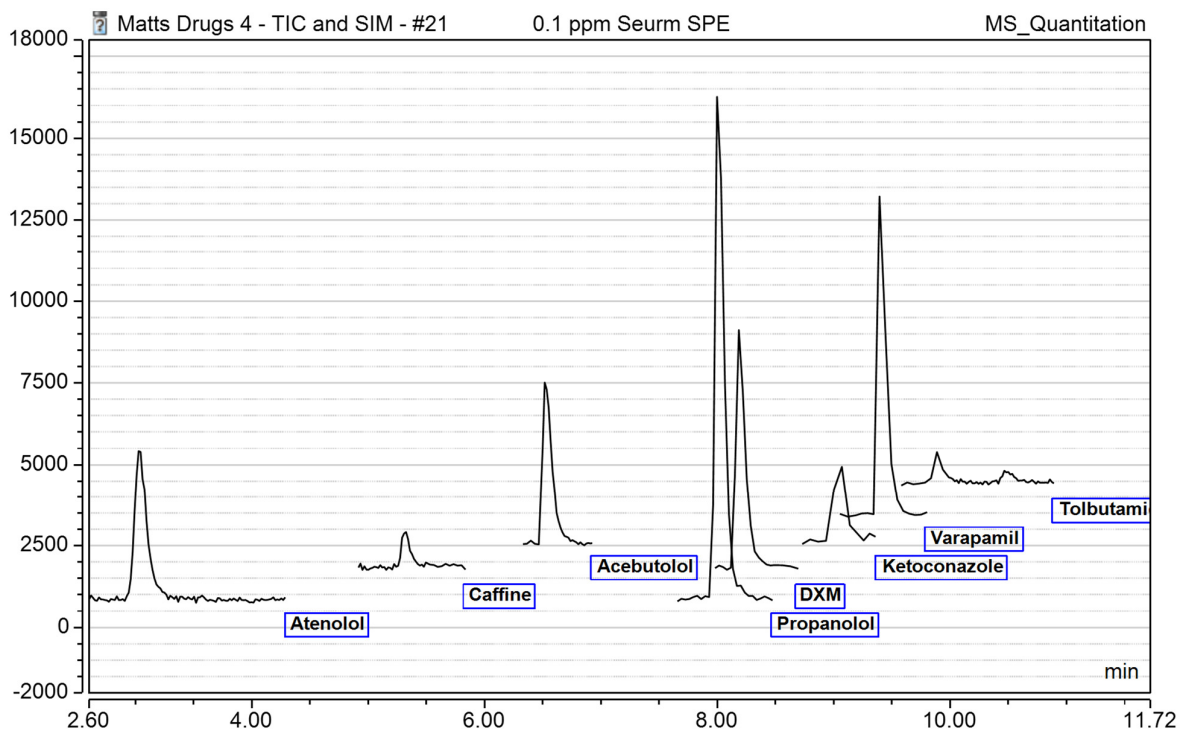


Recoveries from  $\mu$ SPE of each compound in elution order.

Compound	2ppm	1ppm	0.2ppm	0.1ppm	0.02ppm	0.002ppm
Atenolol	103.58%	105.88%	116.10%	117.74%	111.94%	
Caffeine	99.95%	94.53%	95.85%	97.80%	98.67%	
Acebutolol	109.53%	117.95%	105.35%	138.27%	88.52%	
Propranolol	94.78%	94.52%	105.02%	98.74%	115.13%	
DXM	110.85%	121.42%	155.27%	156.75%	184.09%	
Ketoconazole	100.59%	154.63%	118.22%	94.05%	60.06%	
Varapamil	134.41%	124.57%	150.63%	154.41%	116.89%	
Tolbutamide	102.31%	102.12%	119.97%	146.54%	151.10%	

### Concentration Extraction of Spiked Serum Sample using $\mu$ SPE

Selective Ion Monitoring at 1:5 Concentrated 0.02ppm Serum  $\mu$ SPE sample



Recoveries of compounds in SPE serum sample using Selective Ion Monitoring.

Compound	2ppm	1ppm	0.2ppm	0.1ppm	0.02ppm	0.002ppm
Atenolol	82.41%	84.93%	95.17%	93.89%	91.94%	
Caffeine	76.57%	70.17%	78.02%	83.83%	98.86%	
Acebutolol	92.84%	101.54%	86.76%	121.97%	83.41%	
Propranolol	81.24%	82.08%	92.93%	86.35%	103.67%	
DXM	96.11%	107.63%	139.58%	137.19%	161.85%	
Ketoconazole	73.08%	135.55%	106.25%	76.57%	56.13%	
Varapamil	107.89%	100.28%	127.97%	128.80%	91.11%	
Tolbutamide	83.94%	80.84%	99.63%	156.68%	179.62%	

## CONCLUSION

### Calibration Standards

There was most likely a sample prep error in the 1ppm standards for verapamil and ketoconazole, as seen in the reduced R2 value. Even including this all calibrations are highly linear and each curve passes almost exactly through the middle showing there is not carry over from other runs. Several of the calibration points down at 0.002 ppm were indistinguishable from noise

### Extaction of Calibration Standards using $\mu$ SPEed

Recoveries of all compounds were good with recoveries generally being close to 100%. There are exceptions to this, DXM and verapamil both showed significantly more single response in the  $\mu$ SPE samples, this may have been due to insufficient washing steps within the SPE cartridge preparation.

Another factor contributing to the poor recoveries in lower concentrations would be due to the difficulty integrating the poor peak shape. As can be seen in the 0.02ppm samples the peak shapes for compounds deteriorated due to low signal intensity and insufficient scan time while scanning for multiple ions.

### Extaction of Spiked Serum Sample using $\mu$ SPEed

The Serum  $\mu$ SPE samples show good recoveries across the board for all compounds, which is to be expected. The method itself provided a rapid way to clean and analyse what would otherwise be a dirty sample.

## ACKNOWLEDGEMENTS

Matthew Diplock (PhD Candidate), Prof Philip Doble<sup>1</sup>,

<sup>1</sup>University of Technology, Sydney - School of Mathematical and Physical Sciences, Faculty of Science.

## $\mu$ SPEed Ordering Information

Part Number	Code	Description
<b><math>\mu</math>SPEed Cartridges</b>		
01-10110	$\mu$ SPEed, C18RPS-3 $\mu$ m/120Å (Pkt 10)	3 $\mu$ m/ 120Å ODS spherical silica packing with high acidic resistance suitable for general organic compound applications.
01-10115	$\mu$ SPEed, Silica-3 $\mu$ m/120Å (Pkt 10)	3 $\mu$ m/120Å spherical bare silica packing. High purity silica for normal and hilly applications
01-10150	$\mu$ SPEed, PS/DVB -3 $\mu$ m/ 300Å (Pkt 10)	3 $\mu$ m/ 300Å spherical, crosslinked polystyrene divinyl benzene
01-10151	$\mu$ SPEed, PS/DVB RP-3 $\mu$ m/ 300Å (Pkt 10)	3 $\mu$ m/ 300Å Phenyl (RP) spherical, crosslinked polystyrene divinyl benzene
01-10155N	$\mu$ SPEed, PS/DVB SAX-3 $\mu$ m/ NP (Pkt 10)	3 $\mu$ m/Non Porous SAX spherical, crosslinked polystyrene divinyl benzene
01-10156N	$\mu$ SPEed, PS/DVB SCX-3 $\mu$ m/ NP (Pkt 10)	3 $\mu$ m/Non Porous SCX spherical, crosslinked polystyrene divinyl benzene

