

Extraction of Barbiturates from Oral Fluid using ISOLUTE® SLE+ after Collection with the Quantisal™ Collection Device prior to GC/MS Analysis

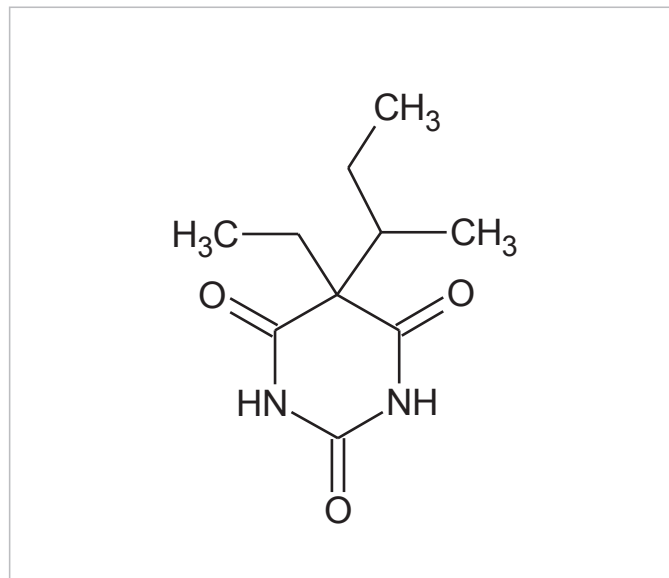


Figure 1. Structure of Butabarbital

Introduction

This application note describes the extraction of Butalbarbital, Butabarbital, Amobarbital, Pentobarbital, Secobarbital, Hexobarbital and Phenobarbital from oral fluid matrix collected using Quantisal™ collection devices (Immunoanalysis), 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 400 µL and 1 mL sample capacity formats. 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

Sample pre-treatment:	Following collection, add concentrated ammonium hydroxide (15 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 (400 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- <i>tert</i> -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 pre-treated oral fluid (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 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	1 st Qual Ion	2 nd 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

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 88–106% as shown in **Figure 2**. RSDs were below 6% for all analytes.

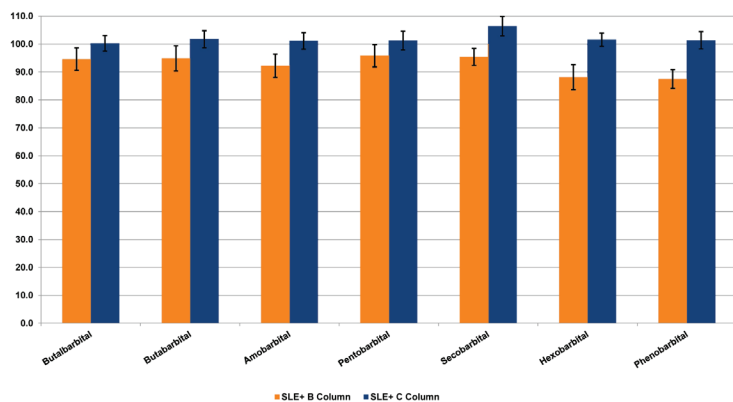


Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol.

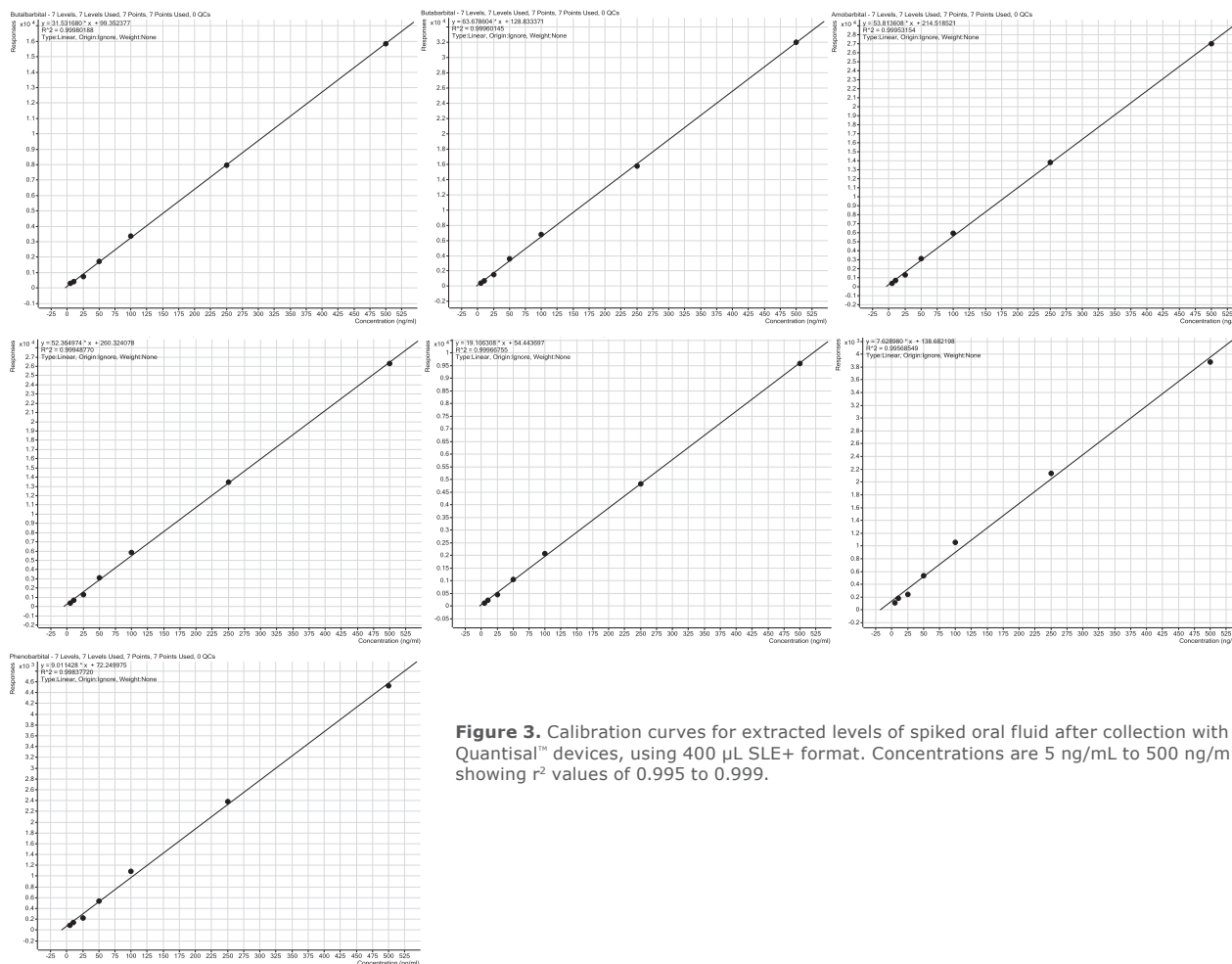


Figure 3. Calibration curves for extracted levels of spiked oral fluid after collection with Quantisal™ devices, using 400 µL SLE+ format. Concentrations are 5 ng/mL to 500 ng/mL showing r^2 values of 0.995 to 0.999.

Table 2. Lower Limits of Quantitation (LLOQ) using Quantisal 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	10	4
Pentobarbital	10	4
Secobarbital	25	10
Hexobarbital	10	4
Phenobarbital	10	4

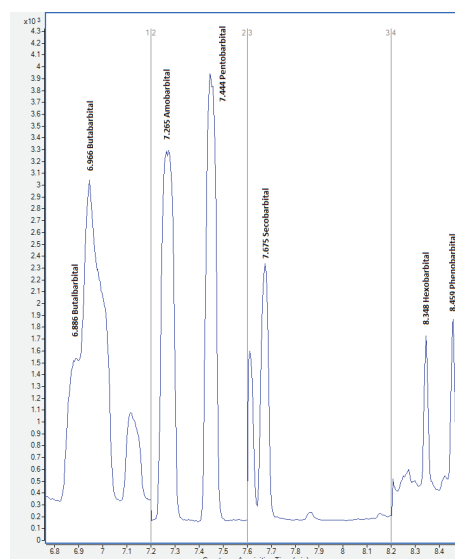


Figure 4. GC/MS chromatography for Quantisal™ collected oral fluid spiked at 40 ng/mL. Early eluting peaks are visually poor in shape due to the acquisition of 6 SIMs but mass spectrometry is able to determine the quantification m/z with no contribution or interference from closely eluting analytes.

Additional Information

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

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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