Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Intercept® Oral Fluid Drug Test Kit Prior to GC/MS Analysis

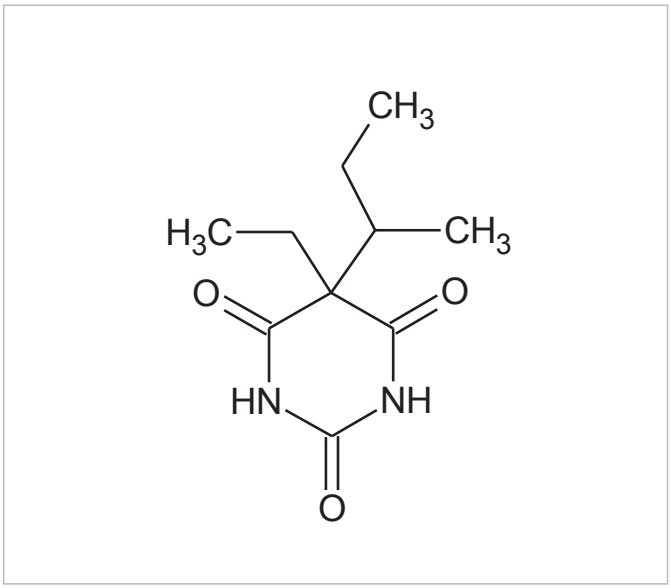

Figure 1. Structure of Butabarbital

Introduction

This application note describes the extraction of Butalbarbital, Butabarbital, Amobarbital, Pentobarbital, Secobarbital, Hexobarbital and Phenobarbital from oral fluid matrix collected using the Intercept Oral Fluid Drug Test Kit (Orasure Technologies), prior to GC/MS analysis.

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

This application note describes an effective and efficient ISOLUTE SLE+ protocol optimized for 400 µL and 1 mL sample capacity formats. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 90% with RSDs lower than 7% for all analytes.

Analytes

Butalbarbital, Butabarbital, Amobarbital, Pentobarbital, Secobarbital, Hexobarbital and Phenobarbital

Sample Preparation Procedure

Sample pre-treatment:	Following collection, add 0.5% ammonium hydroxide (aq) (10 µL) to each collection device (see additional information).
Format:	**ISOLUTE® SLE+ 400 µL sample volume columns, part number 820-0055-B**
Sample loading:	Load the pre-treated oral fluid (300 µL) 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 methyl-*tert*-butyl-ether (MTBE) (1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.
Format:	**ISOLUTE® SLE+ 1 mL sample volume columns, part number 820-0140-C**
Sample loading:	Load the complete contents of the pre-treated oral fluid device 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 MTBE (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.
Post Elution & Reconstitution:	Dry the extract in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap (1.0 bar at 40 °C for 40 mins). Upon dryness, reconstitute with 80 µL ethyl acetate and 20 µL TMAH (trimethylanilinium hydroxide, 0.2M) and vortex for 20 seconds. Transfer to a high recovery glass vial.

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:	150 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection:	1 µL
Wash solvents:	Ethyl acetate
Oven:	Initial temperature 120 °C, hold for 1 minute
	Ramp 12 °C/min to 192 °C,
	Ramp 120 °C/min to 330 °C, hold for 0.85 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

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Butalbarbital	196	195	181
1	Butabarbital	169	184	211
2	Amobarbital	169	184	225
2	Pentobarbital	169	184	225
3	Secobarbital	196	195	181
4	Hexobarbital	235	81	169
4	Phenobarbital	232	146	175

Results

The optimized ISOLUTE®SLE+ protocol demonstrated analyte recoveries ranging from 91–104% as shown in **Figure 2**. RSDs were below 7% for all analytes.

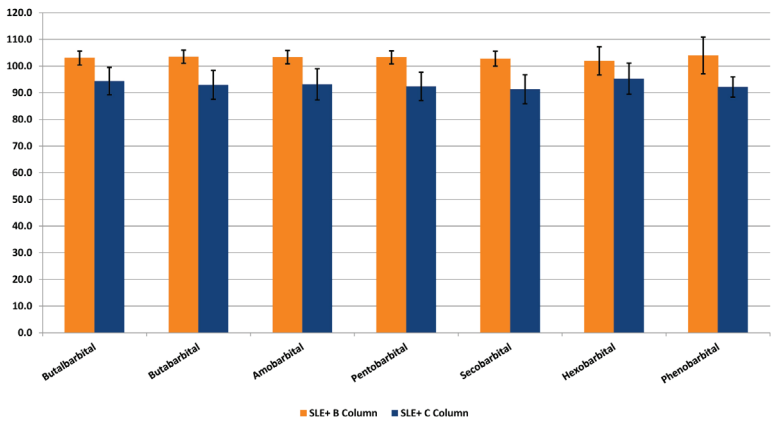

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol.

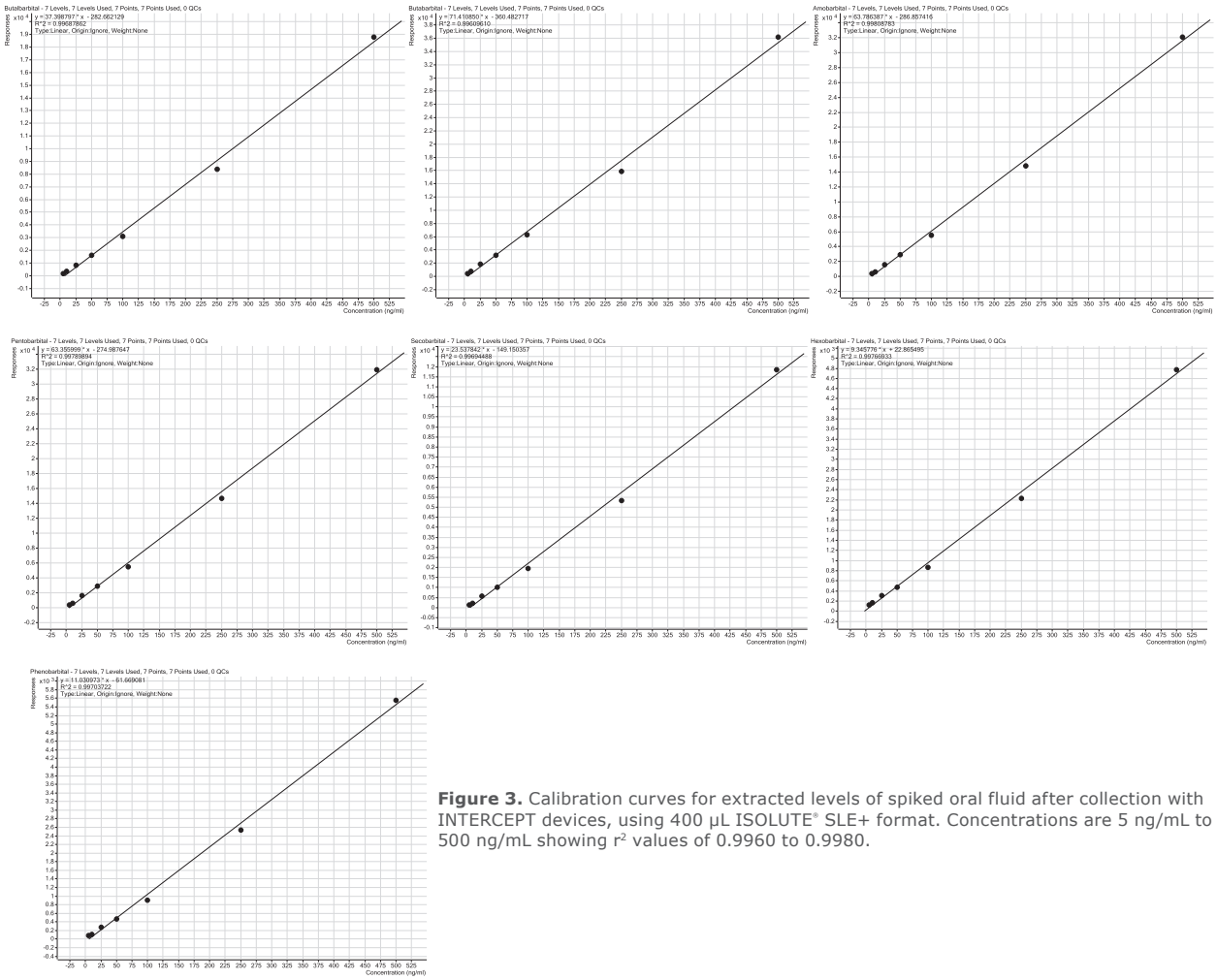

Figure 3. Calibration curves for extracted levels of spiked oral fluid after collection with INTERCEPT devices, using 400 µL ISOLUTE® SLE+ format. Concentrations are 5 ng/mL to 500 ng/mL showing r^2 values of 0.9960 to 0.9980.

Table 2. Lower Limits of Quantitation (LLOQ) using INTERCEPT devices prior to optimized ISOLUTE® SLE+ procedure

Analyte	SLE+ B Format LLOQ (ng/mL)	SLE+ C Format LLOQ (ng/mL)
Butalbarbital	25	10
Butabarbital	10	4
Amobarbital	5	2
Pentobarbital	10	4
Secobarbital	10	4
Hexobarbital	10	4
Phenobarbital	25	10

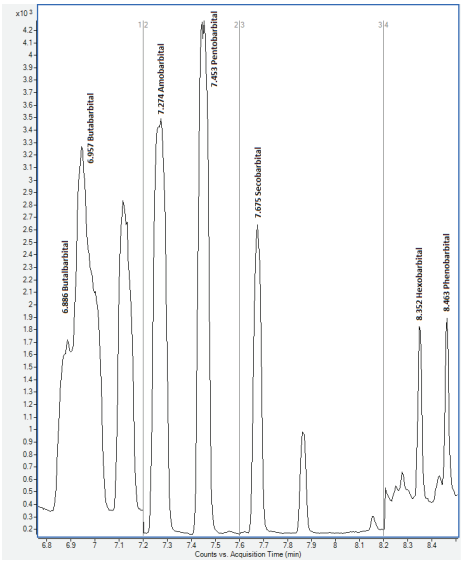

Figure 4. GC/MS chromatography for INTERCEPT® collected oral fluid spiked at 40 ng/mL. Early eluting peaks are visually poor in shape due to the acquisition of 6 SIMs but mass spectrometry is able to determine the quantification m/z with no contribution or interference from closely eluting analytes.

Additional Information

1. 0.5 % ammonium hydroxide is prepared from concentrated stock (28–30%) by adding 50 µL to 10 mL HPLC grade water.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Columns	50
820-0140-C	ISOLUTE® SLE+ 1 mL Supported Liquid Extraction Columns	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold 4	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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