



BATH SALTS IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU – CLEAN SCREEN® DAU

PFAA-0-1 – SELECTRA-SIL® PFAA

SPFPOH-1 – SELECTRA-SIL® PFPOH

SLDA100ID21-5UM – SELECTRA® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5 .
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
1 x 3 mL 100 mM phosphate buffer (pH 6.0)
NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute

4. WASH COLUMN:

1 x 3 mL D.I. H₂O
1 x 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE BATH SALTS:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

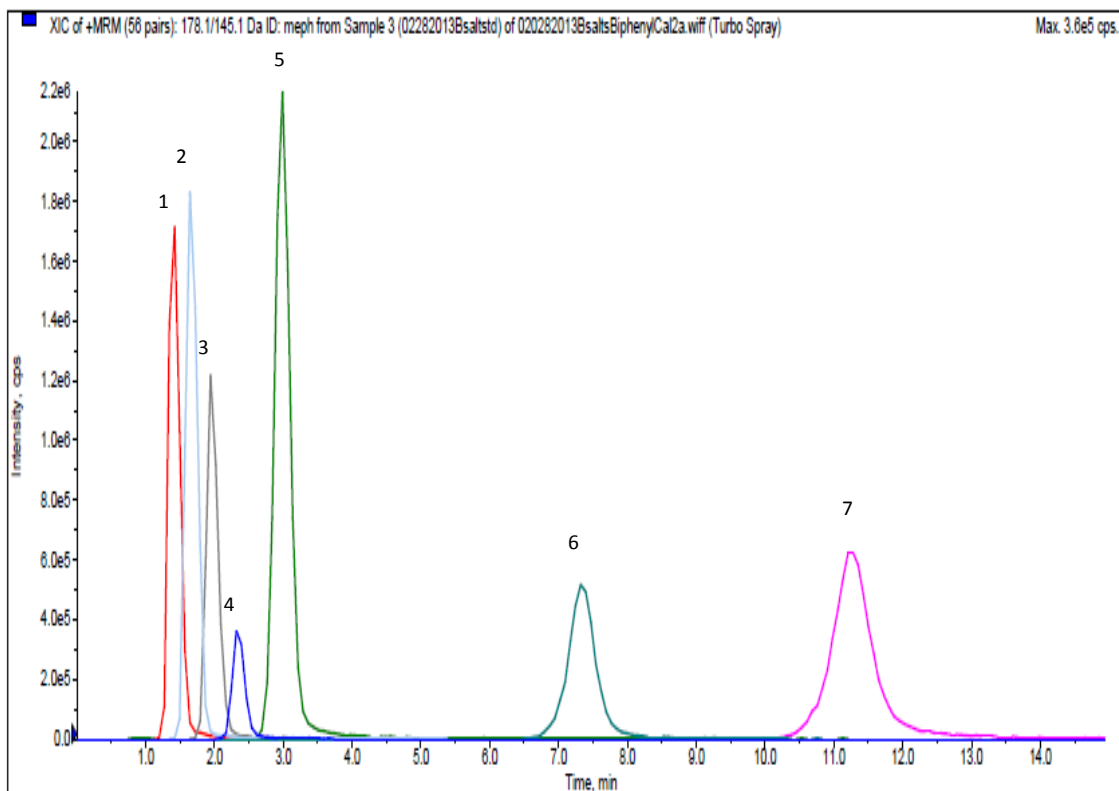
Add 50 µL of 1% HCl in Methanol to each test tube
Evaporate to dryness at < 40 °C

NOTE: A 1% HCl in CH₃OH solution has been used to prevent volatilization by the formation of
The hydrochloric salt of the drugs

7. RECONSTITUTE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 5 µL.
- **GC-MS:** Fluoroacrylate with PFPA (PFAA)
Add 50 µL PFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 µL Ethyl Acetate

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1.Flephedrone	182.1	164.2	1.41
2.Methylone	208.1	160.1	1.66
3.Methadrone	194.1	161.1	1.96
4.Methedrone	178.1	145.1	2.34
5.Methethcathinone	192.2	174.0	2.98
6.MDPV	276.2	126.1	7.34
7.Pyralvalerone	246.2	105.2	11.24

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

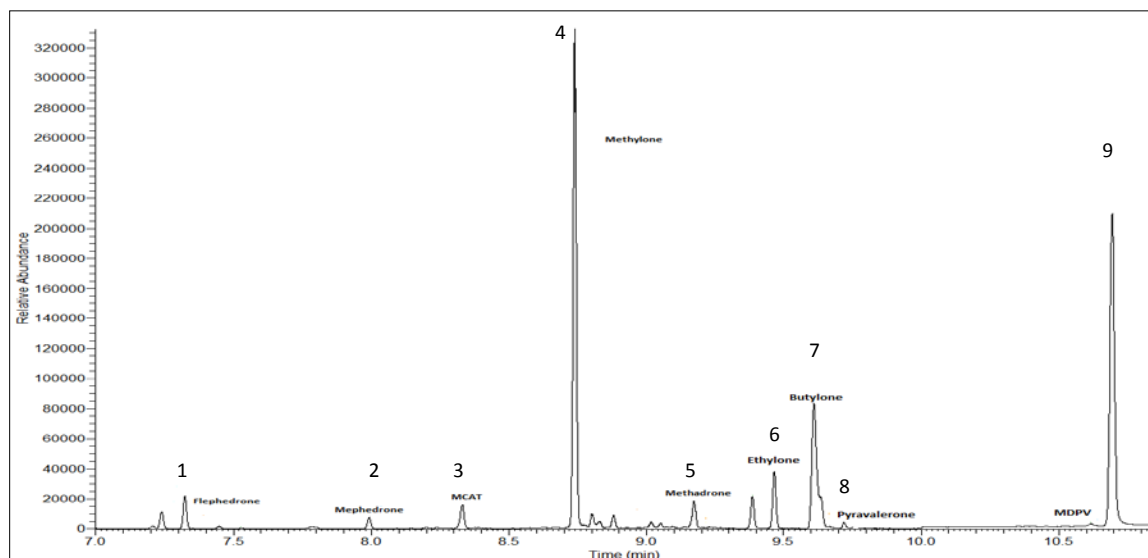
LC Column: Selectra[®] DA HPLC Column 100 x 2.1 mm 5 µm

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	70	30
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):



Fluoroacylate with PFAA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Flephedrone	123	204	160	7.32
2.Mephedrone	204	160	149	7.99
3.MCAT	218	174	91	8.33
4.Methylone	353	204	160	8.74
5.Methadrone	135	160	204	9.17
6.Ethylone	218	190	367	9.47
7.Butylone	218	160	367	9.61
8.Pyravalerone	126	84	91	9.72
9.MDPV	126	96	84	10.62

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30 m x 0.25 mm (0.25 µm) TG-1MS

Injector: 1 µL Splitless, 250 °C

Oven temperature program: 50 °C (1) to 310 °C (25 °C/ minute): hold (3.6 minute)

Carrier gas: Helium (1.2 mL/ minute)

MSD condition: Aux temperature: 280 °C, MS Source: 250 °C, MS Quad: 150 °C

Reference:

Comprehensive Forensic Toxicological Analysis of Designer Drugs; NIJ Grant

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Document No.: 244233

Date Received: December 2013