

Comparison of Solid Phase Extraction Methods for Reduction of Matrix Induced Ion-Suppression of Clenbuterol by Linear Ion Trap

**Craig R. Aurand, Carmen T. Santasania,
Daniel Shollenberger, and Olga Shimelis**

Supelco, 595 North Harrison Road, Bellefonte, PA 16823

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Abstract

In this study we investigate the effectiveness of three different SPE phases for the recovery of clenbuterol from urine and the reduction of matrix induced ion suppression. Here we compare the high selectivity of a molecular imprinted polymer (MIP) with the universal application of a generic polymeric phase and a mixed mode SPE phase. This study examines the degree of sample cleanup from each of the three phases and how this affects the ionization and ultimately the limits of detection and quantitation of clenbuterol.

Analysis of Clenbuterol was performed using a chiral separation to enable quantitation of the S and R enantiomers. Samples were analyzed on the Applied Biosystems 3200 Q Trap Linear Ion traps which enable MRM experiments to be performed for quantitation while simultaneously performing full scan experiments for monitoring matrix interference.

Introduction

Clenbuterol belongs to the class of beta-agonists and was previously used for human and veterinary therapeutics, mainly as anti-asthmatic and bronchodilator. The abuse of clenbuterol-containing products in food-producing animals stems from the ability to induce weight-gain and greater proportion of muscle to fat. Clenbuterol residues can affect lung and heart function in persons who have consumed organ or muscle tissue of animals given the drug. To decrease the exposure and the potential risk to the population, clenbuterol compounds are now banned for use as growth promoters in the EU and in the USA.

Experimental

instrument: Agilent 1100 Stack with Applied Biosystems 3200 Linear Ion Trap
column: Astec CHIROBIOTIC™ T, 10 cm × 2.1 mm I.D., 5 µm particle size
mobile phase: 10 mM ammonium formate in methanol
flow rate: 300 µL/min.
temp.: 30 °C
injection : 1.0 µL
source conditions: Turbo Ion Spray ESI+
CUR: 35.00
IS: 3200.00
TEM: 425.00
GS1: 45.00
GS2: 40.00
ihe: ON
DP 70.00
EP 10.00

Experimental (contd.)

3200QT Method Parameter Table (Period 1 3 Experiments)

Period 1 Experiment 1:

Scan Type: MRM (MRM)

Polarity: Positive

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)
277.20	203.10	150.00
Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)
277.20	168.20	150.00

Period 1 Experiment 2:

Scan Type: MRM (MRM)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)
277.20	203.10	150.00
Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)
277.20	168.20	150.00

Period 1 Experiment 3:

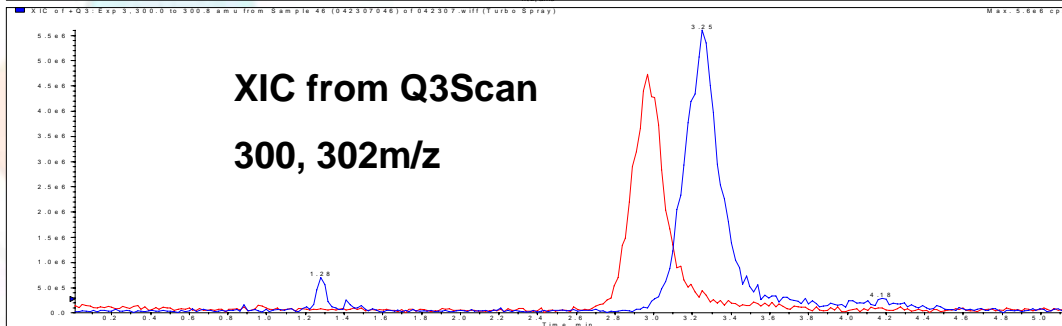
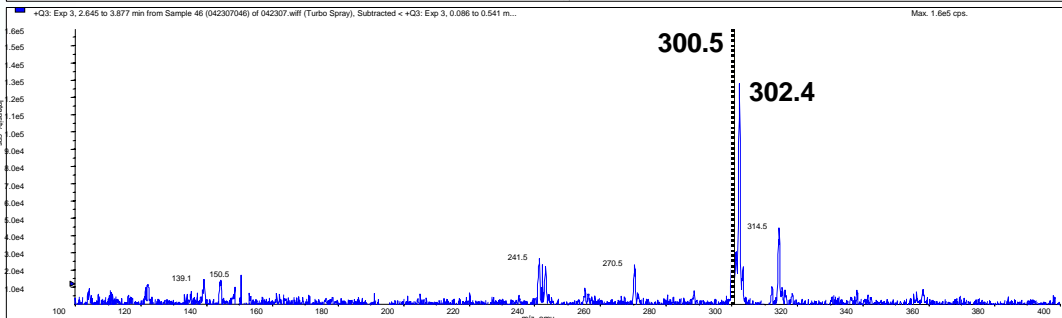
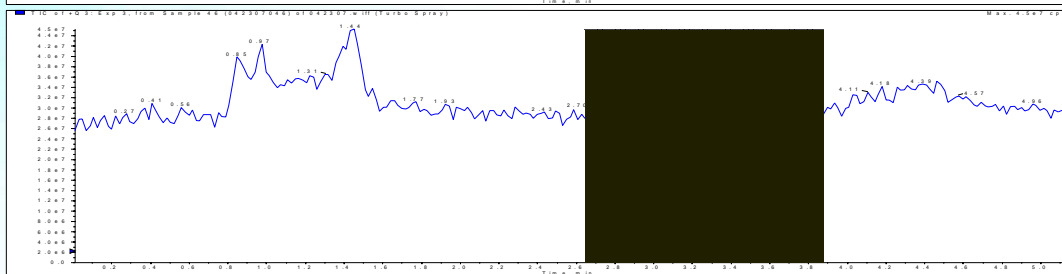
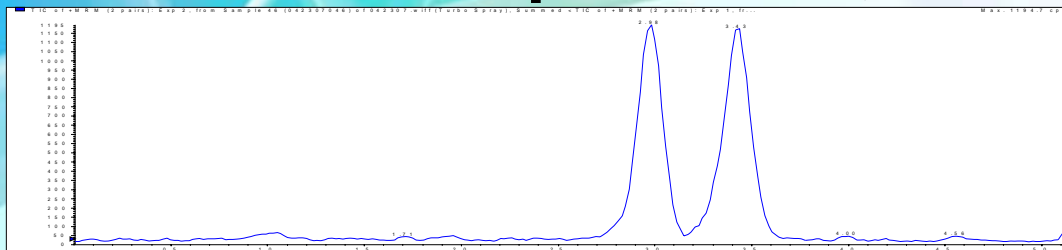
Scan Type: Q3 MS (Q3)

Start (amu)	Stop (amu)	Time (sec)
100.00	400.00	0.50

Experimental (contd.)

- Experiment 1:** MRM Transition for S_enantiomer of Clenbuterol. This experiment monitors two transitions, 277.2-203.1 and 277.2-168.2 for use with summed multiple ions quantitation.
- Experiment 2:** MRM Transition for R_enantiomer of Clenbuterol. This experiment monitors two transitions, 277.2-203.1 and 277.2-168.2 for use with summed multiple ions quantitation.
- Experiment 3:** Q3 Scan for monitoring matrix. This is a scan from 100-400m/z in quadrupole 3 to monitor matrix that may interfere with quantitation of the S_R enantiomers of Clenbuterol. From the Q3 Scan, corresponding XIC from matrix ions are generated to monitor degree of sample cleanup.

Period 1 Experiments, One Injection of Sample



MRM Experiment
(Quantitation)

TIC from Q3 Scan Experiment

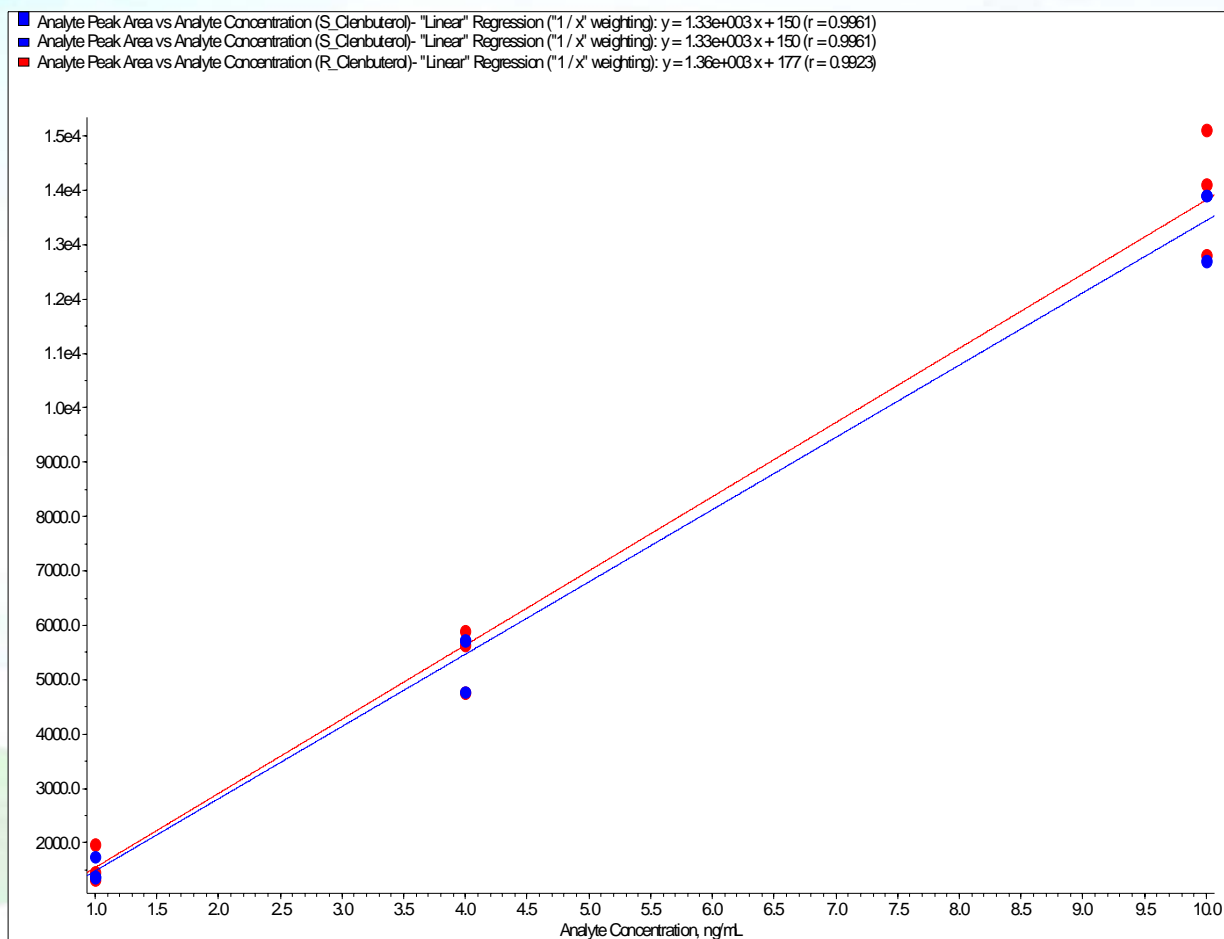


Q3 Scan Data



XIC from Q3 Scan Experiment
(Interference from Matrix)

Calibration Curve for Chiral Separation of R_S Clenbuterol



n=3

Experimental (contd.)

Using chiral chromatographic conditions, three separate sample prep methods were compared for recovery of Clenbuterol along with sample clean up. Each of the three SPE phases has a different selectivity requiring separate wash and elution protocols for each phase.

Quantitation of Clenbuterol was performed using summed multiple ions, where two separate transitions are monitored and integrated resulting in a summed response for both transitions. This enabled an increase in response, up to 70%, over a single monitored MRM and enhanced compound identification.

SPE Methods

Molecular Imprinted Polymer

Cartridge: SupelMIP™-Clenbuterol, 25 mg/10mL Large Reservoir Cartridge

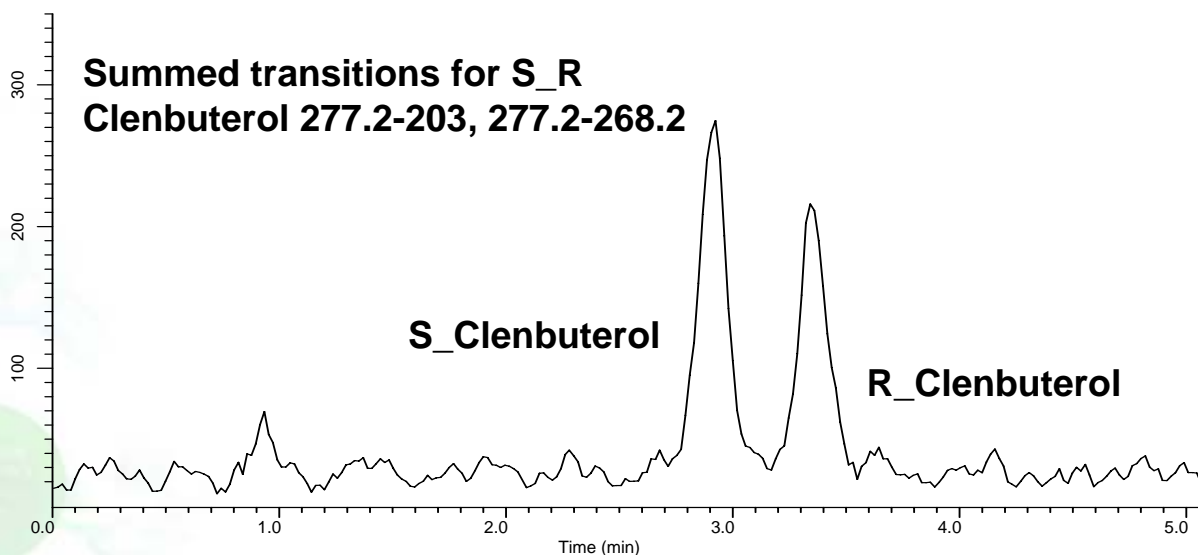
Condition: 1.0 mL methanol, 1.0 mL water, 1.0 mL 25 mM ammonium acetate

Load: 1.0 mL human urine: 1.0 mL 25 mM ammonium acetate

Wash: 1.0 mL 25 mM ammonium acetate, 1.0 mL water, 1.0 mL acetonitrile

Elute: 2 x 1.0 mL 10% formic acid:methanol

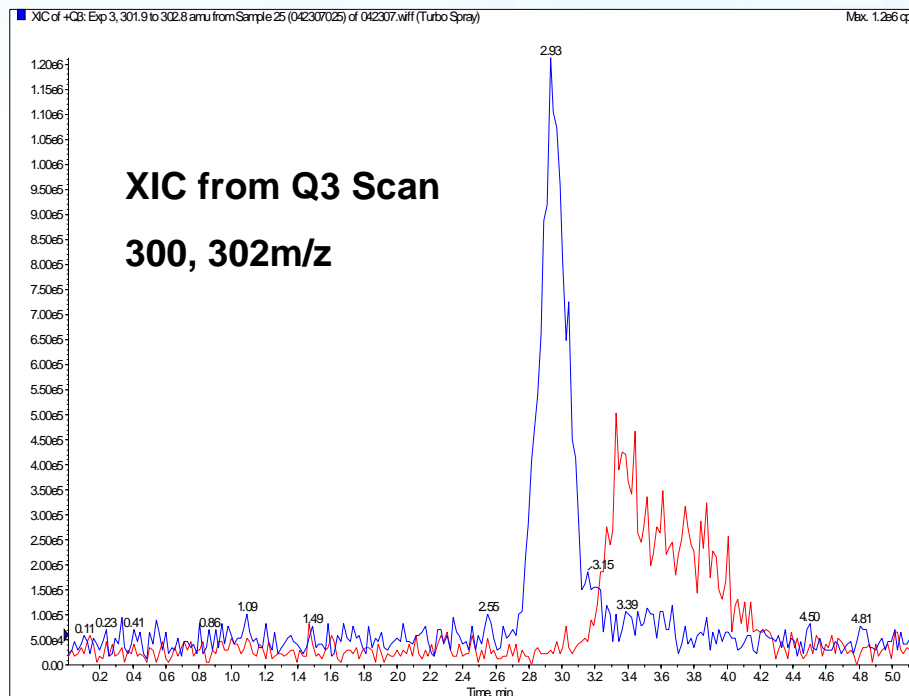
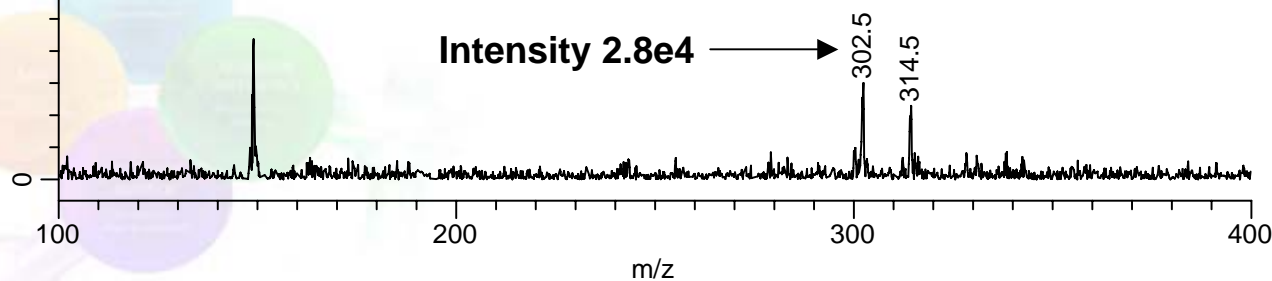
Evaporate and reconstitute in 1.0 mL of mobile phase



1.0 ng/mL spiked human urine

1.0 ng/mL S_R Clenbuterol Spiked Urine MIP SPE

Interference Detected from
Q3 Scan over Clenbuterol
Retention Time (2.6 - 4.0 min.)



Hydrophilic Polymer¹

Cartridge: Leading Hydrophilic Polymer 30 mg/1.0 mL cartridge

Condition: 1.0 mL methanol, 1.0 mL water

Load: 1.0 mL human urine: 1.0 mL 2% ammonium hydroxide in water

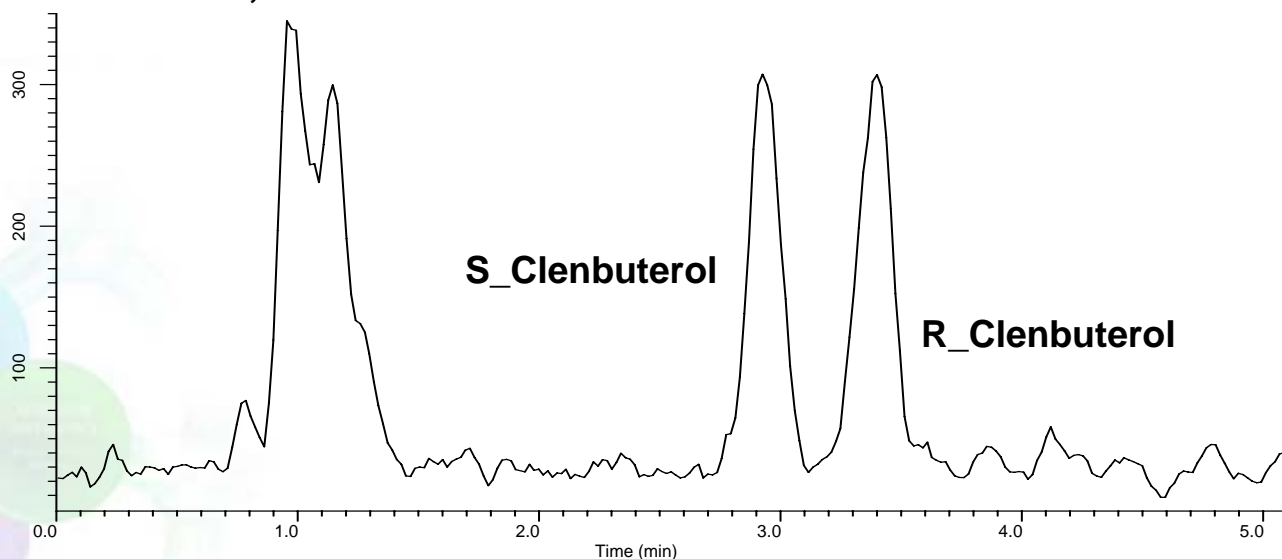
Wash: 0.5 mL 2% ammonium hydroxide in water

Elute: 2 x 1.0 mL methanol

Evaporate and reconstitute in 1.0 mL of mobile phase

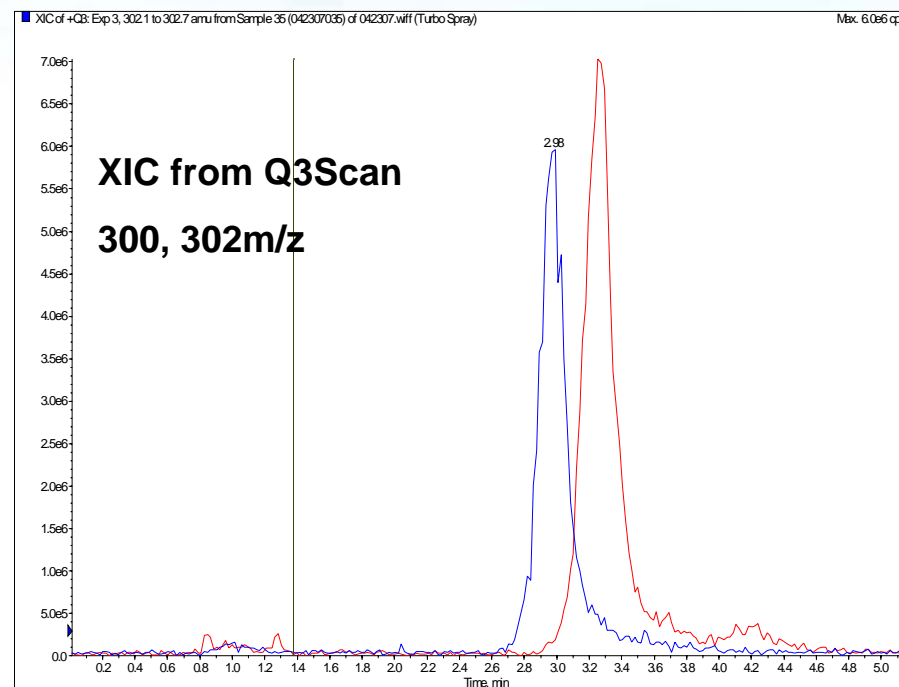
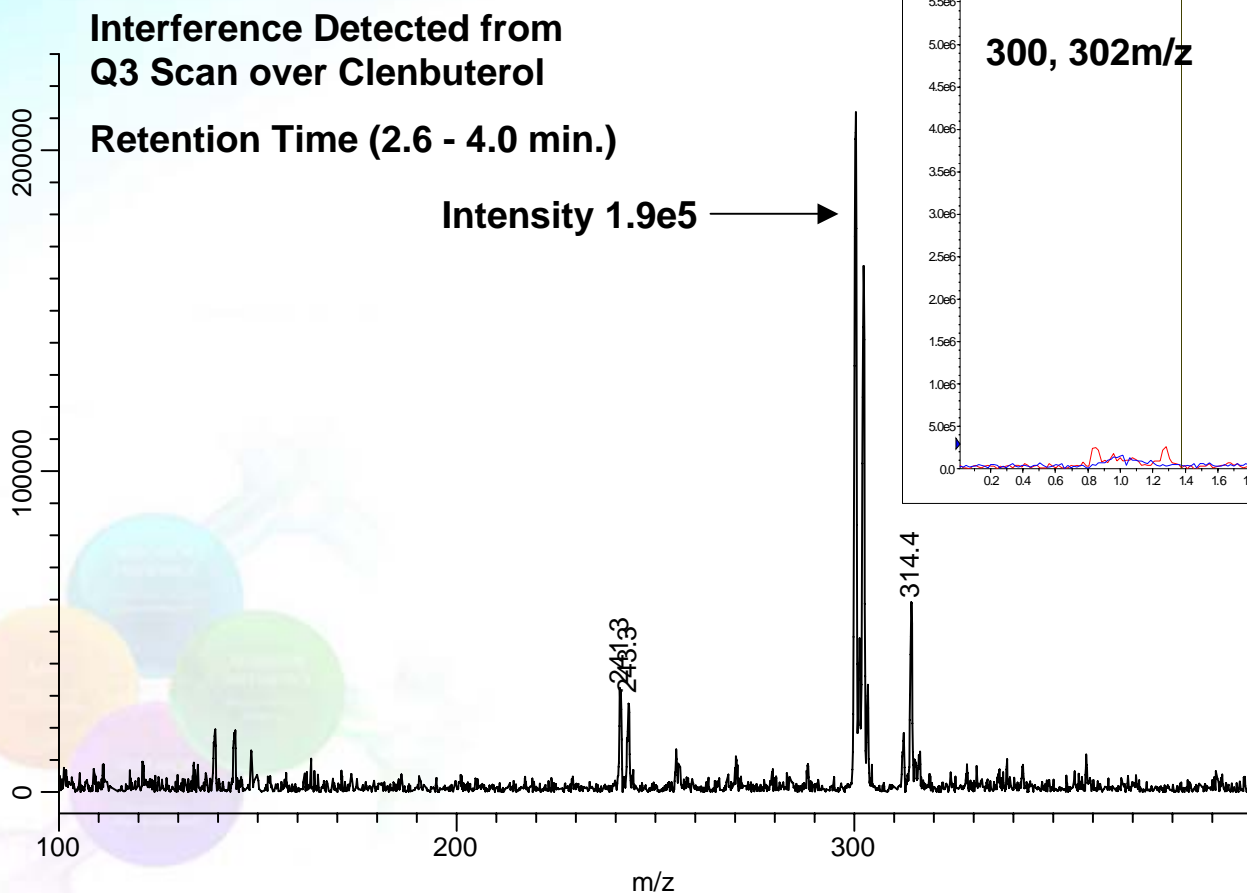
Summed transitions for S_R Clenbuterol

277.2-203, 277.2-268.2



1.0 ng/mL spiked human urine

1.0 ng/mL S_R Clenbuterol Spiked Urine Hydrophilic Polymer SPE



Mixed Mode

Cartridge: Supelco Discovery® MCAX 25 mg/1.0 mL cartridge

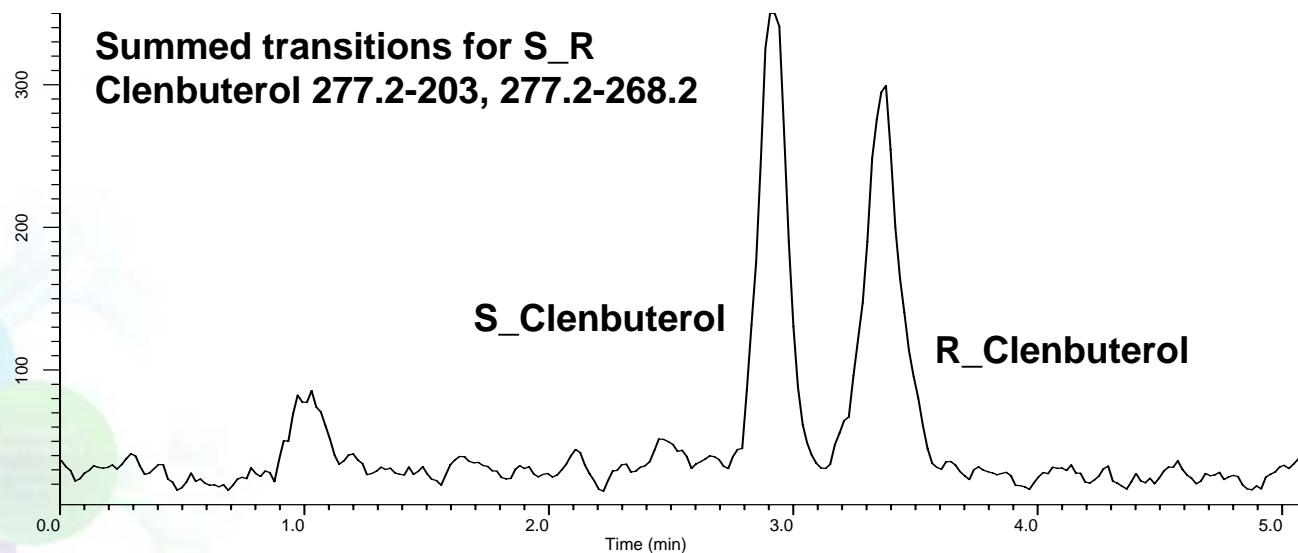
Condition: 2.0 mL methanol, 2.0 mL 10 mM ammonium acetate

Load: 1.0 mL human urine: 1.0 mL 10 mM ammonium acetate

Wash: 2.0 mL 10mM ammonium acetate, 2.0 mL 10% acetic acid,
2.0 mL methanol

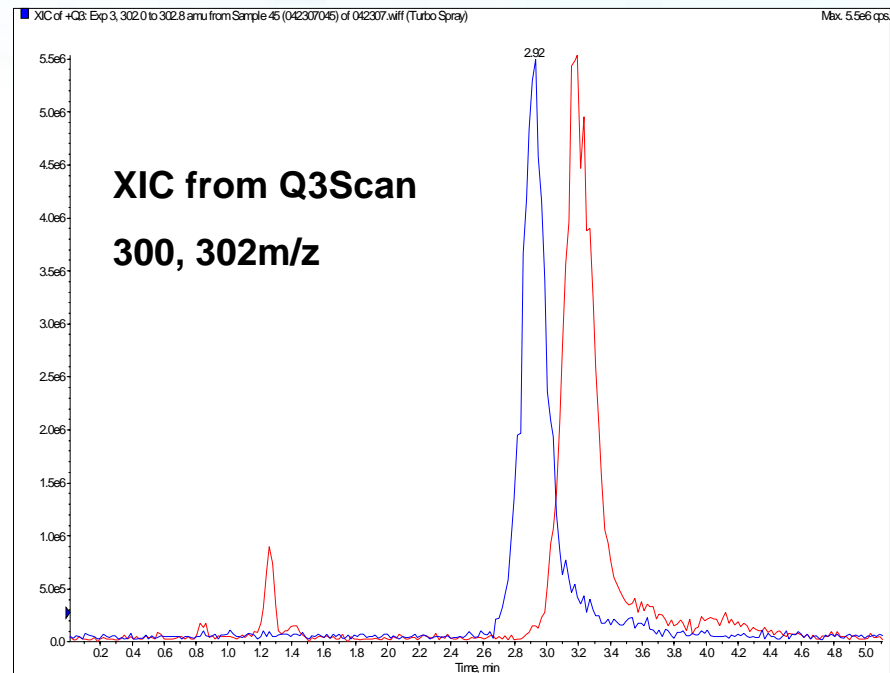
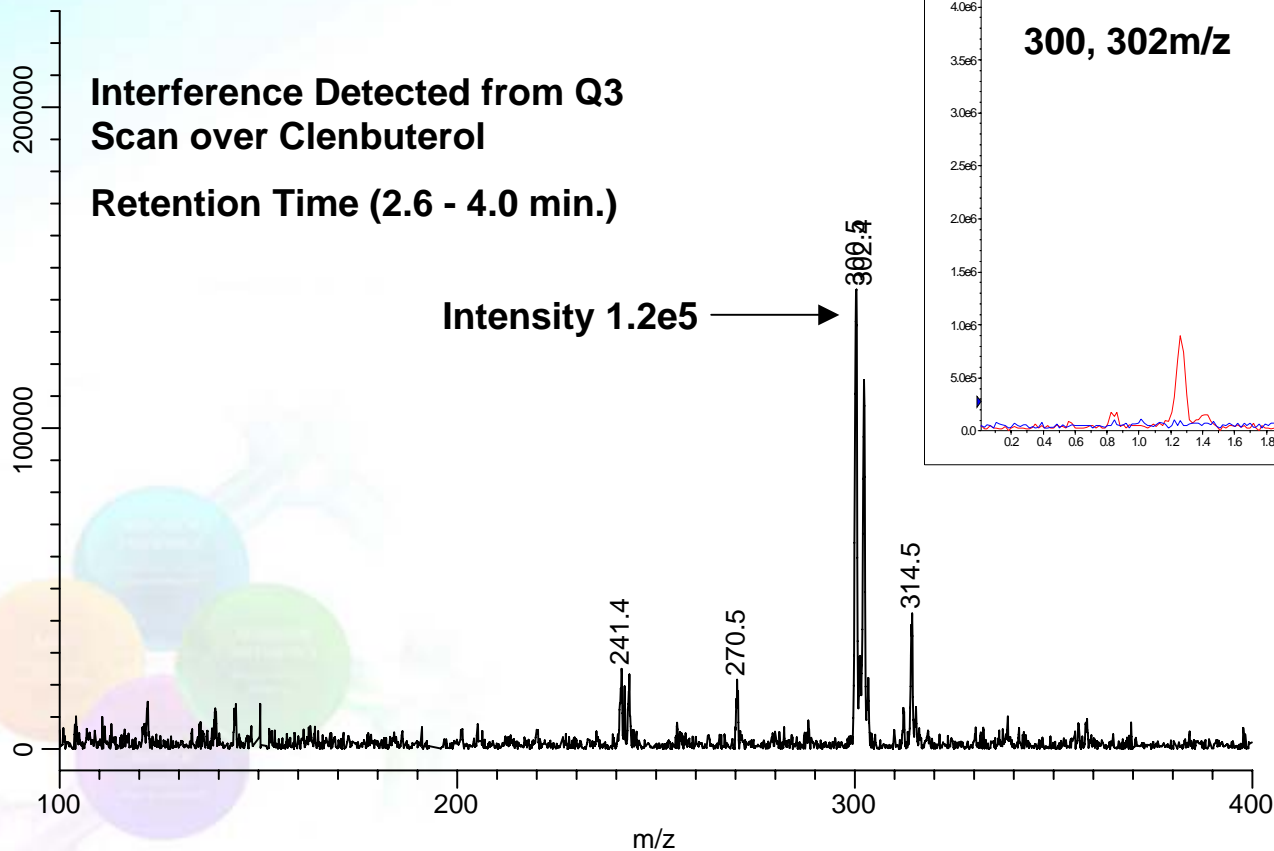
Elute: 2 x 1.0 mL 2% ammonium hydroxide in methanol

Evaporate and reconstitute in 1.0 mL of mobile phase



1.0 ng/mL spiked human urine

1.0 ng/mL S_R Clenbuterol Spiked Urine Mixed Mode SPE



Results

R_S Clenbuterol Recovery

Sample I.D.	Analyte Peak Name	MIP Recovery	Hydrophylic Polymer	Mixed Mode
1 ng Spiked Urine	S_Clenbuterol	105	119	57
1 ng Spiked Urine	R_Clenbuterol	107	130	95
5 ng Spiked Urine	S_Clenbuterol	95	138	86
5 ng Spiked Urine	R_Clenbuterol	96	138	88
10 ng Spiked Urine	S_Clenbuterol	95	111	96
10 ng Spiked Urine	R_Clenbuterol	98	104	98

Relative recovery calculations were used to determine the matrix affect from SPE methods. This corrected for any loss of material on the SPE phase that could be considered suppression of signal.

Results (contd.)

The degree of sample cleanup varied greatly for each solid phase extraction techniques. The hydrophilic polymer SPE method exhibited the least amount of sample cleanup resulting in the greatest amount of matrix ions. The referenced SPE conditions did not sufficiently remove the matrix. Also an interference from the hydrophilic polymer was also observed in the quantitative MRM experiment. This contributed to the elevated response for S_R Clenbuterol

The Mixed Mode SPE method also allowed a substantial amount of matrix to be eluted. Because the primary retention mechanism is ion exchange, this would enable ionic species in the matrix to be retained along with the S_R Clenbuterol. This interference is coeluted in the elution step.

Results (contd.)

The molecular imprinted polymer exhibited the highest degree of sample cleanup (of all the techniques). Because the retention mechanism is primarily hydrogen bonding along with ion exchange, this enables strong reverse phase washes to be performed with still retaining the Clenbuterol. Absolute recovery was slightly lower than from the other phases.



Summary

The flexibility of the linear ion trap system allowed for full scan data to be collected simultaneously with quantitative MRM experiments, this proved to be extremely valuable for the SPE method development. Though each SPE method utilized different retention mechanism, the degree of sample clean up greatly depended on the ability to perform strong wash conditions to remove matrix while still retaining S_R Clenbuterol. As in all sample prep methods, a cleaner final sample results in a more rugged analytical method resulting in less down time and greater sample through put.

References

1. M. Josefsson, A. Sabanovic, Journal of Chromatography A 1120 (2006) 1-12.

