Extraction of Barbiturates From Human Urine Using ISOLUTE® SLE+ Columns with GC-MS Analysis

Introduction

This application note describes the extraction of a range of barbiturates from human urine using ISOLUTE SLE+ supported liquid extraction columns followed by GC-MS analysis.

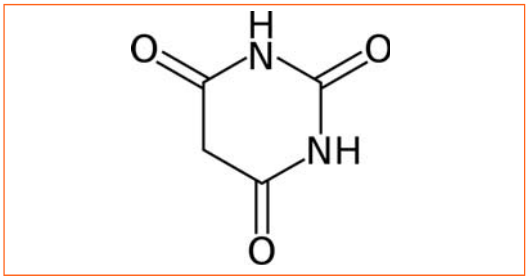

Figure 1. Structure of Barbituric acid, the basic structure of all barbiturates

This method describes the use of ISOLUTE SLE+ supported liquid extraction 1 mL sample volume columns to extract a range of barbiturates from human urine. The analysis of these analytes was carried out by GC-MS. This simplified and efficient extraction method has significant analyte recoveries ranging from 103-108% with LOQs of 10 ng/mL and RSDs <10%.

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 time.

Analytes

Butabarbital, butalbarbital, amobarbital, pentobarbital, secobarbital, hexobarbital, phenobarbital.

ISOLUTE SLE+ procedure

ISOLUTE SLE + 1 mL sample volume column, part number 820-0140-C

Sample Pre-treatment: To 500 µL of urine add 100mM ammonium acetate pH 5 (500 µL, 1:1, v/v).

Sample Load: Load pre-treated sample (1 mL) to column followed by a pulse of vacuum to initiate flow and allow to absorb for five minutes.

Analyte Extraction: Elute with dichloromethane (2.5 mL). Leave to flow under gravity for 5 minutes, then follow with a further aliquot of dichloromethane (2.5 mL) and allow to flow under gravity for a further five minutes, to complete extraction apply a short pulse of vacuum.

Post extraction: Evaporate to dryness at room temperature (80 L/min) and reconstitute in ethyl acetate (200 µL).

Reagents

Ethyl acetate from Fisher Scientific, Loughborough.

Ammonium acetate from Sigma-Aldrich, Gillingham.

Trimethylphenylammonium hydroxide (TMAH) from Sigma-Aldrich, Gillingham.

Application Note

GC Conditions

Carrier: Helium 2 mL min^{-1} (constant flow)

Inlet: Splitless, 150 °C

Injection: In-port flash alkylation: 1 µL sample + 1 µL 0.2M TMAH (trimethylphenylammonium hydroxide) in MeOH

Oven: 120 °C to 290°C at 15 °C min^{-1}, hold 2min

Transfer Line: 280 °C

Mass Spectrometry Conditions

Source temp: 230 °C.

Quadropole temp: 150 °C.

Solvent delay: 7 min.

MSD mode: SIM.

SIM Groups: 1 - 7.4 min to 7.8 min / 2 - 7.8 min to 8.1 min /

3 - 8.1 min to 8.4 min / 4 - 8.4 min to 9.8 min /

5 - 9.8 min to 10.4 min / 6 - 10.4 min to 16.0 min

Table 1. SIM parameters

Scan function	Compound	Quant Ion	1st Qual Ion	2nd Qual Ion	Dwell / ms
1	Butalbarbital	196	181	25	100
1	Butabarbital	169	211	37	100
2	Amobarbital	169	225	29	100
3	Pentobarbital	169	225	33	100
4	Secobarbital	196	181	25	100
5	Hexobarbital	235	81	27	100
6	Phenobarbital	232	175	33	100

Results

Figure 2 shows the mass chromatograms for all the extracted barbiturates spiked at 10 ng/mL. Figure 3 shows average analyte recoveries ranging from 103-108% for barbiturates spiked at 10 ng/mL, with RSDs < 10% (n=3).

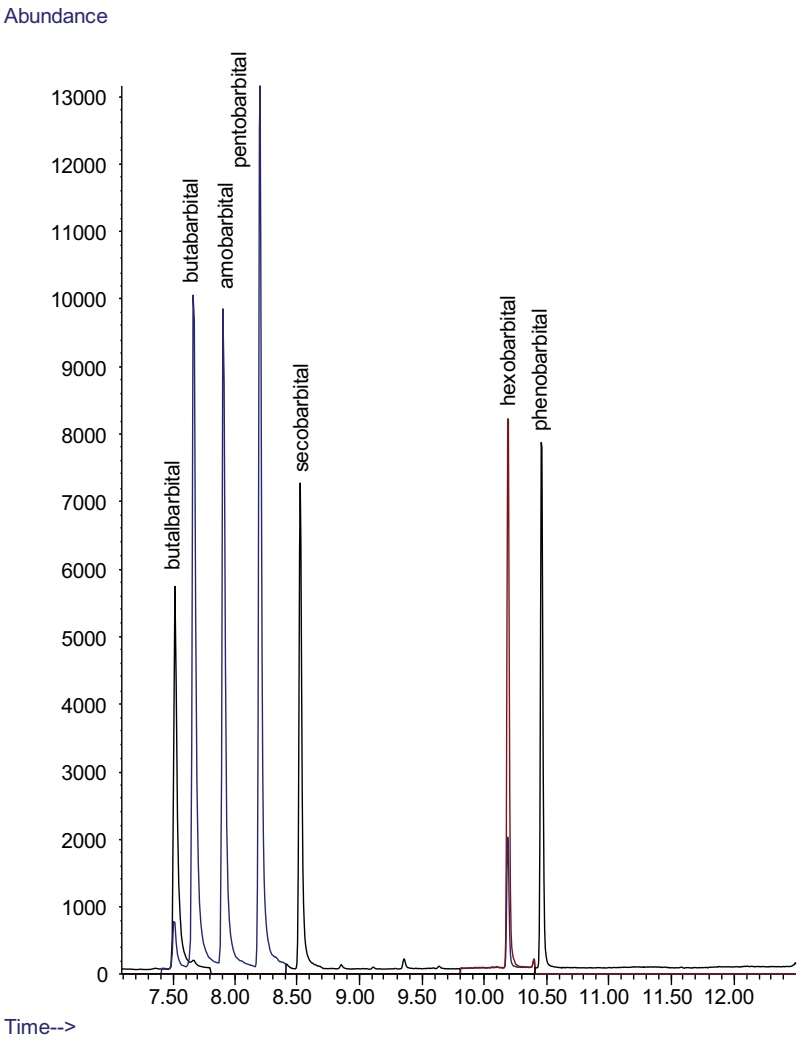

Figure 2. Typical chromatograms for all Barbiturate analytes at 10 ng/mL

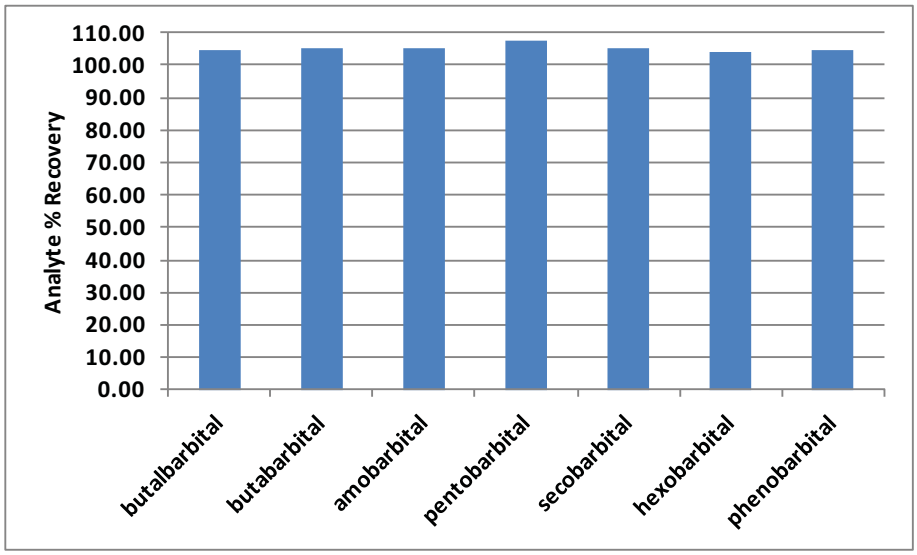

Figure 3. Average analyte recoveries of a range of Barbiturate analytes at 10 ng/mL (n=3).

Ordering information

Part number	Description	Quantity
820-0140-C	ISOLUTE SLE + 1 mL sample	30

NORTH AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
1-pointsupport@biotage.com

EUROPE
Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

Japan
Tel: +81 3 5627 3123
Fax:+81 3 5627 3121
jp_order@biotage.com

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