Extraction of Amphetamine and Methamphetamine from Urine using ISOLUTE® HCX Columns

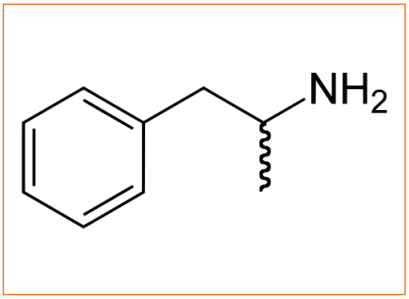

Figure 1. Structure of Aphetamine

Introduction

This method was developed for the extraction of amphetamine and methamphetaminefrom urine for drugs of abuse applications. Typical absolute recoveries for these drugs are > 80%.

The analytes have a non-polar structure, with an ionizable amine group. Both these characterisics are utilized in this mixed-mode extraction, allowing a clean final extract.

Application Note

Extraction Procedure

ISOLUTE® SPE Column: Confirm HCX 130 mg/3 mL, Part number 902-0013-B

Matrix: The matrix is of high ionic strength and contains many possible interference compounds.

Pre-treatment: Add 0.5 mL of 0.05 M phosphate buffer, pH 6.0 and 100 µL of internal standard (methamphetamine-D5 for GC-MS) to 1 mL urine. Sample pH should be in the range of 5-6.

Condition: Condition the column with 1 mL methanol at 2–4 mL/min

Equilibration: Rinse the column with 1 mL of deionized water at 2–4 mL/min, followed by 1 mL of 0.05 M phosphate buffer, pH 6.0 at 2–4 mL/min

Sample loading: Apply sample at a flow rate of 1–2 mL/min

Interference elution: Rinse column with 1 mL deionized water at 2 to 4 mL/min, followed by 1 mL 0.01 M HCl at 2–4 mL/min, and then 2 mL of methanol at 2–4 mL/min. Dry column on vacuum box for 1 minute at -20 psig.

Analyte elution: Before elution, add 0.1 mL of tartaric acid solution to the collection vial, so that the column eluate mixes with the tartaric acid solution on elution. This forms a less volatile 'semi-tartrate' complex with the eluted amphetamine, reducing loss on subsequent evaporation steps. Elute amphetamines with 1.2 mL of methanol/ammonium hydroxide 98:2 at 1 mL/min.

Derivatization:

1. Evaporate the column eluate to dryness at 40 °C.
2. Add 50 µL of pentafluoropropionic anhydride and 50 uL of ethyl acetate to the collection vial.
3. React at room temperature for 5 minutes.
4. Evaporate to dryness with nitrogen at room temperature.
5. Reconstitute in 50 µL ethyl acetate and inject 1–2 µL into GC.

Analytical method: GC, GC-MS

Column: DB-5, 15 m x 0.25 mm I.D. x 0.25 µm
Initial temp: 70 C for 1 min
Temp. ramp: 15 C/min
Final temp: 250 °C for 1 min

Reagents:

1. Methanol
2. 0.05 M sodium or potassium phosphate buffer at pH=6
3. 0.01 M HCl
4. Tartaric acid solution: 10 mg tartaric acid in 100 mL ethyl acetate.
5. Methanol/ammonium hydroxide 98:2
6. Pentafluoropropionic anhydride
7. Ethyl acetate

Aditional Information:

If the addition of tartaric acid solution is not compatible with your analytical methodology, an alternative approach is to use a keeper solvent during evaporation.

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