

The Class-Selective Extraction and Analysis of β -Receptor Agonist and Antagonists using Molecularly Imprinted Polymer SPE

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Beta-adrenergic blocking agents (beta-blockers) are a class of drugs used to treat various cardiac disorders such as hypertension, angina, congestive heart failure and arrhythmia. Beta-2-adrenergic receptor agonists (beta-agonists) have been clinically used to treat asthma and other breathing disorders. However, because of key side effects associated with the drugs, they are heavily regulated by government agencies worldwide. Beta-blockers have been used as a performance enhancer among athletes by lowering heart rate and reducing tremor. Consequently, the International Olympic Committee has banned the use of beta-blockers. Beta-agonists are an illegal muscle growth promoter due to its anabolic effects. As a result, the drugs have been internationally banned for use in humans, livestock, and racehorses. Also, because these drugs are not completely eliminated from the body upon ingestion, they are often excreted in wastewaters after therapeutic use. As a result, there has been concern for the longterm subtle and chronic effects of these drugs on humans and the ecosystem.

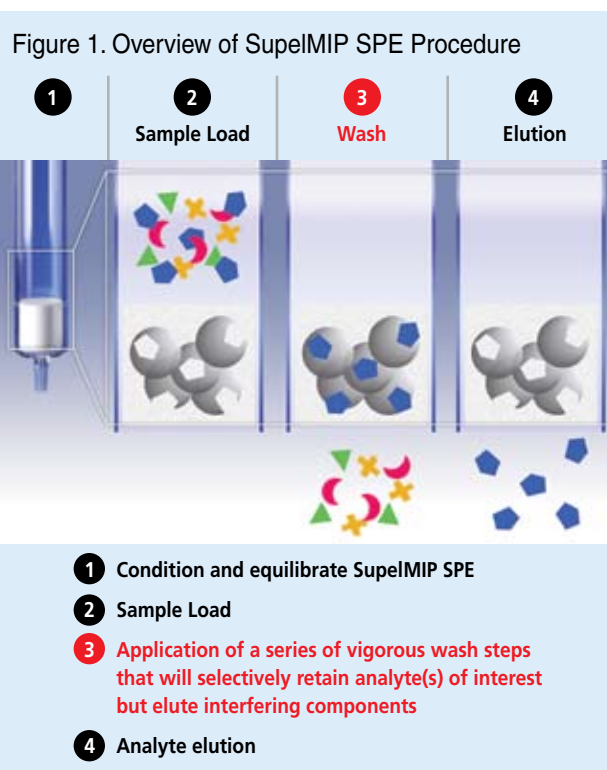
Because the drugs are heavily regulated and often analyzed in difficult sample matrixes such as biological fluids and waste water, a highly selective and sensitive extraction and analytical method are required to achieve targeted lower limits of detection and quantitation. For example, maximum residue limits for beta-agonists in Europe are 0.1 and 0.3 ppb (EU Council regulation ECC No. 2377/90).

In previous issues of the Reporter we demonstrate the use of molecularly imprinted polymer (MIP) SPE for the highly selective extraction of single analytes such as chloramphenicol, clenbuterol, and NNAL from difficult sample matrixes such as biological fluids. These applications are thoroughly discussed in US Reporter Issues 25.1, 25.2, and 25.3, respectively. In this report, we describe the use of MIP based SPE for the simultaneous extraction (class-selective) of both beta-agonists and beta-blockers for subsequent LC-MS-MS analyses.

Improving Selectivity with SupelMIP SPE

MIPs are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compounds or a class of structurally related compounds with high selectivity. By careful design of the imprinting site, the binding cavities can be engineered to offer multiple interactions with the analyte(s) of interest (combination of hydrogen bonding, hydrophobic and ionic interactions, and Van der Waals) allowing for stronger and more specific analyte retention. Improved selectivity is introduced through the use of harsher wash conditions during sample prep methodology (Figure 1). Because extraction selectivity is significantly improved, lower background is observed allowing analysts to achieve lower detection limits relative to other less selective sample prep techniques.

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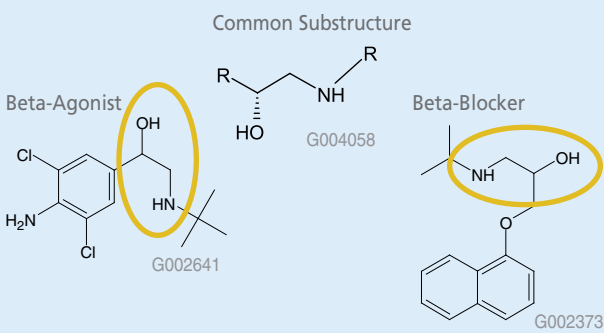


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Using SupelMIP SPE for Class-Selective Retention

Although the specificity and selectivity of MIPs are often compared to the interactions observed in antibody-antigen interactions, the MIP binding site often offers a range of interaction types (e.g., ion-exchange, reversed-phase, hydrogen bonding, etc.) that can be exploited to offer selective retention during sample load and/or wash. Very often, selective interaction between a MIP phase and analyte occurs at the substructure for the analyte. When conducting class-selective extraction, the MIP-analyte interaction occurs with a substructure common between a class of analytes. In the case of beta-agonists and beta-blockers, selective MIP retention is dominated by ion-exchange and hydrogen bonding, and specifically targeted towards the beta-alcohol and secondary amine common across both these classes of compounds (Figure 2).

Figure 2. Beta-Alcohol and Secondary Amine Sub-structure Common Between Beta-Agonists and Beta-Blockers



Extraction and Analysis of Beta-Blockers and Beta-Agonists Using SupelMIP SPE

In this study, a selection of 10 beta-blockers and beta-agonists were extracted from both horse urine and wastewater using SupelMIP SPE - Beta-Receptor via the extraction procedure described in Table 1. Analysis of the resulting eluate was conducted by LC-MS-MS using the procedure described in Table 2.

Lower Limits of Quantitation in Horse Urine and Wastewater

Using the SupelMIP SPE and LC-MS-MS described in Tables 1 and 2, trace levels of beta-agonists and beta-blockers were determined in spiked urine and wastewater samples, and lower limits of quantitation (LLOQ) values were determined for each of the analytes tested relative to sample matrix in which the signal-to-noise ratio of each analyte response was 10. The LLOQ values were summarized in Table 3, and an example chromatogram of a spiked urine sample is depicted in Figure 3.

Table 1. SupelMIP Extraction Procedure for Beta-Agonists and Beta-Blockers

Sample Pre-Treatment:

Horse urine was centrifuged at 3000 g for 10 min., diluted with DI water 1:1 (v/v), adjusted to pH 7.

Wastewater was filtered with 1 μ m filter paper and adjusted to pH 6-7.

SPE Procedure:

SupelMIP SPE – Beta-Receptor, 25 mg/10 mL (LRC) (Cat. No.53223-U)

1. Condition and equilibrate MIP phase with 1 mL acetonitrile and 1 mL DI water.
2. Load 1 mL pre-treated urine sample.
3. Wash (elute interferences) using the following wash scheme:
 - 3 x 1 mL DI water (elution of salt and matrix interferences)
 - Apply 2 min. of full vacuum to dry the tube.
 - 1 mL acetonitrile (selective removal of hydrophobic interferences)
 - 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrophilic interferences)
 - Apply 2 min. of full vacuum to dry the tube.
4. Elute beta-agonists and beta-blockers with 2 x 1 mL 1% formic acid in acetonitrile. Evaporate and reconstitute with LC mobile phase prior to analysis.
5. Evaporate under nitrogen and reconstitute with 150 μ L 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis

Table 2. LC-MS/MS Conditions for Beta-Agonists and Beta-Blockers

column: C18, 5 cm x 3 mm I.D., 3 μ m,
instrument: API3200 MS-MS
mobile phase: (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile

gradient:	Min.	% A	% B
	0	95	5
	2	90	10
	5	50	50
	6	50	50
	7	95	5

flow rate: 0.5 mL/min.

Detection (MS/MS):	Analyte	Rt (min.)	Q1/Q3	DP	EP	CEP	CE	CXP
	Atenolol	3.0	267.2/145	45	5	15	38	4
	Carazolol	6.2	299.1/194.2	50	5	20	37	5
	Metoprolol	5.6	268.2/133	45	4	15	35	4
	Propranolol	6.5	260.2/154.9	50	4	15	34	4
	Timolol	5.5	317.2/188.1	50	7	20	32	6
	Clenbuterol	5.6	277.1/202.9	26	3	10	22	7
	Ritodrine	3.9	288.2/121	39	5	11	31	4
	Salbutamol	2.6	240.2/147.9	38	4	12	24	4
	Terbutaline	2.6	226.2/152	36	4	10	24	4
	Tulobuterol	5.6	228.2/154.1	41	5	10	20	5

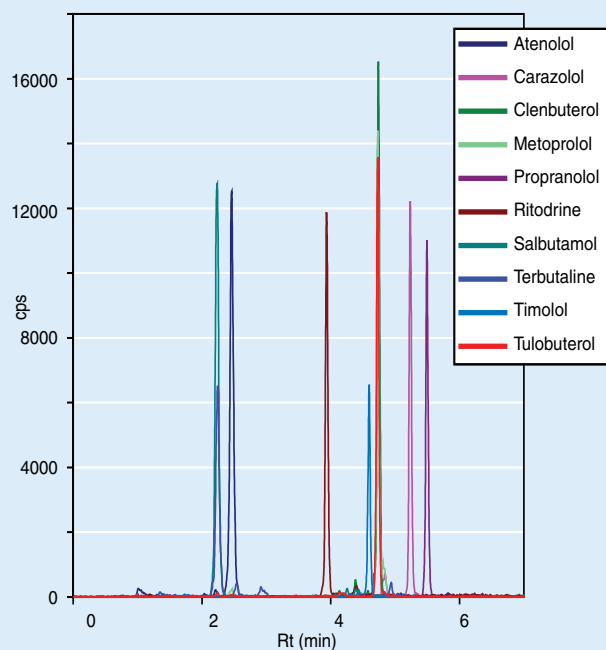
dwel time (MS): 50
ion mode: Positive
ion source: Turbospray
ion spray voltage: 5500 V
source temperature: 500 $^{\circ}$ C
curtain gas: 10 psi
gas 1: 50 psi
gas 2: 60 psi
injection: 20 μ L

Using the SupelMIP SPE protocol and LC-MS-MS conditions described in this report, lower quantitation limits of 0.1 ng/mL and 0.01 ng/mL were achieved for horse urine and wastewater, respectively. Lower limits of detection for beta-blockers were estimated to be < 0.1 μ g/L for urine and 0.01 μ g/L for wastewater.

Table 3. LLOQ Values of Beta-Agonists and Beta-Blockers in Urine and Wastewater

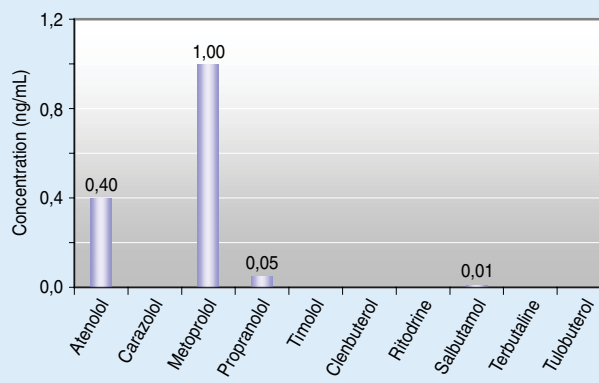
Analyte	Lower Limit of Quantitation (ng/mL, ppb, or µg/kg)	
	1 mL Horse Urine	10 mL Wastewater
Atenolol	0.1	0.01
Carazolol	0.1	0.01
Metoprolol	0.1	0.01
Propranolol	0.1	0.01
Timolol	0.1	0.01
Clenbuterol	0.02	0.002
Ritodrine	0.05	0.005
Salbutamol	0.1	0.01
Terbutaline	0.2	0.02
Tulobuterol	0.005	0.0005

Figure 3. Total Ion Chromatogram of Urine Sample Spiked with 1 ng/mL Beta-Blockers and Beta-Agonists



Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

Table 4. Determined Concentrations of Beta-Agonist and Beta-Blockers in Wastewater



An actual wastewater sample was collected from a sewage treatment plant located in Sweden, and extracted and analyzed using the SupelMIP procedure and LC-MS-MS conditions described in this report. Using this procedure, four analytes were detected and quantitated. The other analytes were below the limits of quantitation for this assay. Determined concentration values are described in Table 4.

Conclusion

In this report, we demonstrated the trace level determination of beta-agonists and beta-blockers in both horse urine and wastewater using class-selective molecularly imprinted polymer SPE phase. The SupelMIP SPE – Beta-Receptor assay described in this report took less than two hours to complete and offered the selectivity necessary to achieve quantitation limits of 0.1 ng/mL and 0.01 ng/mL for horse urine and wastewater, respectively. This procedure was further demonstrated by analyzing an actual wastewater sample where 4 out of 10 beta-receptor agonists and antagonists were determined and quantified.

Featured Products

SupelMIP SPE Cartridges	Sorbent Mass (mg)	Cartridge Volume (mL)	Cartridges/Box	Cat. No.
Clenbuterol	25	10	50	53201-U
Beta-agonists (class selective)	25	10	50	53202-U
Beta-agonists (class selective)	25	3	50	53225-U
Beta-blockers (class selective)	25	10	50	53218-U
Beta-blockers (class selective)	25	3	50	53213-U
Full beta receptor (beta agonists and beta blockers)	25	10	50	53223-U
Full beta receptor (beta agonists and beta blockers)	25	3	50	53224-U
Chloramphenicol	25	10	50	53210-U
Chloramphenicol	25	3	50	53209-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	10	50	53206-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	3	50	53203-U
TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	50	10	50	53221-U
TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	50	3	50	53222-U
Riboflavin (vitamin B ₂)	25	10	50	53207-U
Triazines (class selective)	25	10	50	53208-U