

# Analysis of Synthetic Cathinones From Blood and Urine Using Clean Screen® XCEL I on LC-MS/MS



## UCT Part Numbers

**CSXCE103**

Clean Screen® XCEL I  
130 mg, 3 mL

**SCS27-DA521**

SelectraCore® DA Column  
50 x 2.1 mm, 2.7 µm

**SLGRDHLDR-HPOPT**

Selectra® Direct Connect  
Guard Holder

**SCS27-DAGDC21**

SelectraCore® DA Guard Column  
5 x 2.1 mm, 2.7 µm

**SPPHO6001-10**

Select pH Buffer Pouches 100 mM  
Phosphate pH 6.0

## Introduction:

Synthetic cathinones also known as ‘bath salts’ are a group of designer stimulants related to the naturally occurring alkaloid cathinone, which can be found in Khat.<sup>1,2</sup> Stimulants cause an increase in activity in the central nervous system (CNS). At lower doses, some effects of stimulants include an increase in attention, alertness, and energy.<sup>3</sup> Misuse of stimulants can result in some serious adverse effects such as psychosis, paranoia, aggression, and addiction.<sup>3,4</sup> Synthetic cathinones are novel psychoactive substances (NPS), which means these drugs are new and forensic laboratories must continue to update their scope of analysis to include these analytes. Newly discovered synthetic cathinones include N,N-dimethylpentylone and α-PHP.

This application note outlines an extraction procedure and instrument parameters for analyzing new synthetic cathinones and other commonly encountered stimulants from blood and urine. Analytes were extracted from biological matrices using UCT’s Clean Screen® XCEL I solid phase extraction column and structurally similar compounds were resolved with liquid chromatography using a SelectraCore® DA column. UCT’s Clean Screen® XCEL column does not require column conditioning which subsequently reduces solvent usage and extraction time. UCT’s core-shell column line, SelectraCore® efficiently resolves compounds allowing for short run times.



## Sample Preparation:

0.5 mL sample + 1.5 mL 100 mM phosphate buffer pH 6 + ISTDs, vortex and centrifuge

## SPE Procedure:

### 1. Load Sample:

- a) Load sample 1-2 min/mL

### 2. Wash Column:

- a) 1 x 3 mL DI H<sub>2</sub>O
- b) 1 x 3 mL MeOH

### 3. Dry Column:

- a) Dry for at least 5 minutes at full vacuum or pressure

### 4. Elute:

- a) 1 x 3 mL EtOAc:IPA:NH<sub>4</sub>OH (78:20:2)

### 5. Evaporate:

- a) Add 100 µL of 1% HCl in MeOH to help prevent loss of analyte during evaporation
- b) Evaporate samples to dryness at 5 psi and 35°C

### 6. Reconstitute:

- a) Reconstitute samples in 0.5 mL of 5:95 H<sub>2</sub>O:MeOH or other appropriate solvent and volume



### Instrument Parameters

LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® DA Column 50 x 2.1 mm, 2.7 µm
Guard Column	SelectraCore® DA Guard Column 5 x 2.1 mm, 2.7 µm
Column Temperature	45°C
Flow Rate	0.4 mL/min
Injection Volume	1 µL
Mobile Phase A	5 mM ammonium formate + 0.1% formic acid in water
Mobile Phase B	5 mM ammonium formate + 0.1% formic acid in methanol
Gradient	Conc. B 5% (0 min) - 100% (8-10 min) - 5% (10.20 - 13.20 min)

### MRM Table

Analyte	R.T. (min)	Parent Ion (m/z)	Product Ion 1 (m/z)	CE (V)	Product Ion 2 (m/z)	CE (V)
Methamphetamine	2.72	149.8	91.0	-22	119.0	-16
Amphetamine	2.28	136.0	91.0	-21	119.1	-15
Eutylone	3.72	235.7	188.1	-20	218.0	-14
Butylone	3.43	221.9	174.0	-19	204.1	-14
Pentylone	3.99	235.7	188.1	-20	218.1	-14
N,N-dimethylpentylone	4.10	249.8	175.0	-21	205.1	-17
MDMA	3.09	194.0	163.1	-14	105.0	-25
α-PHP Metabolite	4.78	247.8	230.1	-18	72.2	-21
α-PHP	4.63	246.3	91.1	-26	77.0	-49

\*CE = collision energy

## Chromatogram:

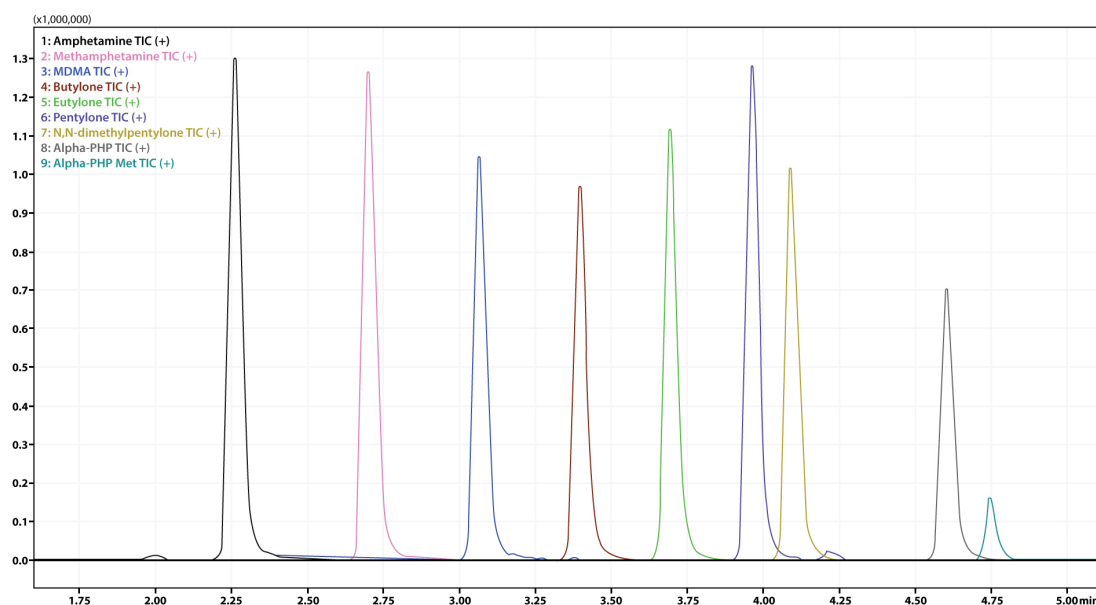
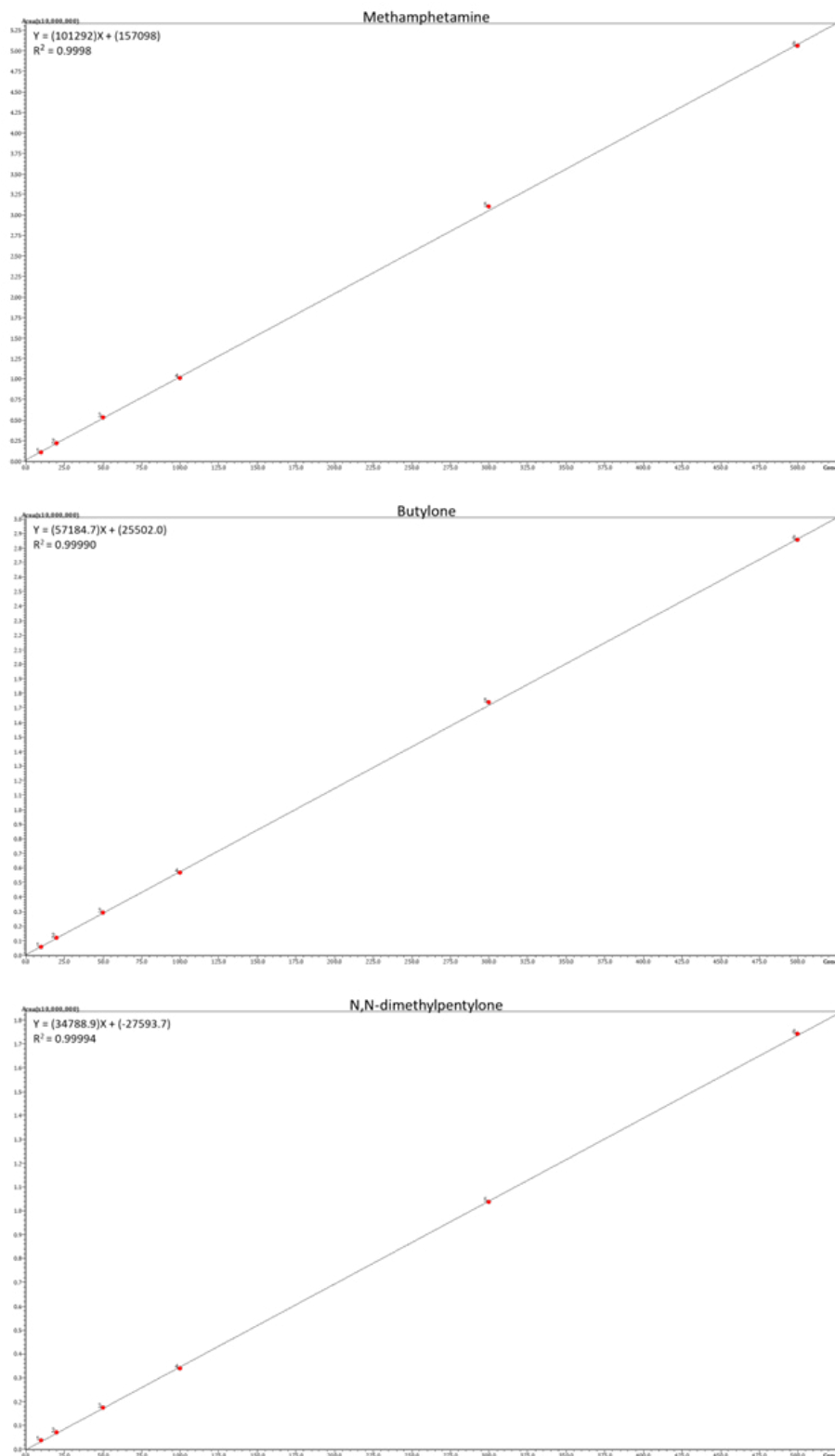


Figure 1: Chromatogram of Extracted Blood Sample at 25 ng/mL



## Calibration Curves:



**Figure 2:** 6-point calibration curve extracted from blood for methamphetamine, butylone, and N,N-dimethylpentylone (10, 20, 50, 100, 300 & 500 ng/mL)

## Results:

Urine						
n=5	25 ng/mL			200 ng/mL		
Analyte	Recovery (%)	Matrix Effects (%)	RSD (%)	Recovery (%)	Matrix Effects (%)	RSD (%)
Methamphetamine	100	-5	6	106	-5	2
Amphetamine	101	-6	5	105	-7	2
Eutylone	99	-2	6	101	-2	3
Butylone	98	-4	6	101	-4	2
Pentylone	98	-3	7	100	-4	2
N,N-dimethylpentylone	97	1	7	102	-3	3
MDMA	99	-2	7	100	0	3
α-PHP Metabolite	105	-3	5	103	-4	2
α-PHP	103	-5	6	105	-3	2

Blood						
n=5	25 ng/mL			200 ng/mL		
Analyte	Recovery (%)	Matrix Effects (%)	RSD (%)	Recovery (%)	Matrix Effects (%)	RSD (%)
Methamphetamine	91	-5	9	102	-16	5
Amphetamine	90	-6	8	101	-14	5
Eutylone	90	-5	8	97	-13	5
Butylone	90	-5	8	98	-13	5
Pentylone	91	-10	8	95	-16	6
N,N-dimethylpentylone	89	-9	9	98	-17	5
MDMA	90	-5	9	96	-12	6
α-PHP Metabolite	84	-32	17	80	-37	7
α-PHP	91	-23	12	99	-29	6

\*Recoveries were calculated using a pre- and post-spike sample technique. Matrix effects were calculated by comparing post-spike samples and solvent standards.



## Conclusion:

The SPE method was evaluated by calculating the recoveries, matrix effects, and relative standard deviations for a low and high concentration (25 ng/mL & 200 ng/mL). For urine (n=5) recoveries ranged from 97% to 106%. Matrix effects and relative standard deviations were low for the two concentrations with ranges of (-7)% to 0% and 2% to 7% respectively. For blood samples, recoveries ranged from 84% to 102% and relative standard deviations ranged from 5% to 17%. Despite having a slightly larger range of matrix effects for blood [-37% to -5%], samples were still successfully extracted and quantitated. With a limit of detection of 5 ng/mL this method can be used to identify and quantify synthetic cathinones in blood and urine.

All samples were analyzed using a LC-MS/MS equipped with UCT's SelectraCore® DA column. The biphenyl phase successfully resolved isomers, eutylone and pentylone. All analytes were separated using a simple linear gradient with a run time of less than 15 minutes.

## References:

- [1] Synthetic cathinones - Alcohol and Drug Foundation. (2021, November 10). Adf.org.au.  
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