Extraction of Designer Stimulants from Urine Using ISOLUTE® SLE+ prior to GC/MS Analysis

This application note describes the extraction of a range of recreational ecstasy-like drugs, designer stimulants and the hallucinogen 2C-B from urine prior to GC/MS analysis. The method described in this application note may also be suitable for extraction of other related analogs and derivatives of these drug compounds, for example the substituted cathinone class (bathsalts). This application note is optimized for extraction of 1 mL sample volumes.

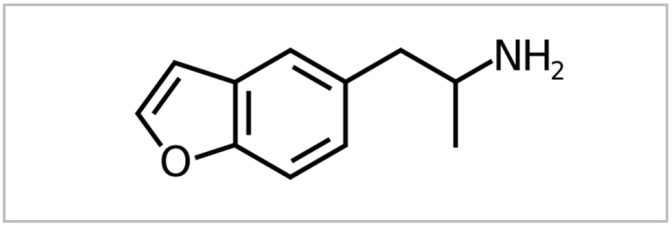

Figure 1. Structure of 5-APB, a chemical found in '*Benzo Fury*'

Introduction

ISOLUTE® SLE+ Supported Liquid Extraction plates and 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

5-APB, 6-APB, pMMA, BZP, TFMPP, 2C-B, mCPP, Amphetamine-d5 as internal standard.

Sample Preparation Procedure

Sample Pre-treatment Spike urine sample with ISTD (10 µL in methanol) and dilute spiked sample with ammonium hydroxide (1%, 1:1, v/v)

ISOLUTE SLE+ 1 mL Sample Volume columns, part number 820-0140-C

Sample Loading: Load the pre-treated urine (1 mL total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte Extraction: Collect extracts into glass tubes containing methanolic HCl (100 µL, 0.2M)

Apply methyl tert-butyl ether (MTBE) (2 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (2 mL) and allow to flow for another 5 minutes.

Apply vacuum or positive pressure for 10–20 seconds to elute any remaining extraction solvent.

Post Elution and Derivatisation: Evaporate to dryness in a stream of air or nitrogen using a SPE Dry (ambient temperature, 20 to 40 L/min) or TurboVap (1.5 bar at ambient temperature for 40 mins).

Reconstitute with 0.2M HCl in ethyl acetate (500 µL) and vortex for 20 seconds. Transfer to a high recovery glass vial and evaporate to dryness.

Add ethyl acetate (25 µL) and pentafluoropropionic acid anhydride (PFPA) (25 µL) and cap with a non-split cap. Vortex for 20 seconds and heat vial in a heating block set to 70 °C for 30 minutes. Remove vial from the block and allow to cool.

Evaporate at ambient temperature and reconstitute with ethyl acetate (50 µL).

GC Conditions

Instrument:	Agilent 7890A with QuickSwap
Column:	Phenomenex Zebron ZB-Semivolatiles, 30 m x 0.25 mm ID x 0.25 µm
Carrier:	Helium 1.2 mL/min (constant flow)
Inlet:	225 °C, Split 10:1, 17.25 psi; Septum purge flow: 3 mL/min
Injection:	1 µL
Wash Solvent:	Ethyl acetate
Oven:	Initial Temperature 55 °C
	Ramp 25 °C/min to 175 °C, hold for 4 minutes
	Ramp 120 °C/min to 200 °C, hold for 3 minutes
	Ramp 120 °C/min to 240 °C, hold for 1 minutes
	Ramp 120 °C/min to 330 °C, hold for 1.5 minutes
Post Run:	Backflush for 2.4 minutes (3 void volumes)
Transfer Line:	280 °C

MS Conditions

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

SIM Parameters

Table 1. Ions acquired in the Selected Ion Monitoring (SIM) mode (all PFPA derivatives)

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion	3rd Qual Ion
1	Amphetamine-d5	194	122	92	N/A
2	5-APB	158	131	190	321
2	6-APB	131	158	190	321
3	pMMA	204	121	148	N/A
4	BZP	231	322	175	N/A
4	TFMPP	200	172	229	N/A
5	2C-B	242	229	231	N/A
6	*m*CPP	166	195	138	429

Results

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 105–109% as shown in **Figure 2**. RSDs were below 10% for all analytes for all donors.

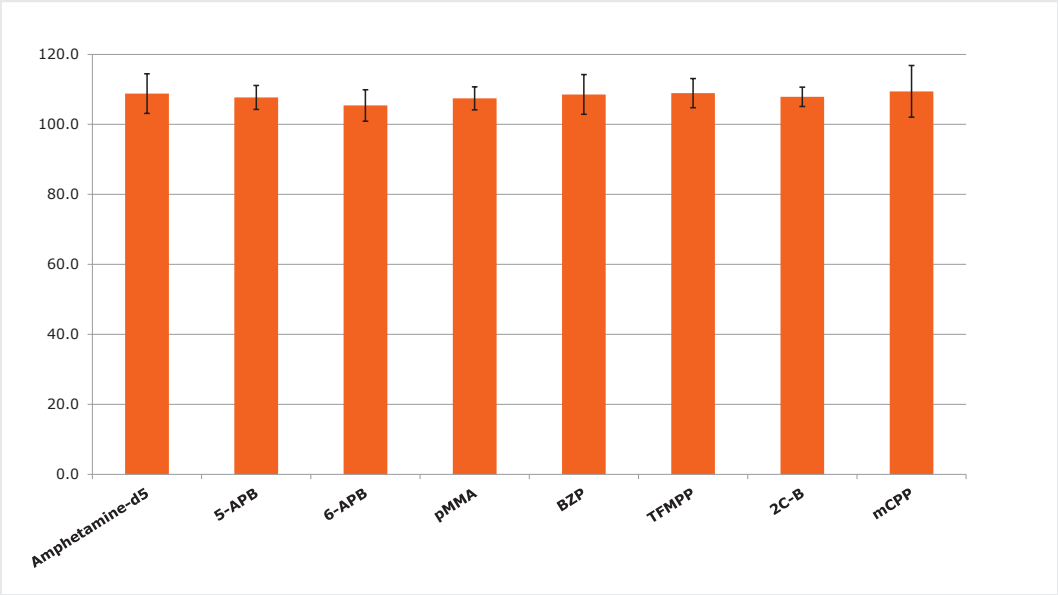

Figure 2. Typical analyte % recoveries for extracted synthetic stimulants (n=7) using this ISOLUTE® SLE+ protocol.

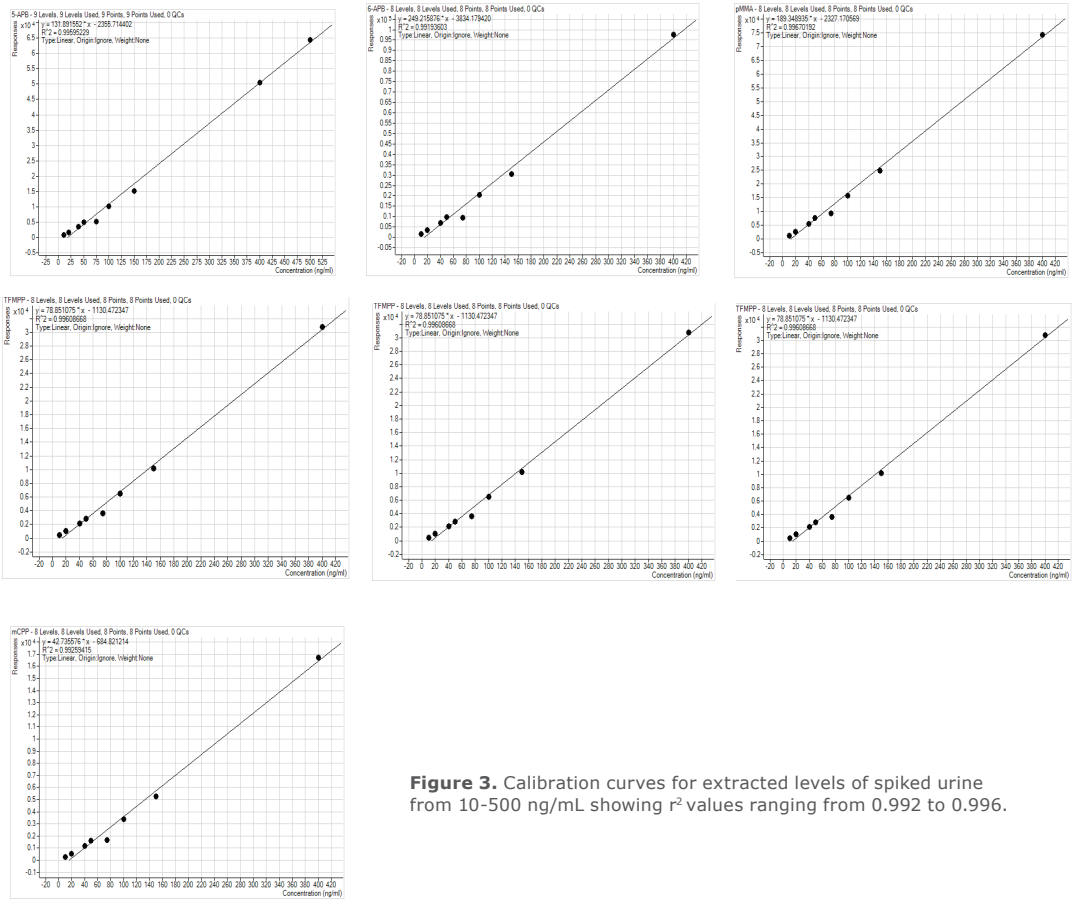

Figure 3. Calibration curves for extracted levels of spiked urine from 10-500 ng/mL showing r^2 values ranging from 0.992 to 0.996.

Table 3. Lower Limits of Quantitation (LLOQ) seen from 500 µL extracted urine using ISOLUTE® SLE

Analyte	Lower Limit Of Quantitation
5-APB	10 ng/mL
6-APB	10 ng/mL
pMMA	10 ng/mL
BZP	10 ng/mL
TFMPP	10 ng/mL
2C-B	20 ng/mL
mCPP	10 ng/mL

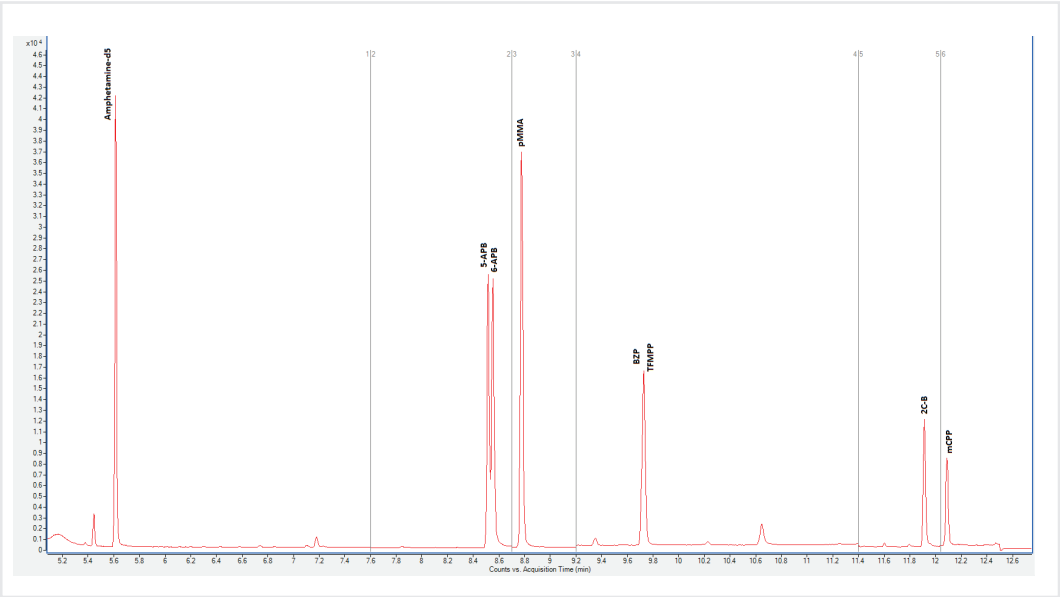

Figure 4. GC/MS chromatography for the application analytes from urine spiked at 100 ng/mL

This application note was developed with minimal modification of Biotage application note AN776: Extraction of Bathsalts (Subsitiuted Cathinones) from Human Urine using ISOLUTE SLE+ columns prior to GC/MS Analysis. Further optimization to this method could be performed with respect to sample pre-treatment and elution solvent selection. Alternative extraction solvents, for example DCM, may be appropriate. For more information, see the ISOLUTE® SLE+ User Guide.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Supported Liquid Extraction Column	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold 48 Position	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103198	TurboVap® 96 without racks 100/120 VAC	1
C103199	TurboVap® LV without racks 220/240 VAC	1

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