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

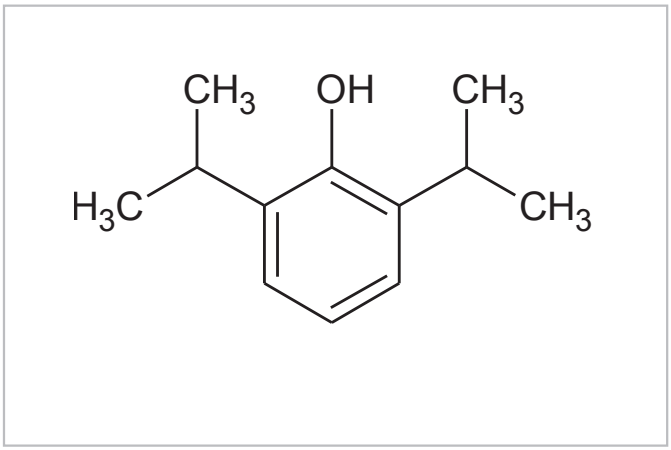

Figure 1. Structure of Propofol

Introduction

This application note describes the extraction of the anaesthetic agent Propofol from whole blood 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 effective and efficient ISOLUTE SLE+ protocols optimized for the 400 µL and 1 mL capacity column format.

Analytes

Propofol & Propofol-d17 as internal standard

Sample Preparation Procedure

Sample Pre-treatment: Spike with ISTD and dilute whole blood 1:1 (v/v) with HPLC-grade water. Mix.

Format: **ISOLUTE SLE+ 400 µL Sample Volume columns, part number 820-0055-B**

Sample loading: Load 0.3 mL of the pre-treated whole blood onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction: Apply 1 mL of methyl *tert*-butyl ether (MTBE) and allow to flow under gravity for 5 minutes into a glass tube containing 0.5% tetrabutylammonium hydroxide (TBAH) in methanol (10 µL).

Apply a further 1 mL aliquot of MTBE and allow to flow for another 5 minutes. Apply vacuum or positive pressure for 10–20 seconds to remove any remaining extraction solvent.

Format: **ISOLUTE SLE+ 1 mL Sample Volume columns, part number 820-0140-C**

Sample loading: Load 0.8 mL 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 2.5 mL of methyl *tert*-butyl ether (MTBE) and allow to flow under gravity for 5 minutes into a glass tube containing 0.5% tetrabutylammonium hydroxide (TBAH) in methanol (25 µL).

Apply a further 2.5 mL aliquot of MTBE and allow to flow for another 5 minutes. Apply vacuum or positive pressure for 10–20 seconds to remove any remaining extraction solvent.

Post Elution and Reconstitution: Dry the extract in a stream of air or nitrogen at ambient temperature using a SPE Dry (20 to 40 L/min) or TurboVap (1.0 bar) for 40 mins.

Upon dryness, reconstitute with 100 µL heptane 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:	275 °C, Splitless, 50 mL/min purge after 60 seconds
Injection Volume:	2 µL
Wash Solvents:	Heptane and Acetone
Oven:	Initial temperature 60 °C, hold for 1 minute Ramp 20 °C/min to 190 °C Ramp 120 °C/min to 330 °C
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	Qualifier Ion
1	Propofol-d17	195	177
1	Propofol	178	163

Results

This optimized SLE+ protocol demonstrated analyte recoveries ranging from 95-104% as shown in **Figure 2**. RSDs were below 6%.

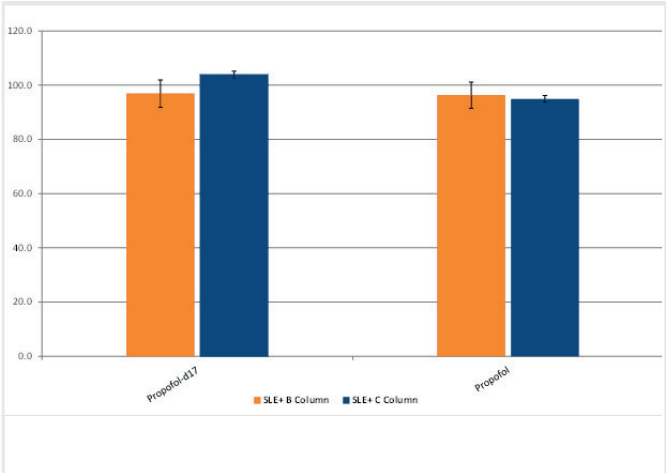

Figure 2. Typical analyte % recoveries for extracted Propofol (n=7) using the ISOLUTE® SLE+ protocols.

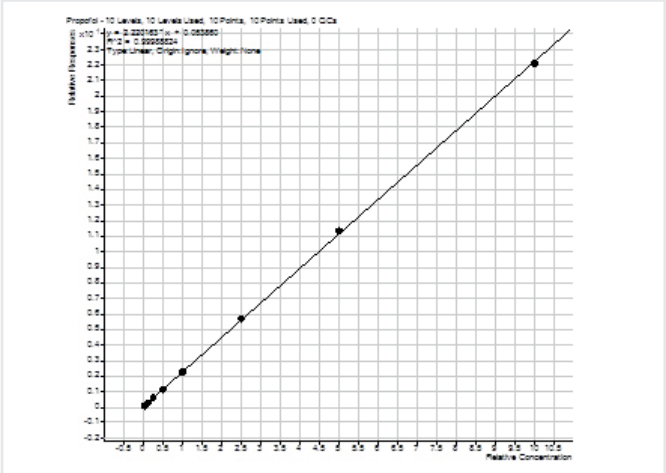

Figure 3. Calibration curve for levels of spiked whole blood extracted using 400 µL SLE+ format, showing r^2 value of 0.999. The spiked concentrations ranged from the Lower Limit Of Quantitation (LLOQ) 2.5 ng/mL up to 1000 ng/mL.

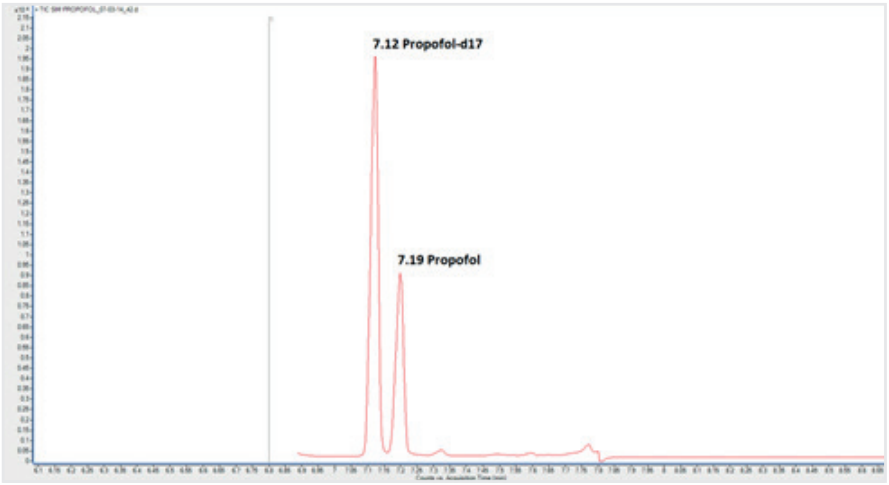

Figure 4. GC/MS chromatography of Propofol-d17 and Propofol, extracted from whole blood at 100 ng/mL and 25 ng/mL respectively.

Extract Cleanliness

An experiment was performed using LC-MS/MS to evaluate the level of residual phospholipids in the final extract. Phospholipids are interfering matrix components which can mask or otherwise interfere with the quantitation of the compounds of interest in LC-MS/MS. Although not directly applicable to GC-MS analysis, it does indicate the level of co-extracted material in extracts prepared using this method. Both formats of ISOLUTE® SLE+ show very clean total ion chromatograms (see **Figure 5**) when the optimized method is used, compared to plasma matrix which has only been precipitated prior to LC-MS/MS. Therefore, this sample preparation method may be appropriate for use in assays where LC-MS/MS, rather than GC-MS, is the endpoint of choice. The presence of TBAH during evaporation prevents ionisation of the analyte, and minimises losses on evaporation.

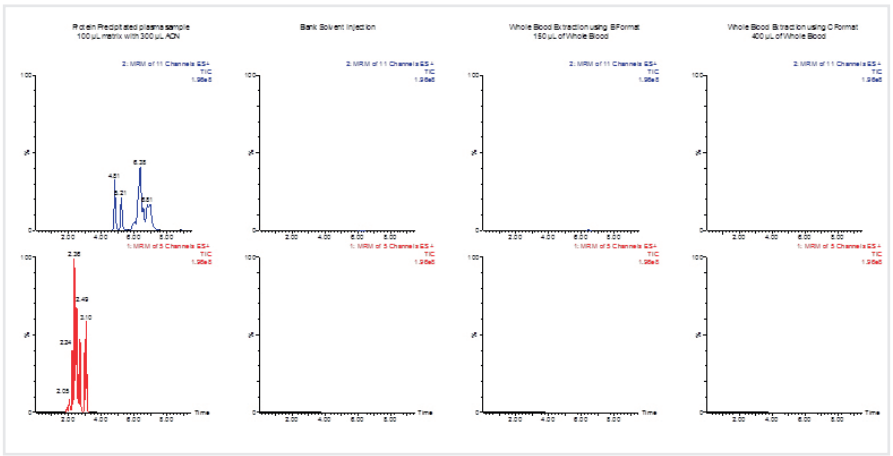

Figure 5. Total Ion Chromatograms demonstrating the content of phospholipids in final extracts and the cleanliness of the optimized method. The abundance is fixed for all charts to the highest point on precipitated plasma matrix

Additional information

0.5% tetrabutylammonium hydroxide (TBAH) in methanol was produced by diluting the commercially available 40% weight in water. Dilute 50 µL of the stock in 4 mL methanol for an 80-fold dilution.

The presence of TBAH during evaporation prevents ionisation of the analyte, and minimises losses on evaporation.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	50
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Columns	30
PPM-48	Biotage® Positive Pressure Manifold 48 Position	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 without racks 100/120V	1
C103199	TurboVap® LV without racks 220/240V	1

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