Extraction of Bath Salts (substituted cathinones) From Human Urine Using ISOLUTE® SLE+ Columns prior to GC-MS Analysis

This application note describes the supported liquid extraction clean-up of a range of substituted cathinones from urine prior to quantitative GC-MS analysis.

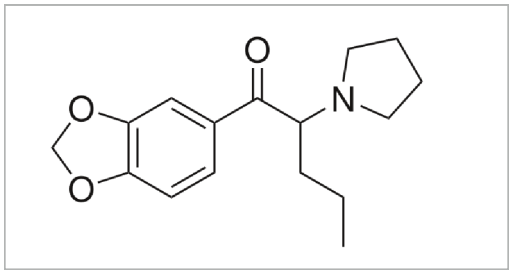

Figure 1. Structure of MDPV

Introduction

'Bath salts' is the street name for a family of designer drugs chemically similar to cathinones that give the user similar effects to amphetamines. The abuse of these drugs is on the increase and regulation against their use and supply has now been implemented in the EU and North America.

Analyte recoveries achieved using this method to extract bath salts from urine ranged from 87-99% with RSDs below 10% for all analytes.

ISOLUTE SLE+ Supported Liquid Extraction columns offer an efficient alternative to traditional liquid liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Methcathinone, Mephedrone, Methedrone, Methylone, Butylone, Ethylone, MDPV, Naphyrone

Sample Preparation Procedure

Column configuration: ISOLUTE SLE+ 1 mL Sample Volume column, part number 820-0140-C

Sample pre-treatment: Dilute urine 1:1 (v/v) with 150 mM ammonium hydroxide.

Sample loading: Load the pre-treated sample (1 mL total volume) onto the column and apply a pulse of vacuum (VacMaster 20 Sample Processing Manifold, 121-2016) or positive pressure (PRESSURE+ 48 Positive Pressure Manifold, PPM-48) to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte extraction: Apply MTBE (2 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (2 mL) and allow to flow under gravity for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent, collecting into a glass culture tube containing 0.2 M hydrochloric acid (100 µL) to add stability during evaporation.

Post extraction: Evaporate the extract to dryness (ambient temperature). Add pentafluoropropionic acid anhydride (PFPA) (50 µL) and ethyl acetate (50 µL) for derivatization. Vortex for 20 seconds, transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block (70 ºC) for 20 minutes. Remove vial and allow to cool. Evaporate the mixture to dryness (ambient temperature). Reconstitute in dichloromethane:isopropanol (95:5, v/v) (100 µL). Cap with a non-split cap and vortex for 30 seconds.

GC Conditions

Instrument:	Agilent 7890A GC
Column	SGE capillary column; 30 m x 0.25 mm ID-BPX5 x 0.25 µm
Carrier:	Helium 1.2 mL/min (constant flow)
Inlet:	250 °C, Split (ratio 20:1), 24 mL/min Septum purge flow: 3 mL/min
Injection:	1 µL, wash solvents: ethyl acetate and DCM:IPA (95:5, v/v)
Oven:	100 °C initial, 4 °C/min to 190 °C then 100 °C/min to 250 °C, hold 4 min
Transfer Line:	280 °C

Mass Spectrometry Conditions

Instrument:	Agilent 5975C MSD
Source:	230 °C
Quadrupole:	150 °C
MSD mode:	SIM

Table 1. SIM Parameters

SIM Group	Analyte	Quant Ion	1st Qual Ion	2nd Qual Ion	3rd Qual Ion	Dwell (ms)
1	Methcathinone	105	204	160	77	40
2	Mephedrone	119	204	91	160	40
3	Methedrone	135	204	136	77	40
4	Methylone	204	149	160	121	40
5	Butylone	149	218	121	160	40
6	Ethylone-d5	223	191	150	121	25
6	Ethylone	218	190	121	-	25
7	MDPV	127	126	149	-	50
8	Naphyrone	126	127	96	-	50

Results

This ISOLUTE SLE+ protocol demonstrates analyte recoveries ranges from 87-99% as shown in figure 3 (page 3) with RSDs below 10% for all analytes. Robustness testing was carried out across three days using three different sources of urine. Figure 2 shows the chromatogram for the full range of extracted bath salts at a concentration range of 100 ng/mL.

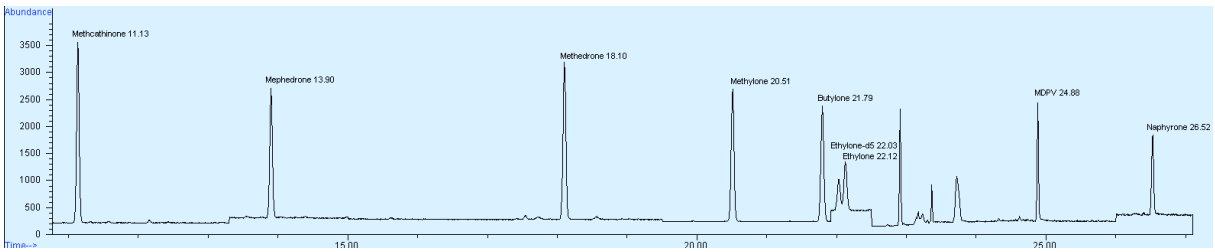

Figure 2. Zoomed chromatogram showing extracted bath salts analytes at 100 ng/mL

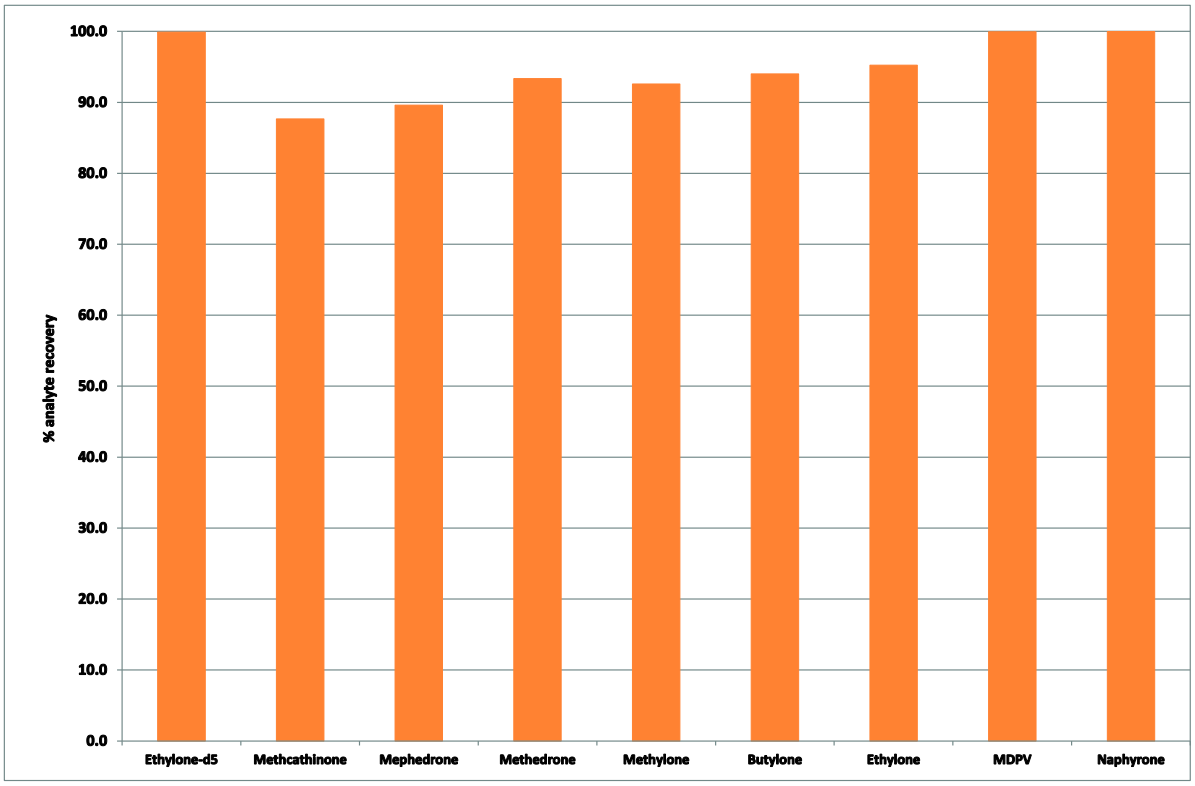

Figure 3. Typical analyte % recoveries for a range of extracted bath salts (n=7) using the ISOLUTE SLE+ protocol

Typical extractions showed limits of quantitation ranging from 5-10 ng/mL dependent upon analyte and required detection limit as shown in table 2. Figure 4 shows the calibration curves for methcathinone and mephedrone, demonstrating linearity over the range from 5-250 ng/mL.

Table 2. Limits of Quantitation for extracted bath salts using the ISOLUTE SLE+ protocol

Analyte	LOQ (ng/mL)
Methcathinone	5
Mephedrone	10
Methedrone	5
Methylone	5
Butylone	10
Ethylone	10
MDPV	5
Naphyrone	10

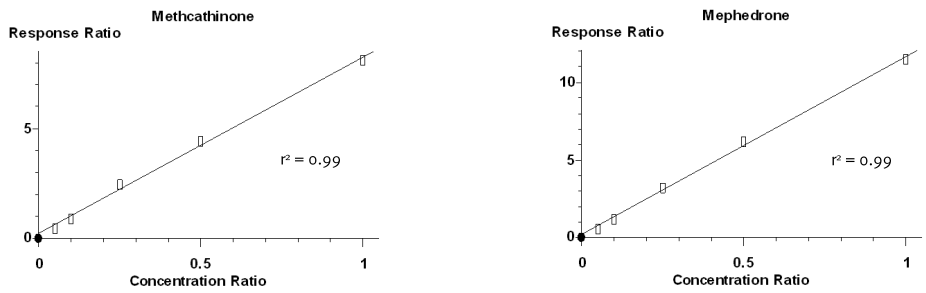

Figure 4. Calibration curves for methcathinone and mephedrone over the range of 5-250 ng/mL

Ordering information

Part Number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume column	30
PPM-48	PRESSURE+48 Positive Pressure Manifold	1
121-2016	VacMaster 20 Sample Processing Manifold	1

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