Determination of Amphetamines in Urine Samples Using Solid Phase Extraction and LC-MS/MS



Procedure:

1. Sample Preparation

- a) Add 1 mL of pH 6 phosphate buffer (100 mM) into a clean sample tube and add internal standards.
- b) Add 1 mL of urine and mix.
- c) 2 mL of pH 6 phosphate buffer (100 mM) and mix.
- d) Centrifuge for 10 minutes at 3000 rpm.

2. SPE Column Conditioning

- a) 1 x 3 mL Methanol.
- b) 1 x 3 mL D.I. H₂O.
- c) 1 x 1 mL 100 mM phosphate buffer (pH 6.0).

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

a) Load sample at 1 to 2 mL/minute

4. Wash Column

- a) 1 x 3 mL D.I. H₂O.
- b) 1 x 3 mL 1 M Acetic Acid.
- c) 1 x 3 mL Methanol.
- d) Dry column (5 minutes at > 10 inches Hg).

UCT Part Numbers

Clean Screen® DAU 200 mg, 6 mL **SLPFPP50ID21-3UM** Selectra® PFPP (50 x 2.1 mm, 3 μm) HPLC Column

SPHPHO6001-5 Select pH buffer pouch 100 mM phosphate pH 6.0

5. Elute Amphetamines

a) Add 1 x 3 mL methylene chloride:

Isopropanol: ammonium hydroxide (78:20:2). **Note:** Prepare a fresh solution of the methylene chloride: isopropanol: ammonium hydroxide mixture by adding 2 mL of ammonium hydroxide to 20 mL of isopropanol. Mix and add 78 mL of methylene chloride.

- b) Collect eluate at a rate of 1 to 2 mL / minute.
- c) Add 100 µL of 1% HCl in methanol to eluate.
- d) Vortex mix.

6. Dry Eluate

- a) Evaporate to dryness under nitrogen < 40°C.
- b) Add 100 μL of mobile phase and vortex mix.





Instrument Conditions						
Column	Selectra [®] PFPP 50 x 2.1 mm; 3 μm					
Column Temperature	40 °C					
Gradient/Mobile Phase	Isocratic - H₂O with 1% Formic Acid: MeOH with 1% Formic acid (70:30)					
Flow Rate	0.3 mL/ minute					
Detector	API 4000 MS/MS					

Mass Spec Table											
Compound	RT (min)	Precursor lon	Product Ion 1	Product Ion 2	DP	EP	CE 1	CE 2	CXP 1	CXP 2	
Amphetamine	5.51	136.1	91.2	119.1	51	10	23	13	16	8	
Methamphetamine	6.46	150.1	91.2	119.1	61	10	29	17	16	8	
MDA	6.46	180.2	163.1	105.1	46	10	15	31	10	6	
MDMA	7.54	194.2	163.1	105.1	76	10	19	63	12	14	

Chromatogram of Select Amphetamines







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