

Comparison of SupelMIP™ SPE – Beta-agonists and Mixed-mode SPE for the Extraction of beta-agonists from Urine Samples



Contributed Article

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Introduction

Beta-2-adrenergic receptor agonists (Beta-agonists) have been clinically used in the treatment of cardiovascular and breathing disorders in veterinary and human medicine. However, beta-agonists are also used as an illegal muscle growth promoter due to its anabolic effects both in humans and in animals. Although the US Food and Drug Administration, US Department of Agricultural and European Union have banned the use of beta-agonists for humans and livestock, illegal use of this class of drugs still frequently occurs. For example, the beta-agonist clenbuterol is widely used among body builders and athletes due to its anabolic effects. In addition, beta-agonists are readily used by farmers to give animals a competitive advantage. Due to the potential health risks and competitive advantage associated with beta-agonist used in livestock and human performance enhancement, residue screening programs are conducted worldwide to monitor the drug. It is therefore critical to develop a highly selective and sensitive analytical assay to monitor beta-agonist residues in difficult biological matrices such as urine, retina, tissues, etc. Table 1 offers an overview of the minimum required performance levels (MRPLs) required of an assay across common sample matrices.

Table 2. SupelMIP SPE – Beta-agonist Extraction Method

Sample Pre-Treatment:

Hydrolyze 5–10 mL calf urine with Glucuronidase/Sulfatase with an activity of 85,000 units/mL (2 h at 37 °C, pH 5) (Sigma-Aldrich (Prod. No.G0876)). Adjust pH to 6–7 followed by centrifugation.

SPE:

SupelMIP SPE – Beta-agonists, 25 mg/10 mL, (Cat. No. 53210-U) Note that a flow rate ~0.5 mL/min was employed for conditioning, sample load and wash. A flow rate of ~0.2 mL/min was used during elution.

1. Column Conditioning:

The columns were equilibrated with 1 mL methanol followed by 1 mL DI water and 1 mL 25 mM ammonium or sodium acetate, pH 6.7.

2. Sample load:

10 mL urine was loaded on the column

3. Washing:

- 1 mL DI water followed by full vacuum through cartridge for 2 min
- 1 mL 1% acetic acid in acetonitrile
- 1 mL 50 mM ammonium acetate, pH 6.7
- 1 mL 60% acetonitrile/40% DI water, followed by full vacuum through cartridge for 2 min. to dry the columns.

4. Elution:

- 2 x 1 mL MeOH/10% acetic acid.
- The eluate was evaporated and reconstituted in mobile phase prior to analysis.

Analytical Method:

Column: Polar Reversed-Phase, Phenyl Phase, 4 µm, 150 x 2 mm

System:

HPLC Agilent 1100 Series

Mobile Phase:

(A) 5 mM ammonium acetate; (B) methanol

Gradient:

10 to 80% methanol in 15 min.

Mass Spec:

API 3000, ESI (+), MRM

In this article, a summary of the work performed at the Veterinary Institute in Oldenburg, Germany is presented. In order to enhance the methods for analyzing beta-agonists, the use of a molecularly imprinted polymer SPE phase (developed specifically for beta-agonist extraction) was explored. More specifically, SupelMIP – Beta-agonist SPE was evaluated for urine samples and compared against a conventional mixed-mode SPE procedure.

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Table 1. MRPLs of Beta-Agonist Assays in Different Matrices

	Beef / Swine [µg/kg]				Poultry [µg/kg]		
	Plasma	Urine	Retina	Liver	Feed	Water	Retina
Brombuterol	< 0,5	< 0,5	< 3,0	< 1,0	< 1,0	< 1,0	< 3,0
Chlorbrombuterol	< 1,0	< 0,5	< 3,0	< 0,2	—	—	—
Cimaterol	< 2,0	< 3,0	< 10	< 3,0	< 3,0	< 3,0	< 10
Clenbuterol	< 0,2	< 0,2	< 3,0	< 0,2	< 0,2	< 0,2	< 3,0
Clenproperol	< 1,0	< 3,0	< 10	< 1,0	< 1,0	< 1,0	< 10
Mabuterol	< 0,5	< 0,5	< 3,0	< 0,2	< 0,2	< 0,2	< 3,0
Ractopamin	< 3,0	< 3,0	< 10	< 3,0	—	—	—
Salbutamol	< 0,5	< 0,5	< 3,0	< 0,2	< 0,2	< 0,2	< 3,0
Terbutalin	< 3,0	< 1,0	< 20	< 2,0	< 2,0	< 2,0	< 20
Zilpaterol	< 0,5	< 1,0	< 0,5	< 1,0	—	—	—

(continued from page 13)

Methodology

In this work, beta-agonist spiked calf urine samples were extracted and compared using both SupelMIP SPE – Beta-agonist, 25 mg/10 mL LRC and Clean Screen DAU SPE, 500 mg/6 mL (United Chemical Technologies, PA, USA). The SupelMIP extraction and analysis method is detailed in Table 2.

Results

In order to evaluate the performance of the SupelMIP SPE, beta-agonist spiked urine was extracted and compared against Clean Screen DAU SPE (mixed-mode). Figure 1 depicts ion-chromatograms of salmeterol extracted with both SupelMIP SPE and Clean Screen DAU. From this figure, background for salmeterol using SupelMIP is significantly lower than the mixed-mode approach. In addition, response height was greater using the SupelMIP method.

In Figure 2, ion-chromatograms of clenbuterol and clenproperol are illustrated. Both compounds were extracted from urine using SupelMIP SPE and Clean Screen DAU. Response was increased by a factor of 10 for both beta-agonists using the SupelMIP SPE approach relative to mixed-mode.

Figure 1. Ion-chromatogram of Salmeterol (0.2 $\mu\text{g/L}$ spike, MRM 240/148) extracted via SupelMIP SPE and Clean Screen DAU SPE

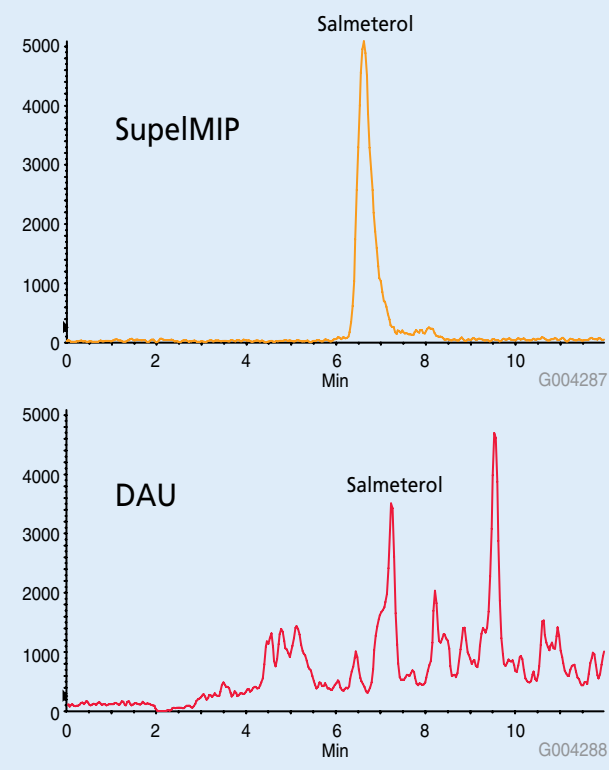


Figure 2. Ion-Chromatograms of Clenbuterol (0.05 $\mu\text{g/L}$ spike, MRM 277/203) and Clenproperol (0.4 $\mu\text{g/L}$ spike, MRM 263/245) extracted via SupelMIP SPE and Clean Screen DAU SPE

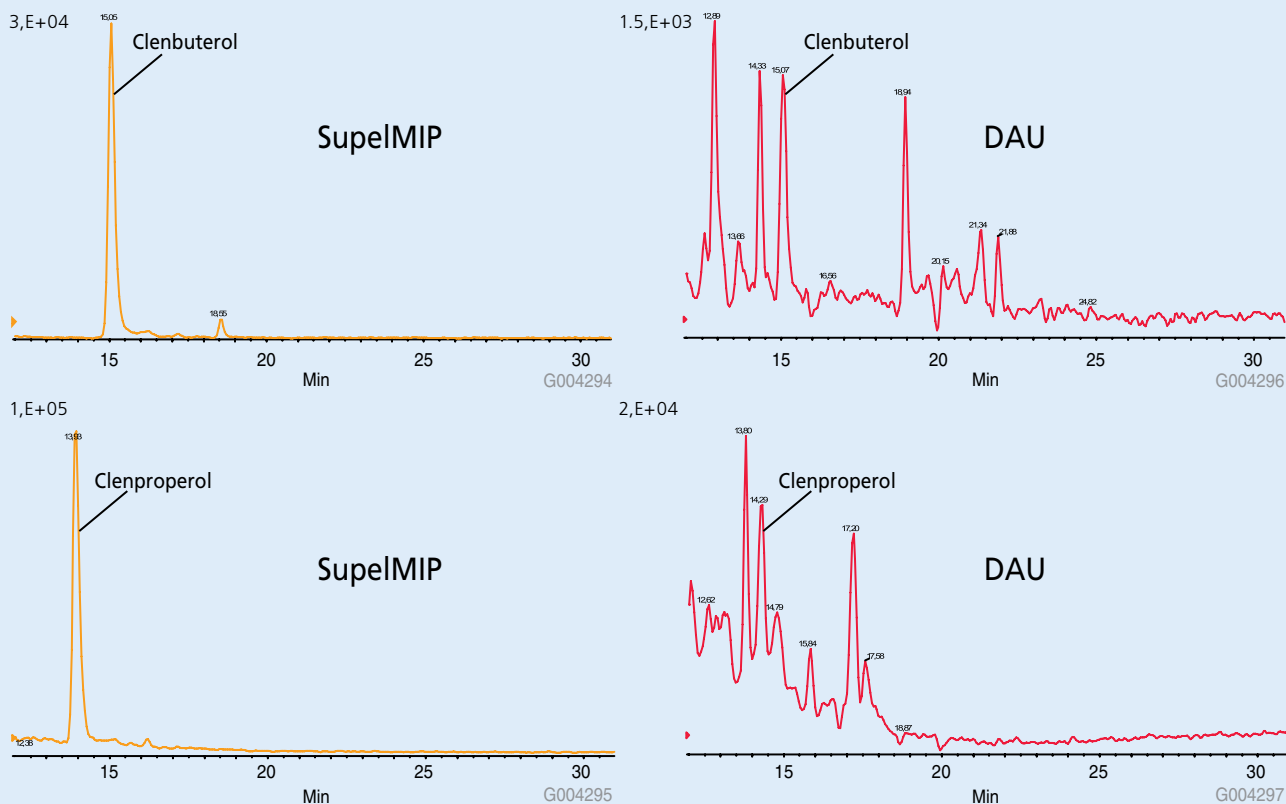


Table 3. Signal-to-Noise Ratio of Beta-agonists

	SupelMIP		Clean Screen DAU	
	Target	Qualifier	Target	Qualifier
Brombuterol	5200	1900	90	30
Chlorbrombuterol	2500	370	120	40
Cimaterol	560	710	100	380
Clenbuterol	1300	140	120	50
Clenproperol	430	1100	100	30
Mabuterol	3000	1100	260	60
Ractopamin	3100	720	100	70
Salbutamol	330	40	40	40
Terbutalin	1300	230	20	70
Zilpaterol	140	220	60	40

In Table 3, the signal-to-noise (S/N) ratio for a broad range of beta-agonists (target ion and qualifier) are presented. For each of the beta-agonists, the SupelMIP approach provided a cleaner and more selective extraction. As a result S/N was significantly higher (often by orders of magnitude)

Conclusion

In this work it was concluded that the SupelMIP SPE – Beta-agonists is a highly selective phase for Beta-agonists. This high selectivity for the class of compounds allows for

very clean extracts with low levels of interfering contaminants. Comparing the performance with general mixed-mode phases, a clear enhancement in the signal to noise ratio is obtained using the SupelMIP phase, allowing for increased analytical sensitivity and lower detection levels.

The method as described fulfills all criteria of the EU Commission Decision 2002/657/EC for confirmatory analysis of substances listed in group A of Annex I of Council Directive 96/23/EC and has been adopted for routine analysis by the Veterinary Institute Oldenburg.

+ Featured Products

Description	Cat. No.
SupelMIP™ SPE – Beta-agonists, 25 mg/10 mL (LRC), Pk. 50	53202-U
β-Glucuronidase from <i>Helix pomatia</i> , Type H-2, aqueous solution, ≥ 85,000 units/mL	G0876

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For more information on SupelMIP SPE, please visit our website: sigma-aldrich.com/supelmip

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