



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

UCT Part Numbers:

CSDAU206: Clean Screen DAU, 200mg / 6 mL tube

SLPFPP50ID21-3UM: Selectra PFPP (50 x 2.1 mm, 3 µm) HPLC Column

SPPHO6001-5: Select pH Buffer Pouch, phosphate buffer pH 6

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Procedure

1) Sample Preparation

- a) Add 1mL of pH 6 phosphate buffer (0.1 M) 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 (0.1 M) and mix
- d) Centrifuge for 10 minutes at 3000 rpm

2) SPE Column Conditioning

- a) 1 x 3 mL CH₃OH.
- b) 1 x 3 mL D.I. H₂O
- c) 1 x 1 mL 0.1 M 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.

3) Wash Column

- b) 1 x 3 mL D.I. H₂O
- c) 1 x 3 mL 1 M Acetic Acid
- d) 1x 3 mL Methanol

e) Dry column (5 minutes at > 10 inches Hg).

4) 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 mobile phase to eluate.

d) Vortex mix.

5) 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

Mobile phase: Isocratic - H₂O with 1% Formic Acid: MeOH with 1% Formic acid (70:30)

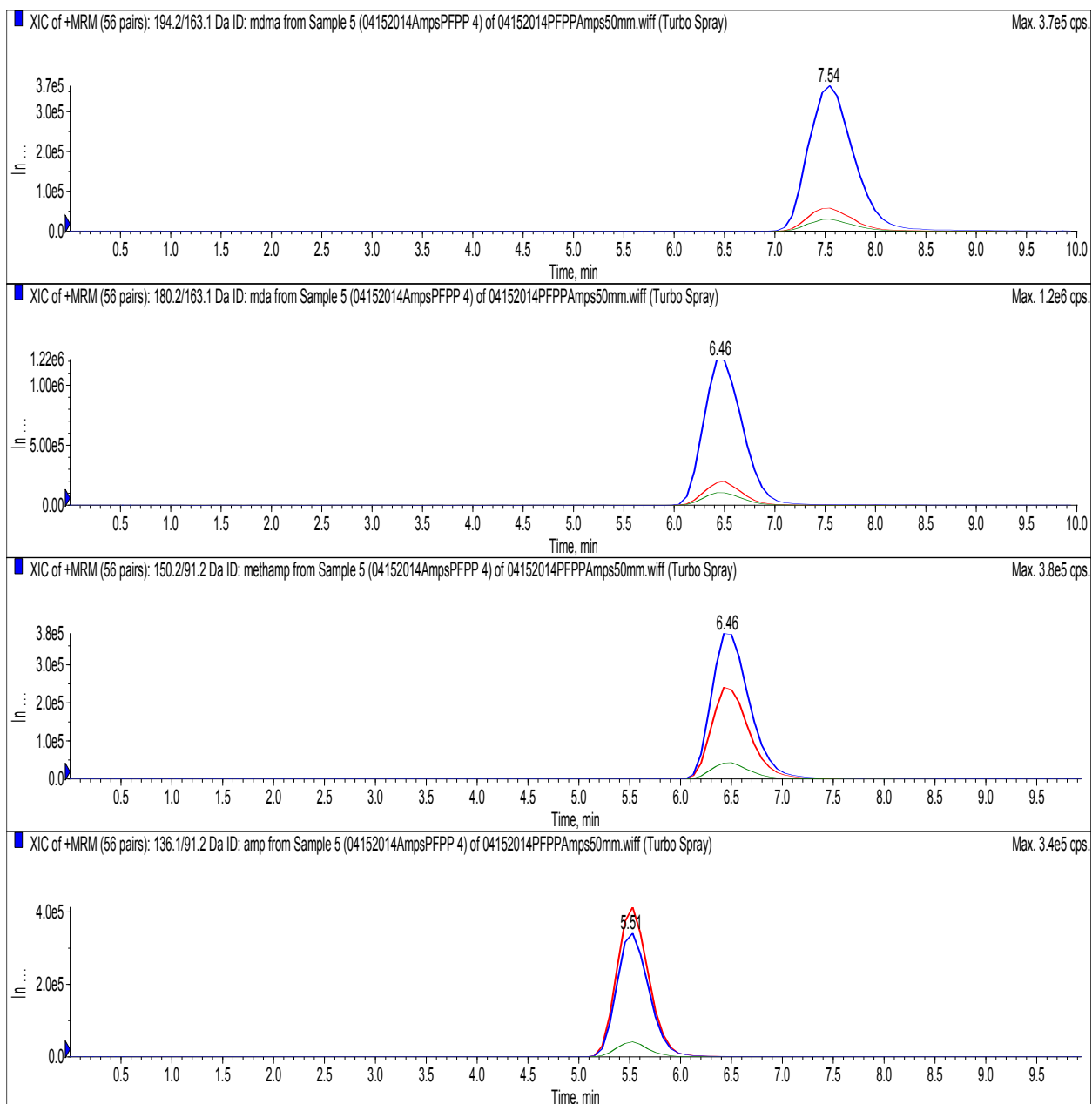
Flowrate: 0.3 mL/ minute

Detector: API 4000 MS/MS

Mass Spec Table

Compound	RT / min	Precursor Ion	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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