Extraction of Phencyclidine (PCP) from Urine Using ISOLUTE® SLE+ Prior to GC/MS Analysis

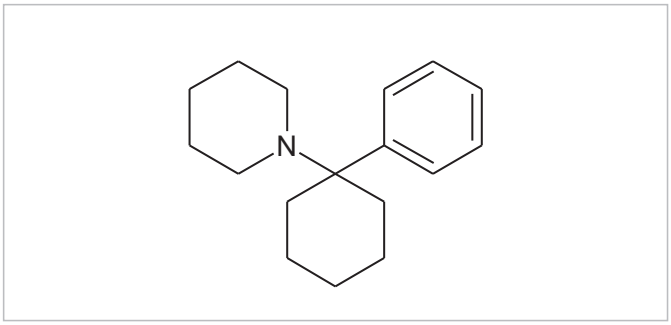

Figure 1. PCP structure

Introduction

This application note describes the extraction of PCP from urine using supported liquid extraction and subsequent analysis by GC/MS.

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.

Analytes

Phencyclidine (PCP) & PCP-D5

Sample Preparation Procedure

Sample Pre-treatment:	Dilute pre-treated urine (1 mL) with 0.5% ammonium hydroxide (aq) (1 mL). Spike PCP-D5 internal standard and vortex mix thoroughly.
Format:	**ISOLUTE® SLE+ 1 mL Sample Volume Columns, part number 820-0140-C (also available in tabless format, part number 820-0140-CG)**
Sample Loading:	Load pre-treated urine (1 mL) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
Analyte Extraction:	Apply 1-chlorobutane (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of 1-chlorobutane (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).
Post Elution and Reconstitution:	To each sample, add 0.2M methanolic HCl (100 µL). Dry the extract in a stream of air or nitrogen at ambient temperature using a SPE Dry (20 to 40 L/min) or TurboVap (1.0 bar) for 45 mins. Upon dryness, reconstitute with ethyl acetate (200 µL) and transfer to a high recovery glass vial.

Biotage®

GC Conditions

Instrument:	Agilent 7890A with QuickSwap
Column:	Agilent J&W DB-5 30 m x 0.25 mm ID x 0.25 µm
Carrier	Helium 1.2 mL/min
Inlet:	175 °C, Splitless, purge on at 2.0 minute, 50 mL/min
Injection:	1 µL
Wash Solvents:	1: Acetone 2: Ethyl acetate
Oven:	Initial temperature 60 °C, hold for 2 mins
	Ramp 80 °C/min to 200 °C, hold for 6.25 mins
	Ramp 100 °C/min to 280 °C
Post Run:	325 °C, Back-flush 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

SIM Group	Analyte	Quantifier Ion	Quantifier Ion 1	Quantifier Ion 2
1	PCP-D5	205	96	
1	PCP	200	91	130

Results

The optimized protocol demonstrated recoveries of greater than 88% from three unique urine donors, with corresponding RSDs of less than 8% (n=7), illustrated in **Figure 2**. **Figure 3**. shows the calibration line formed from the extraction of spiked urine at levels of 10 ng /mL up to 1000 ng/mL.

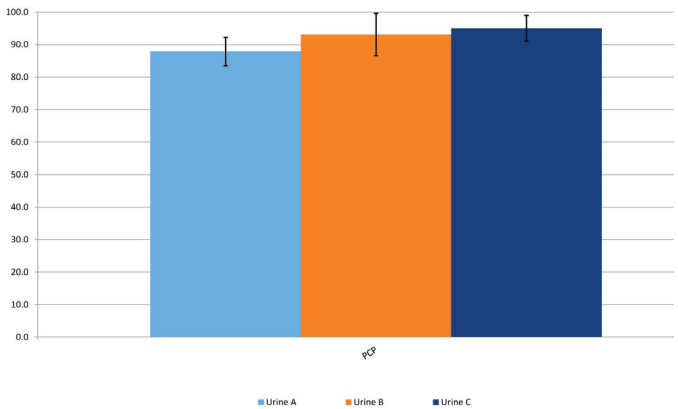

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol on 1 mL C column.

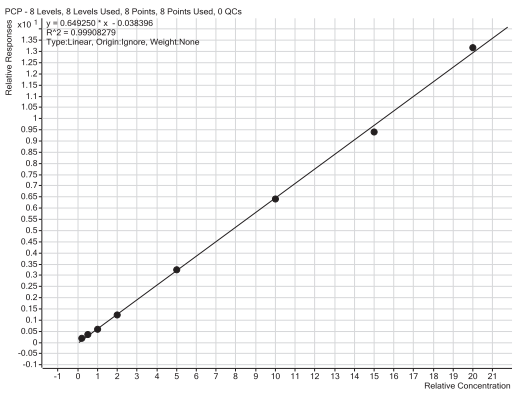

Figure 3. Calibration line for extracted levels of spiked urine, using ISOLUTE® SLE+ protocol described. The coefficient of determination was determined to be greater than 0.999 across concentration values 10, 25, 50, 100, 250, 500, 750 and 1000 ng/mL. The lower limit of quantitation (LLOQ) was determined to be 10 ng/mL.

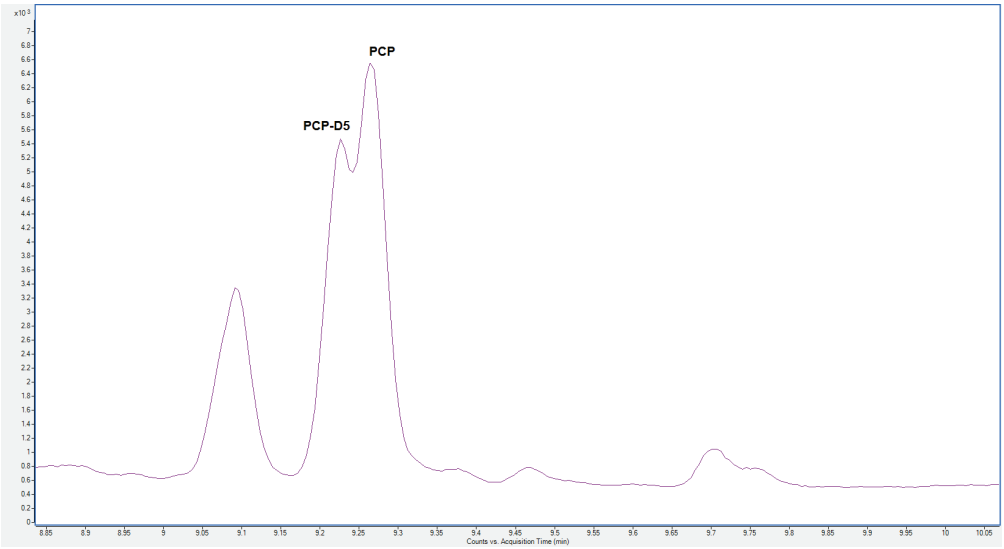

Figure 4. GC/MS chromatography for urine spiked with both PCP and PCP-D5 at 100 ng/mL. TIC acquisition of m/z 205, 96, 200, 91, 130

Additional Information

0.5% ammonium hydroxide (aq) is prepared by adding 0.5 mL concentrated ammonium hydroxide (commercially available 28%–30% NH3 in H2O) to 99.5 mL HPLC grade water.

0.2M methanolic HCl is prepared by adding 200 µL concentrated HCl (commercially available 37%) to 11.8 mL HPLC grade methanol.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1mL Supported Liquid Extraction Column	30
820-0140-CG	ISOLUTE SLE+ 1mL Supported Liquid Extraction Column (Tabless)	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	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® LV, 100/120V	1
C103199	TurboVap® LV, 220/240V	1

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