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Extraction of Barbiturates from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

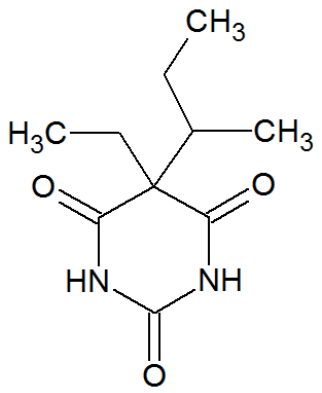

Figure 1. Structure of Butabarbital

Introduction

This application note describes the extraction of barbiturate compounds from whole blood, prior to GC/MS analysis. This protocol also allows the simultaneous extraction of various other drugs of abuse classes: amphetamines, benzodiazepines, cocaine and opiates.

ISOLUTE® SLE+ columns with 1 mL sample capacity are used to extract whole blood samples following a straightforward sample dilution. No protein precipitation or other pre-treatment is required prior to sample loading. The sample preparation procedure delivers clean extracts, good recoveries and RSD values and LLOQs from 20 ng/mL (analyte dependant).

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

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

Sample Preparation Procedure

Format:

ISOLUTE® SLE+ 1mL Sample Volume column, part number 820-0140-C

Sample Pre-treatment

To 1 mL of whole blood, add 10 µL of ISTD (total 100 ng/mL). Allow to equilibrate and add 1 mL of 1% ammonium hydroxide (aq). Vortex.

Sample Loading

Load 750 µL of the pre-treated whole blood 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* (DCM, 2.5 mL) and allow to flow under gravity for 5 minutes. Collect in an appropriate glass tube.

Apply a second aliquot of DCM* (2.5 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure (5-10 seconds) to pull through any remaining extraction solvent into the collection vessel.

* Note that MTBE can be used as an alternative extraction solvent if a non-chlorinated option is required. MTBE also suitable for extraction of other analyte classes (amphetamines, benzodiazepines, opiates). If simultaneous extraction of cocaine and metabolites is required, DCM should be used as extraction solvent.

Post Elution and Reconstitution

Evaporate the extract in a stream of air or nitrogen using a TurboVap® LV (40 °C, 20 to 40 L/min).

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

Reconstitute extracts with ethyl acetate (80 µL) and TMAH (trimethylanilinum hydroxide) (20 µL) and vortex.

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 (constant flow)

Inlet
150 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection
1 µL

Wash Solvents
Acetone & 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

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	Qual Ion 1	Qual Ion 2
1	Butalbarbital	196	181	195
1	Butabarbital	169	184	211
2	Amobarbital	169	184	226
3	Pentobarbital	169	112	184
4	Secobarbital	196	181	195
5	Hexobarbital	235	79	169
5	Phenobarbital	232	117	175

Results

Blank whole blood was spiked at 100 ng/mL for recovery testing; the typical recovery data is shown in **Figure 2.** Both MTBE and DCM protocols gave reproducible data with RSD values <10%.

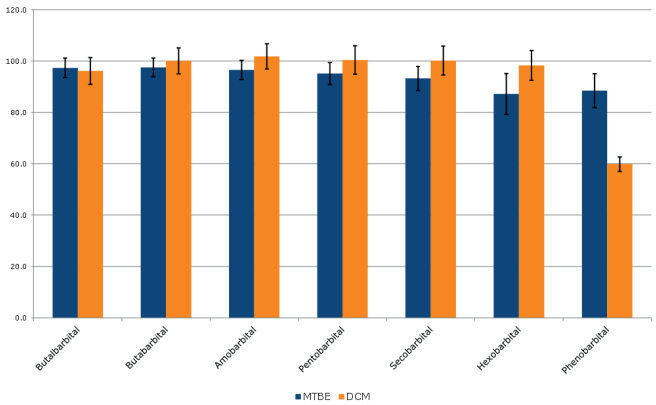

Figure 2. Typical opiate recoveries using MTBE or DCM as extraction solvent.

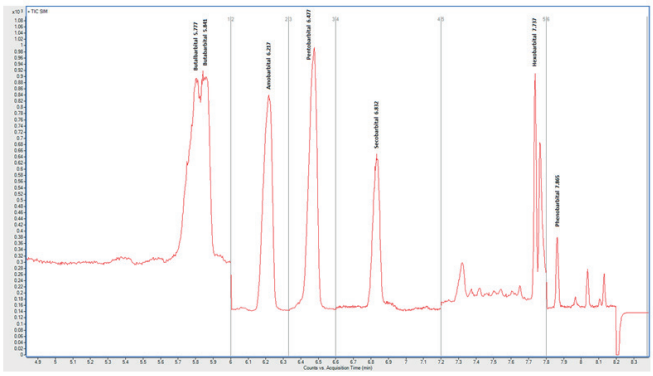

Figure 3. Total Ion Chromatogram of barbiturates at 50 ng/mL using the DCM extraction protocol.

Calibration Curves

Whole blood was spiked prior to extraction, at concentrations of 10, 20, 50, 75, 100, 200 and 500 ng/mL for each analyte to create calibration curves. Morphine-D3 and 6-MAM-D3 were spiked at 100 ng/mL for each level. The curves are shown in **Figure 4**.

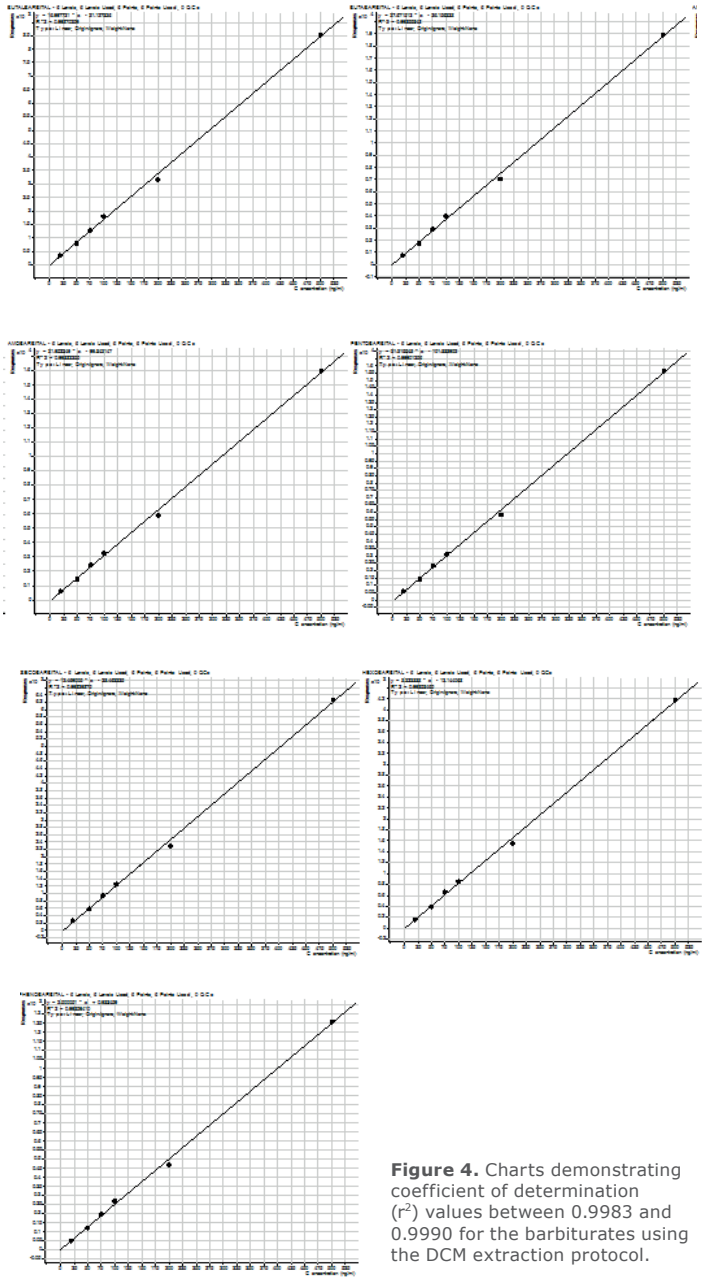

Figure 4. Charts demonstrating coefficient of determination (r^2) values between 0.9983 and 0.9990 for the barbiturates using the DCM extraction protocol.

Table 3.
Lower Limits of Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

Drug Analyte	LLOQ (ng/mL) DCM Extraction	LLOQ (ng/mL) MTBE Extraction
Butalbarbital	50	50
Butabarbital	20	20
Amobarbital	20	20
Pentobarbital	20	50
Secobarbital	50	50
Hexobarbital	20	20
Phenobarbital	100	20

Additional Notes

Solvents and reagent preparation:

- All solvents were HPLC grade.
- 1% ammonium hydroxide (aq): Add concentrated ammonium hydroxide (28–30%) (1 mL) to HPLC grade water (99 mL).

Column loading: ISOLUTE® SLE+ columns are underloaded (750 µL sample on a 1 mL capacity column) to avoid breakthrough of whole blood matrix.

Non-chlorinated extraction solvent alternative: MTBE can be used as an alternative extraction solvent if a non-chlorinated option is required. MTBE also suitable for extraction of other analyte classes (amphetamines, benzodiazepines, opiates), but cannot be used for cocaine and metabolites.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column*	30
820-0140-CG	ISOLUTE SLE+ 1 mL Sample Volume 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

*ISOLUTE SLE+ 1 mL Sample Volume columns are available in the tabless (or flangeless) format for compatibility with the Biotage® Extrahera™ and other sample processing platforms. Bulk packs are also available, visit www.biotage.com for further information.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

To locate a distributor, please visit our website www.biotage.com

Part Number: AN853.V.1

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