Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Oral-Eze® Collection Device Prior to GC/MS Analysis

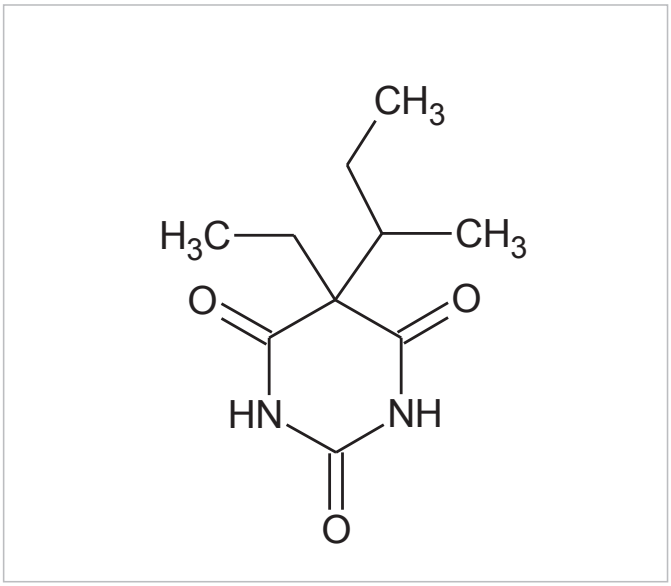

Figure 1. Structure of Butabarbital

Introduction

This application note describes the extraction of Butalbarbital, Butabarbital, Amobarbital, Pentobarbital, Secobarbital, Hexobarbital and Phenobarbital from oral fluid matrix collected using the Oral-Eze® collection devices, 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 the 400 µL sample capacity column format. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 95% with RSDs lower than 9% for all analytes.

Analytes

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

Sample Preparation Procedure

Sample pre-treatment:	Following collection, add 4% ammonium hydroxide (aq) (10 µL) to each collection device (see notes for buffer preparation instructions).
Format:	**ISOLUTE® SLE+ 400 µL Sample Volume Columns, part number 820-0055-B**
Sample loading:	Load 300 µL of the pre-treated oral fluid 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 dichloromethane/isopropanol (95/5, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA (95/5, v/v, 1 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.

Biotage®

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 95–101% as shown in **Figure 2**. RSDs were below 9% for all analytes.

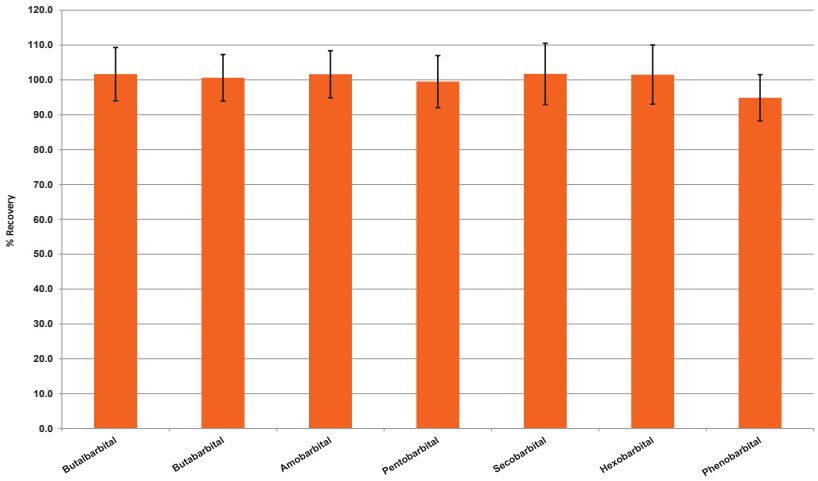

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol, at a concentration of 500 ng/mL of oral fluid.

Additional Information

If a non-chlorinated elution solvent is preferred, MTBE (methyl-tert-butyl-ether) is a suitable substitute solvent.

4% ammonium hydroxide is prepared from concentrated stock (28–30%) by adding 200 µL to 5 mL HPLC grade water.

Pre-treating to basic conditions accommodates extraction of multiple basic drugs of abuse alongside the barbiturates class of drugs, in a single assay, if required.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Columns	50
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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