

# Extraction of the Beta-Agonist Clenbuterol from Urine Using Clenbuterol SupelMIP

## Christine Widstrand, Vice President

MIP Technologies AB, Box 737, 220 07 Lund, Sweden

e-mail: christine.widstrand@miptechnologies.com

## Highly Selective SPE for Trace Analysis in Complex Matrices

### Abstract

Selective sample preparation is now available based on molecularly imprinted polymers. These highly cross-linked polymeric sorbents give significant benefits for trace analysis in complex matrices. They provide lower detection limits, significant time and cost savings and enhanced MS-compatibility.

### Introduction

Trace analysis of compounds from complex biological samples require often very extensive and time consuming sample preparation procedures due to the insufficient selectivity of traditional SPE sorbents.

SupelMIP cartridges are specifically designed to selectively extract analytes or classes of analytes at trace levels from complex matrices. They contain SPE sorbents based on molecularly imprinted polymers (MIPs).

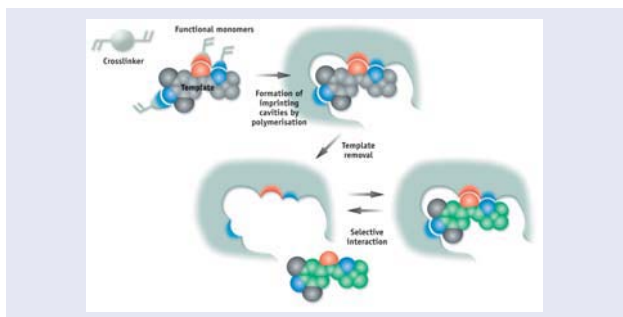


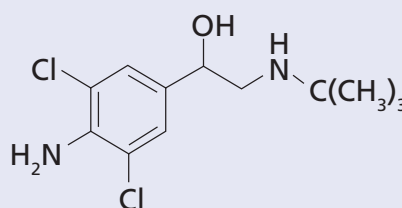
Figure 1. The basic principle of molecular imprinting

Selectivity is introduced during the preparation of the MIP sorbent by choosing one or more functional monomers which in solution will form complexes with a template molecule that mimic the analyte or a sub-fragment of the analyte<sup>1</sup> (Figure 1). Once the polymer is formed the template molecule is removed from the MIP resulting in specific cavities or imprints, sterically and chemically complementary to the analyte or group of analytes of interest. The interactions between the analyte and the functional groups in the cavities are based on hydrogen

bonding, ionic, Van der Waals or hydrophobic interactions. Due to multiple interaction sites binding is stronger compared to random binding to traditional SPE sorbents.

Clenbuterol, a synthetic beta-agonist, is illegally used as a growth promoter both in humans (doping) and in livestock (breeding)<sup>2</sup> (Figure 2).

Figure 2. Chemical Structure of Clenbuterol



Screening programs are executed all over the world to find the banned drug in food and feed samples, due to the potential health risks associated with beta-agonist residues in meat products. A number of food poisoning cases have been reported with consumption of contaminated meat<sup>3</sup>, the latest case very recently in Shanghai, where over 300 people were hospitalized<sup>4</sup>.

The determination of beta-agonists in biological samples is at trace levels (typically < 0.5 ng/mL) and in complex biological matrices, such as urine, muscle, liver etc. Conventional SPE materials are normally not selective enough.

The highly selective Clenbuterol SupelMIP is designed for extraction of the most common beta-agonist, clenbuterol, from complex matrices. Available is also a class selective Beta-agonist SupelMIP, which extracts a broad range of beta-agonists from complex biological samples.

### Experimental

5 mL bovine urine was extracted on a Clenbuterol SupelMIP column and compared with extraction on three different mixed-phase cartridges (C4, C8 and C18 mixed with a strong cation exchanger)

Protocol for mixed-phase cartridges:

The cartridges were conditioned with 2x1 mL MeOH followed by 2x1 mL 50 mM NH<sub>4</sub>Ac buffer pH 6.0. 5 mL urine, pH 6.0 and spiked with 2 ng/mL Clenbuterol was applied to the cartridge. Interferences were eluted with 2x1 mL 50 mM NH<sub>4</sub>Ac buffer pH 6.0, 2x1 mL 1 M HAC (cartridges dried for 30 seconds after this wash) and finally 2x1 mL NaOH. Clenbuterol was eluted with 1 mL MeOH/5% ammonia.

Protocol for Clenbuterol SupelMIP cartridges<sup>5</sup>:

The cartridge was conditioned with 1 mL MeOH, 1 mL water and 1 mL 25 mM NH<sub>4</sub>Ac pH 6.7. 5 mL urine diluted 1:1 with 25 mM NH<sub>4</sub>Ac pH 6.7 was applied to the column. Interferences were eluted by 1 mL of water, followed by 2 minutes of vacuum, 1 mL of acetonitrile/2% acetic acid, 1 mL 0.5 M NH<sub>4</sub>Ac pH 5 and 1 mL 70% acetonitrile in water. Finally Clenbuterol was eluted with 2x1 mL MeOH/1% trifluoro acetic acid (TFA).

Each SupelMIP phase is delivered with a data sheet describing a recommended extraction protocol.

## Results and Discussion

MIP particles synthesized to be specific for clenbuterol show greater specificity for clenbuterol than conventional SPE particles. Clenbuterol can bind to the polymer with a variety of bonds (ionic, hydrophobic and hydrogen). In the selective cavities on the MIP, these binding possibilities are sterically arranged to fit clenbuterol.

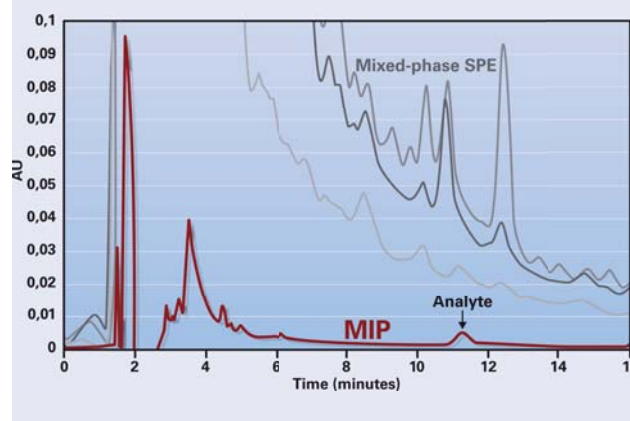
Conditioning consists of wetting the MIP using methanol and water and then adjusting the pH to 6.7 so that the acidic monomer is in a negatively charged state for ionic bonding. During sample loading, clenbuterol is non-selectively retained, together with substances from the urine matrix. In a water environment, interactions between the selective cavities of the MIP and clenbuterol are not well established. Bonding of clenbuterol occurs not only in the selective cavities, but also to the backbone of the polymer. Non-selective binding enables a high total loading capacity of the material. It has been proven that a dilution of urine 1:1 with 25 mM buffer pH 6.7 was optimal for loading<sup>5</sup>. Undiluted urine might decrease the recovery probably due to the high ionic strength of urine that reduces the amount of clenbuterol adsorbed on the polymer during sample loading. To reveal the interactions between clenbuterol and the selective cavities of the MIP the polymer has to be in an acetonitrile environment. By adding 2% acetic acid to acetonitrile it was shown that the MIP phase still retained clenbuterol while interfering compounds were effectively removed. It was observed that some of the clenbuterol was lost if the water content of the polymer was too high and therefore a few minutes of

vacuum was necessary to semi-dry the MIP before the selective wash in order to obtain high recoveries. The interference elution steps described in the protocol for Clenbuterol SupelMIP cartridges in the experimental section must be performed in the described order. Using this protocol, determination of 0.5 ng of clenbuterol/mL urine is possible when using UV detection. Depending on how selective the detector is some of the steps could be left out, but the selective wash (acetonitrile/2% acetic acid) must always be performed.

Elution of clenbuterol was effective by the use of methanol mixed with 1% TFA. Stronger concentrations of TFA can lead to degradation of clenbuterol.

The chromatograms show the SPE extraction of clenbuterol from a 5 mL urine sample on Clenbuterol SupelMIP vs. conventional, mixed-mode SPE particles, which gave high background and misleading responses (Fig 3).

Figure 3. Extraction of Clenbuterol on Mixed-Mode Phases (C4, C8 and C18 in grey) and Clenbuterol SupelMIP (in red). Analysis by HPLC-UV.



The precision and accuracy of the method were determined by analyzing spiked urine samples at 0.6 and 6.0 ng clenbuterol/mL (n=6). The results are presented in Table 1. The results show that the performance of this MIP-based method is well within the limits of that expected of current bioanalytical methods.

Table 1. Precision and Accuracy for the Analysis of Clenbuterol in 5 mL Spiked Urine Samples

	Within-day		Between-days	
	0.6 ng/mL	6.0 ng/mL	0.6 ng/mL	6.0 ng/mL
n	5	6	9	10
Mean + SD	0.58+0.025	5.8+0.12	0.61+0.039	5.9+0.24
SD (%)	4.3	2.1	6.4	4.1
Accuracy (%)	96.7	96.7	101.7	98.3
No. analyses	1	1	3	3

## Conclusions

The Clenbuterol SupelMIP exhibits high selectivity for clenbuterol. When the selective binding of clenbuterol is established the MIP can be washed harshly to elute interfering compounds from the matrix without any loss of clenbuterol. This result in much cleaner extracts than those obtained with mixed-phase SPE. Quantitation by HPLC-UV down to 0.5 ng clenbuterol/mL urine is possible with Clenbuterol SupelMIP cartridges. With more selective detectors such as MS even lower detection limits are expected to be reached. Furthermore clenbuterol is extracted with high recoveries and the SPE method is robust with high accuracy and precision. Compared with conventional SPE sorbents such as mixed-phase SPE, the SupelMIP sorbents result in increased cleanliness, improved detection limits, significant time and cost savings and improved MS compatibility.

## References

1. B. Sellergren (Ed.), Molecularly Imprinted Polymers. Man-made mimics of Antibodies and Their Applications in Analytical Chemistry, Techniques and Instrumentation in Analytical Chemistry, Vol 23, Elsevier, Amsterdam, 2001.
2. Council Directive 86/469/EEC, European Union, Brussels, 1988.
3. New type of "angel dust" found, The Irish Times, 1 May 1995.
4. New Food Poisoning Case Hits China. Food productiondaily.com, Sep 19, 2006
5. A. Blomgren, C. Berggren, A. Holmberg, F. Larsson, B. Sellergren and K. Ensing, J. Chrom. A, 975, 157-164 (2002)

## ! Related Information

For more information please visit us on our web site  
[sigma-aldrich.com/supelmip](http://sigma-aldrich.com/supelmip)

## + Featured Products

SupelMIP For	Bed weight	Column volume	Pk of	Cat. No.
Clenbuterol	25 mg	10 mL	50	53201-U

## + Related Products

SupelMIP For	Bed weight	Column volume	Pk of	Cat. No.
<b>Beta-agonists</b> (class selective)	25 mg	10 mL	50	53202-U
	25 mg	3 mL	50	53225-U
<b>NNAL</b> (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25 mg	10 mL	50	53206-U
	25 mg	3 mL	50	53203-U
<b>Riboflavin</b> (vitamin B2)	25 mg	10 mL	50	53208-U
<b>Triazines</b> (class selective)	25 mg	10 mL	50	53207-U
<b>Chloramphenicol</b>	25 mg	10 mL	50	53210-U
	25 mg	3 mL	50	53209-U
<b>Beta blockers</b> (class selective)	25 mg	10 mL	50	53218-U
	25 mg	3 mL	50	53213-U
<b>TSNAs</b> (NNK, NNN, NAB, NAT)	25 mg	10 mL	50	53221-U
	25 mg	3 mL	50	53222-U
<b>Full beta receptor</b> (beta agonists and beta blockers)	25 mg	10 mL	50	53223-U
	25 mg	3 mL	50	53224-U