

This Data Sheet Contains Important Information About This Product.

SupelMIP™ SPE – Beta-agonists

Product Description:

Molecular imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). It is therefore critical for analysts to use the methodology described below when using this phase. Conventional generic methodologies employed with conventional SPE chemistries (e.g., reversed-phase C18) will yield sub-optimal results when employed with this phase.

The following methods have been developed for the selective extraction of beta-agonists from biological matrices. Methods have been developed for both biological tissues (e.g. bovine muscle) and fluids (e.g. urine). The methods are highly reproducible and offer beta-agonist recoveries in the range of 35-90%. Since the methods are amenable to the extraction of a wide range of beta-agonists, recoveries may vary for each specific molecule. It is recommended to use the prescribed method as a screening tool to identify which beta-agonists are present. Once specific beta-agonists are identified, conditioning, wash, and elution steps can be further optimized to offer higher recoveries if required.

Extraction Procedure: Recommended flow rate is ~0.5 mL/min. For analyte elution, a flow rate at ~0.2 mL/min. is recommended.

Application Name:	Extraction of Beta-agonists from bovine muscle and other tissues ¹	Extraction of Beta-agonists from urine and other biological fluids ²
Analyte:	Beta-agonists	Beta-agonists
Sample Matrix:	Validated for bovine muscle but is amenable with other tissues such as rabbit, duck, turkey, liver, and fish	Urine
General Comments:	The compounds cimaterol, cimbuterol, ractopamine, clenproperol, clenbuterol, brombuterol, mabuterol, mapenterol and isoxsuprine meet the requirements for quantitative determination. Screening is reliable to below 1 µg/kg.	Typical recoveries are over 70% for ritrodriene, clenbuterol, formoterol, salmeterol, ractopamine, tulobuterol, brombuterol, and mapenterol; between 40-70% recovery is observed for terbutaline, metaproterenol and cimbuterol. Isoproterenol, salbutamol, fenoterol, and isoxsuprine cannot be determined via HPLC-UV due to interfering peaks.
SupelMIP SPE –Beta-agonists:	25 mg/10 mL (LRC) (Cat. No. 53202-U)	25 mg/10 mL (LRC) (Cat. No. 53206-U); or 25 mg/3 mL (Cat. No. 53225-U)
Sample Pre-treatment:	<ul style="list-style-type: none"> ◆ Combine 5 g thawed mince muscle; 50 µL of 0.1 ng/µL of internal standard (deuterated analog in methanol); 5 mL Tris buffer, pH 9.5; and ~ 5 mg protease ◆ Digest samples overnight at 60 °C ◆ After cooling to room temperature, hydrolyze conjugates by adding: 15 µL concentrated acetic acid; 1 mL 2 M acetic buffer, pH 5.2; and 40 µL suc <i>d'Helix Pomatia</i> (Roche Diagnostics) ◆ Incubate for 2 hours at 37 °C. Adjust to pH > 12 with 10 M NaOH ◆ Liquid-liquid extract the mixture with 10 mL ethyl acetate. Isolate upper organic layer, and extract mixture again with 5 mL ethyl acetate. Combine the organic layer from both extractions, and evaporate under N₂ at 55 °C. ◆ Reconstitute with 4 mL 20% methanol in water. Adjust to pH 1 with concentrated HCl. ◆ Remove fats from the sample by adding 1 mL heptane, shake vigorously, centrifuge at 4000 g, and remove/discard the upper and intermediate layers³. Repeat the fat removal procedure with an additional 1 mL heptane. Neutralize the sample (lower aqueous layer) with 50 µL 10 M NaOH and 2 mL 0.1 M phosphate buffer, pH 6 	Urine (centrifuged at 3000 x g for 10 min.) diluted 1:1 (v/v) with DI water. For β-glucuronidase treatment, please refer to Widstrand, 2004; and Fiori., 2005.

1. Condition/equilibrate cartridge with:	<ul style="list-style-type: none"> ◆ 1 mL methanol ◆ 1 mL DI water ◆ 1 mL 25 mM ammonium or sodium acetate, pH 6.7 																															
2. Load sample: Note: recommended flow rate ~0.5 mL/min.	Apply sample to the cartridge.																															
3. Wash (interference elution): Note: Apply gentle vacuum between each wash step.	<ul style="list-style-type: none"> ◆ Apply 2 min. of full vacuum to remove residual moisture from the cartridge. ◆ 1 mL acetonitrile ◆ 1 mL 0.5% acetic acid in acetonitrile (selective removal of hydrophobic interferences)⁴ ◆ 1 mL 50 mM ammonium acetate, pH 6.7 (selective removal of electrostatically bonded interferences)⁴ ◆ 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrogen bonded interferences)⁴ ◆ Apply full vacuum through cartridge for 2 min. to remove residual solvent. 	<ul style="list-style-type: none"> ◆ 1 mL DI water (selective elution/removal of salts and hydrophilic matrix components) ◆ Apply full vacuum through cartridge for 2 min to remove residual moisture from cartridge. ◆ 1 mL 1% acetic acid in acetonitrile (selective removal of hydrophobic interferences)⁴ ◆ 1 mL 50 mM ammonium acetate, pH 6.7 (selective removal of electrostatically bonded interferences)⁴ ◆ 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrogen bonded interferences)⁴ ◆ Apply full vacuum through cartridge for 2 min. to remove residual solvent. 																														
4. Analyte elution: Note: recommended flow rate ~0.2 mL/min.	Elute beta-agonists with 2 x 5 mL 10% acetic acid in methanol. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with LC mobile phase prior to analysis.	Elute beta-agonists with 2 x 1 mL 10% acetic acid in methanol. Apply a gentle vacuum between each fraction. Evaporate and reconstitute with LC mobile phase prior to analysis.																														
Recommended Analytical Technique: HPLC-UV or LC-MS	<p> column: Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particle size (53822-U) instrument: Applied Biosystems 3200 Q-TRAP mobile phase: 10 mM ammonium acetate (pH unadjusted) in methanol (A) and MS-grade water (B) flow rate: 0.2 mL/min. temp.: 35 °C det.: MS/MS MRM transitions: <table style="margin-left: 20px; border-collapse: collapse;"> <tr><td>1. Metaproterenol</td><td>212.19/152.10</td></tr> <tr><td>2. Terbutaline</td><td>226.21/152.20</td></tr> <tr><td>3. Formeterol</td><td>345.21/121.00</td></tr> <tr><td>4. Salmeterol</td><td>416.33/91.20</td></tr> <tr><td>5. Salbutamol</td><td>240.23/148.30</td></tr> <tr><td>6. Ritodrine</td><td>288.14/121.20</td></tr> </table> </p> <p> ion mode: Positive ion source: Turbospray Ion spray voltage: 2700 V source temp.: 400 °C collision gas: 40 psi inj.: 5 µL gradient: <table style="margin-left: 20px; border-collapse: collapse;"> <thead> <tr> <th>Min.</th> <th>A%</th> <th>B%</th> </tr> </thead> <tbody> <tr><td>0.00</td><td>25</td><td>75</td></tr> <tr><td>2.00</td><td>100</td><td>0</td></tr> <tr><td>4.00</td><td>100</td><td>0</td></tr> <tr><td>4.10</td><td>25</td><td>75</td></tr> <tr><td>8.00</td><td>25</td><td>75</td></tr> </tbody> </table> </p>		1. Metaproterenol	212.19/152.10	2. Terbutaline	226.21/152.20	3. Formeterol	345.21/121.00	4. Salmeterol	416.33/91.20	5. Salbutamol	240.23/148.30	6. Ritodrine	288.14/121.20	Min.	A%	B%	0.00	25	75	2.00	100	0	4.00	100	0	4.10	25	75	8.00	25	75
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Product Information:

Description

SupelMIP SPE - Clenbuterol

25 mg/10 mL (LRC)

Pkg. Qty. Cat. No.

50 **53201-U**

SupelMIP SPE - Beta-agonists (class selective)

25 mg/10 mL (LRC)

50 **53202-U**

25 mg/3 mL

50 **53225-U**

SupelMIP SPE – NNAL

25 mg/10 mL (LRC)

50 **53206-U**

25 mg/3 mL

50 **53203-U**

SupelMIP SPE - Riboflavin (Vitamin B2)

25 mg/10 mL (LRC)

50 **53207-U**

SupelMIP SPE - Triazine 10

25 mg/10 mL (LRC)

50 **53208-U**

SupelMIP SPE - Chloramphenicol

25 mg/10 mL (LRC)

50 **53210-U**

25 mg/3 mL

50 **53209-U**

SupelMIP SPE - Beta-blocker (class selective)

25 mg/10 mL (LRC)

50 **53218-U**

25 mg/3 mL

50 **53213-U**

SupelMIP SPE - TSNAs (NNK, NNN, NAB, NAT)

50 mg/10 mL (LRC)

50 **53221-U**

50 mg/3 mL

50 **53222-U**

SupelMIP SPE - Full Beta-receptors (beta-blockers & beta-agonists)

25 mg/10 mL (LRC)

50 **53223-U**

25 mg/3 mL

50 **53224-U**

1. Procedure based on:
The analysis of beta-agonists in bovine muscle using molecular imprinted polymers with ion trap LCMS screening, Kootstra PR, CJPF Kuipers, KL Wubs, D van Doorn, SS Sterk, LA van Ginkel and RW Stephany, 2005, Anal. Chim. Acta, 529:75-81
2. Procedure based on:
Multi-residue liquid chromatography/tandem mass spectrometric analysis of beta-agonists in urine using molecularly imprinted polymers. Van Hoof et al., Rapid Commun. Mass Spectrom. 2005; 19: 2801-2808

Evaluation of MISPE for the multi-residue extraction of beta-agonist from calves urine. Withstrand et al., J Chromatogr B Analyt Technol Biomed Life Sci. 2004, May 5; 804(1):85-91

Evaluation of two different clean-up steps, to minimize ion suppression phenomena in ion trap liquid chromatography-tandem mass spectrometry for the multi-residue analysis of beta agonists in calves urine. Fiori M. et al., Analytica Chimica Acta 529 (2005) 207-210
3. If the intermediate layer is very viscous, only the top uppermost layer is removed, and an additional 4 mL 20% methanol is added prior to further extraction with 1 mL hexane.
4. The prescribed wash procedure has been optimized to maximize sample clean-up prior to analysis. To increase recovery, reduce the acetic acid content of the second 1 mL 1% acetic acid in acetonitrile wash step. Recovery can be further improved by eliminating the last two 50 mM ammonium acetate and 60% acetonitrile wash step.

SupelMIP SPE developed by MIP Technologies AB

SupelMIP is a trademark of Sigma-Aldrich Co.