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

Part #:

CSXCE111 – CLEAN SCREEN XCEL® 130 mg, 1 mL Tube

PFAA-0-1 – SELECTRA-SIL® PFAA

SPFPOH-1 – SELECTRA-SIL® PFPOH

SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 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. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

Add 50 µL of 1% HCl in CH_3OH to each tube
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

NOTE: A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs.

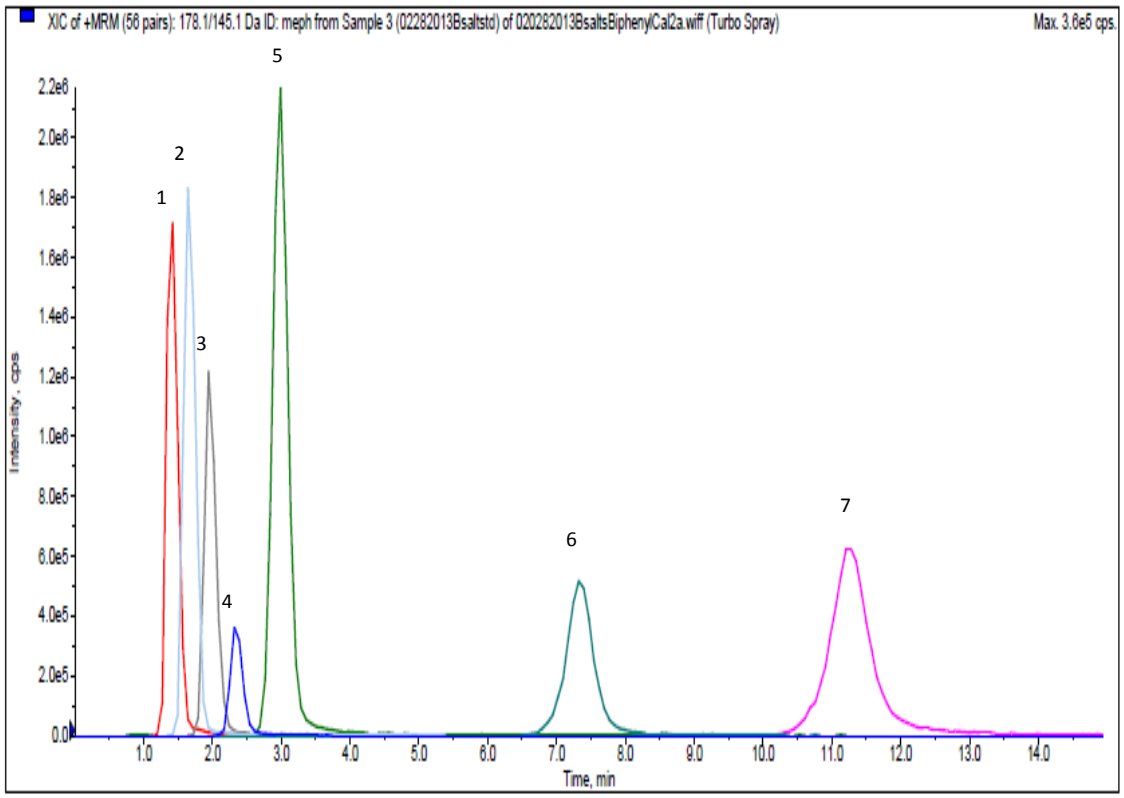
6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 5 µL.

- **GC-MS**: Fluoroacylate with PFPA (PFAA)
  Add 50 µL PFPA. Over lay with N_2 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

NOTES: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.*

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. Pyravalerone	**246.2**	**105.2**	**11.24**

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

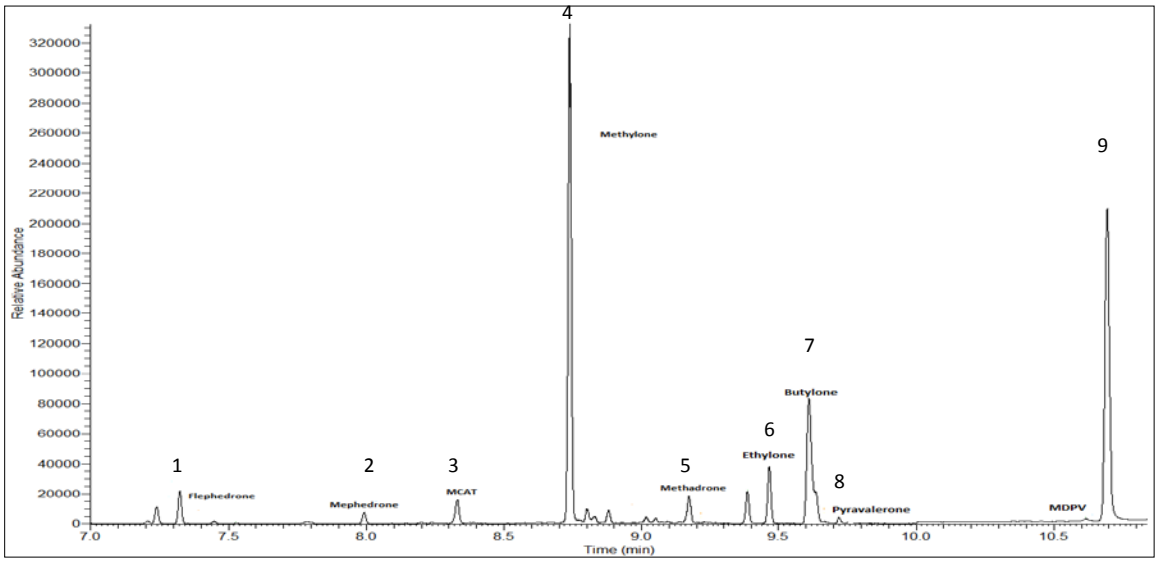
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

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 PFPA (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 ºC, MS Source: 250 ºC, MS Quad: 150 ºC

Reference:

Comprehensive Forensic Toxicological Analysis of Designer Drugs; NIJ Grant
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