Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ After Collection with the NeoSal™ Collection Device Prior to GC/MS Analysis

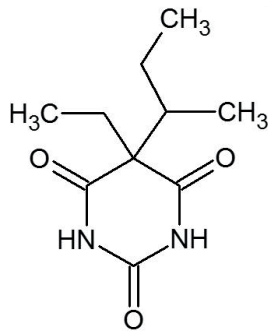

Figure 1. Structure of Butabarbital.

Introduction

This application note describes the extraction of barbiturates from oral fluid matrix collected using the NeoSal 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 bioanalytical 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 1 mL sample volumes. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 88% with RSDs lower than 6% for all analytes.

Analytes

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

Sample Preparation Procedure

Format:

ISOLUTE SLE+ 1 mL Sample Volume Column, Part Number 820-0140-C

Sample Pre-treatment:

Following saliva collection, add 18 µL concentrated ammonium hydroxide to the matrix buffer contained in each collection device.

Sample Loading:

Load 1 mL of the pre-treated oral fluid device buffer matrix 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 2.5 mL of DCM/IPA (95/5, v/v) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA (95/5, v/v, 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:

Dry the extract in a stream of air or nitrogen using a TurboVap® (10 psi at 40 °C for 40 mins).

Reconstitute the extracts with 250 µL ethyl acetate and vortex for 20 seconds before transferring to high recovery GC vials. Dry the extract in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap® (10 psi 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.

Biotage®

GC Conditions

Instrument
Agilent 7890A with QuickSwap

Column
Agilent J&W DB-5ms, 30 m x 0.25 mm ID x 0.25 µm

Carrier
Helium 1.2 mL/min (constant flow)

Inlet
260 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection
1 µL

Wash Solvents
Methanol and ethyl acetate

Oven
Initial temperature 120 °C, hold for 1 minute

Ramp 12 °C/min to 192 °C

Ramp 100 °C/min to 330 °C, hold for 0.5 minutes

Post Run
Backflush for 1.6 minutes (2 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
3	Pentobarbital	169	184	225
4	Secobarbital	196	195	181
5	Hexobarbital	235	81	169
6	Phenobarbital	232	146	175

Results

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 102–107% as shown in **Figure 2**. RSDs were below 6% for all analytes.

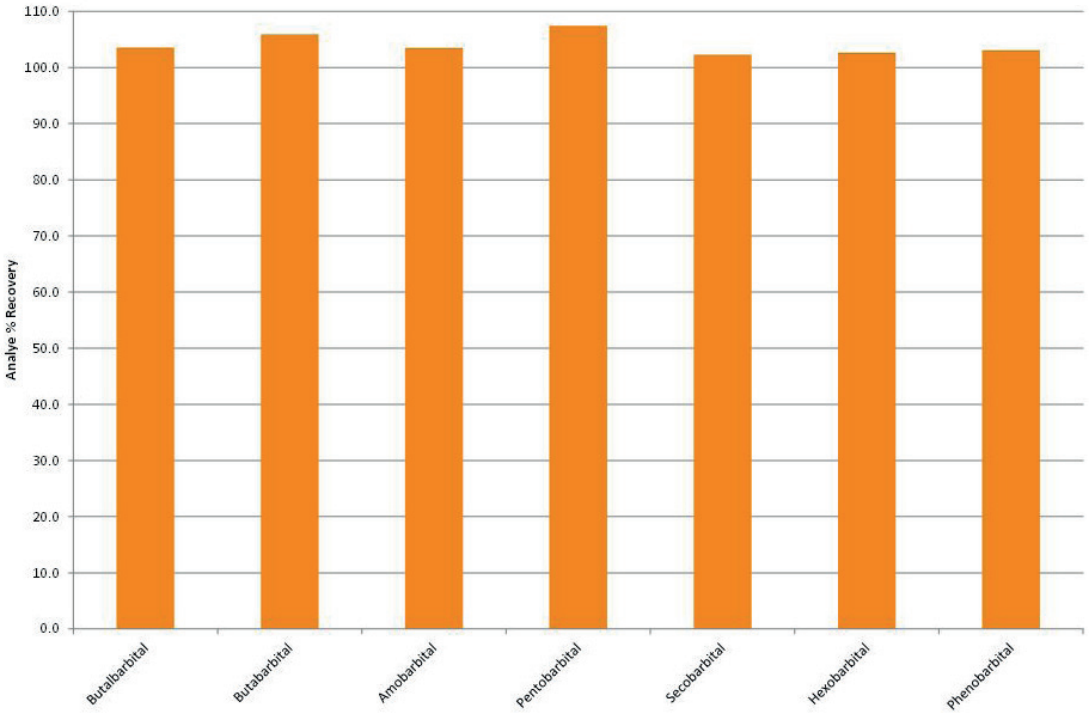

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol.

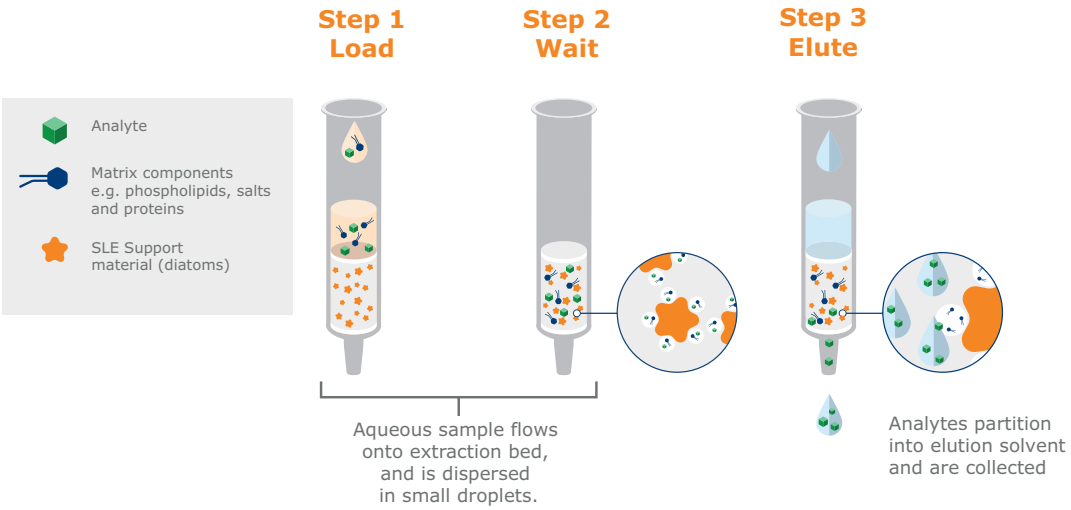

Figure 3. Typical ISOLUTE® SLE+ procedure.

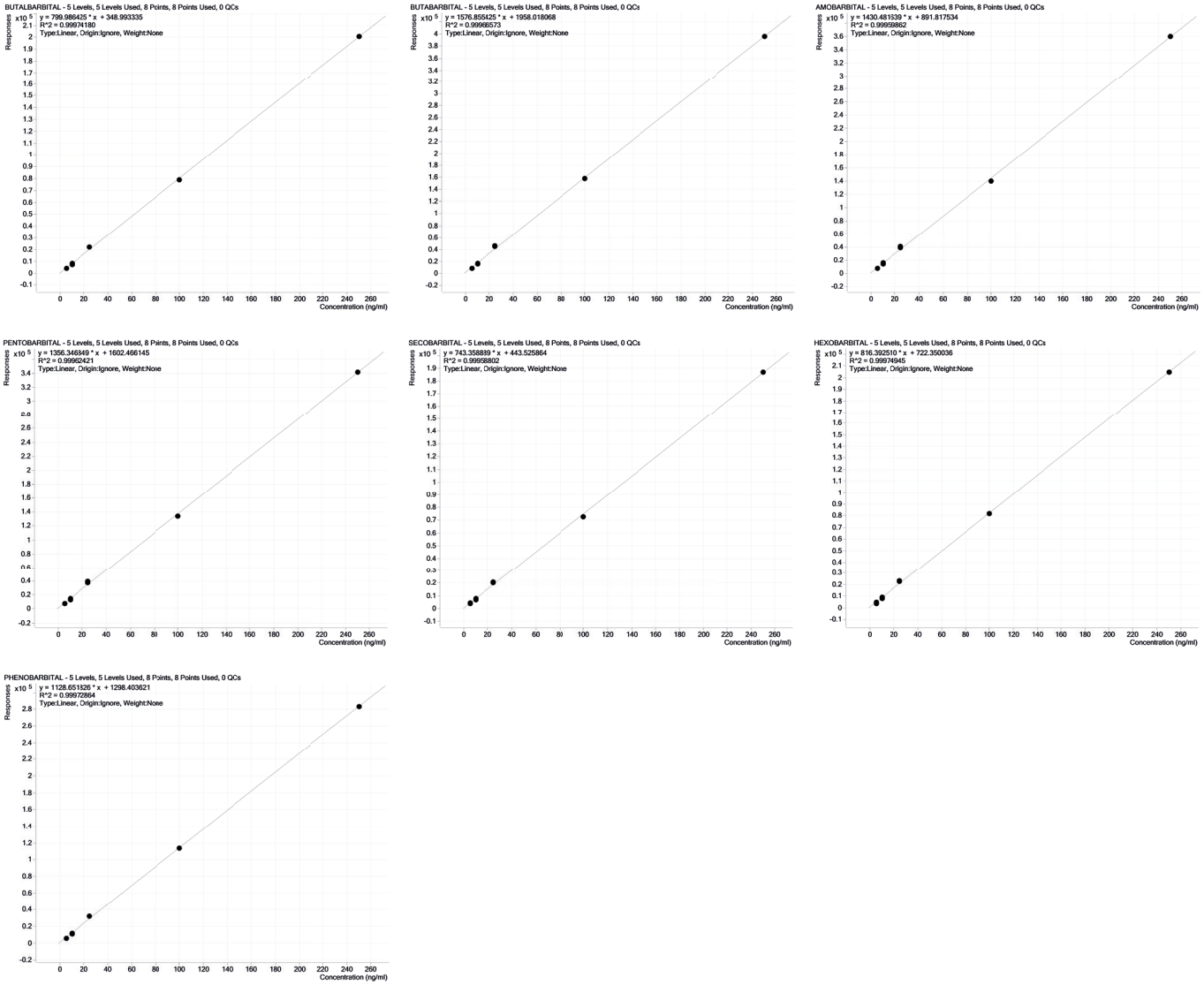

Figure 4. Calibration curves for extracted levels of spiked oral fluid after collection with NeoSal devices, using 1 mL ISOLUTE ®SLE+ format. Analyte concentrations spiked into each device are 5, 10, 25, 100 and 250 ng/mL showing r^2 values of 0.995 to 0.999.

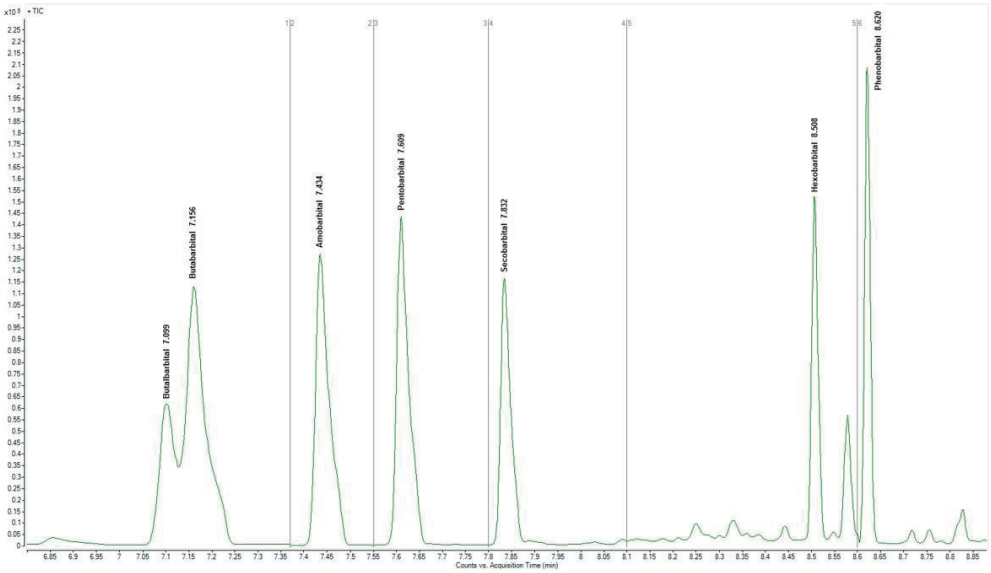

Figure 5. GC/MS chromatography for NeoSal collected oral fluid spiked at 250 ng/mL. Early eluting peaks are visually poor in shape due to the acquisition of 6 SIMs but mass spectrometry is able to determine the quantification m/z with no contribution or interference.

Reagent Preparation

Concentrated ammonium hydroxide: Concentrated stock used to modify pH prior to extraction is commercially available (28–30%).

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

Drug Analyte	LLOQ (ng/mL)
Butalbarbital	5
Butabarbital	5
Amobarbital	5
Pentobarbital	5
Secobarbital	5
Hexobarbital	5
Phenobarbital	5

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry 96 Sample Concentrator System 220/240V	1
SD-9600-DHS-NA	Biotage® SPE Dry 96 Sample Concentrator System 100/120V	1
C103199	TurboVap® LV Evaporator	1

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