

This Application Note contains important information about this product

AFFINILUTE™ MIP – Clenbuterol

| Description | Quantity | Part Number |
|--|----------|-------------|
| AFFINILUTE MIP Clenbuterol 25 mg/10 mL | 50 | M01-0002-G |

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymers- 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.

Extraction of clenbuterol from urine

The following methods have been developed for the selective extraction of clenbuterol from calf urine. The method is highly reproducible and offers clenbuterol recoveries of $\geq 70\%$.

Extraction Procedure:

A flow rate of ~ 0.5 mL/min. is recommended. For analyte elution a flow rate of ~ 0.2 mL/min. is recommended.

| | |
|---|--|
| Analyte: | Clenbuterol |
| Sample Matrix: | Calf urine |
| General Comments: | The method is optimized for the extraction of clenbuterol, but can also be used to extract structurally similar beta-agonists such as cimaterol, cimbuterol, clenproperol, mabuterol, mapenterol and ractopamine. Using the exhaustive wash steps detailed in this method, clenbuterol detection levels of 0.5 ng/mL urine are achievable via HPLC-UV analysis. |
| Sample Pre-treatment: | Urine (centrifuged at 3000 x g for 10 min.) diluted 1:1 (v/v) with 25 mM ammonium acetate (NH ₄ Ac), pH 6.7 (final pH ~ 7) |
| 1. Condition/equilibrate cartridge with: | <ul style="list-style-type: none"> • 1 mL methanol • 1 mL DI water • 1 mL 25 mM ammonium acetate (NH₄Ac), pH 6.7 |
| 2. Load sample: Note: recommended flow rate ~ 0.5 mL/min. | For UV detection, apply 10 mL sample to the cartridge. For MS detection, apply 1 mL pre-treated sample to the cartridge. |
| 3. Wash (interference elution): Note: Apply gentle vacuum between each wash step. | <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 2% acetic acid in acetonitrile (selective removal of hydrophobic interferences). • 1 mL 0.5 M ammonium acetate, pH 5 (selective removal of electrostatically bonded interferences). • 1 mL 70% acetonitrile/30% DI Water (selective removal of hydrogen bonded interferences). • Apply full vacuum through cartridge for 2 min. to remove residual solvent. |
| 4 Analyte elution: Note: recommended flow rate ~ 0.2 mL/min. | Elute clenbuterol with 2 x 1 mL 1% TFA 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

Standard conditions:

Column: Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particle size (53822-U)
Instrument: Waters Micromass ZQ
Mobile phase: 10 mM ammonium acetate (pH unadjusted); acetonitrile (80:20)
Flow rate: 1.0 mL/min., split to MS
Temperature: 35 °C
Detection: MS, ESI(+) in selected ion recording (SIR)
Injection volume: 10 µL

Peak ID:

1. clenbuterol (M+H)⁺ : 277.07

Trace Conditions:

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 MRM
Mobile phase: 10 mM ammonium acetate (pH unadjusted) in 10% acetonitrile: acetonitrile (80:20)
Flow rate: 0.2 mL/min. temp.: 35 °C
Detection: MS/MS
MRM transitions: (227.2/203.1 and 277.3/168.2)
Ion mode: positive
Ion source: turbospray
Ionspray voltage: 3200 V
Source temp.: 425 °C
Collision gas: 45 psi
Injection: 5 µL

Ordering Information

| AFFINILUTE MIP - Clenbuterol | | |
|-------------------------------------|----|------------|
| 25 mg/10 mL (LRC) | 50 | M01-0002-G |

Related Products

| Description | Quantity | Part Number |
|--|-----------------|--------------------|
| AFFINILUTE MIP β-agonists (class selective) | | |
| 25 mg/10 mL (LRC) | 50 | M02-0002-G |
| 25 mg/3 mL | 50 | M02-0002-B |

| Description | Quantity | Part Number |
|---|-----------------|--------------------|
| AFFINILUTE MIP - β-blocker (class selective) | | |
| 25 mg/10 mL (LRC) | 50 | M18-0002-G |
| 25 mg/3 mL | 50 | M18-0002-B |

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