Extraction of Propofol from Whole Blood Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

Biotage®

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Introduction

Propofol is used in clinical environments to induce states of anesthesia or reduced sensitivity during surgical procedures. However, it also has the potential to be abused for short-term hallucinations and euphoric effects, and with a very small therapeutic range, unanticipated fatal results are possible.

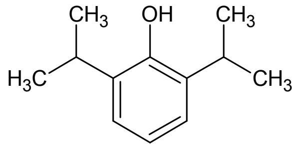

Figure 1. Propofol structure

Experimental

Reagents

Propofol, Propofol-D17 and tetrabutylammonium hydroxide (40% weight in H_2O) were purchased from Sigma-Aldrich (Dorset, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water was used throughout. Blank whole blood was purchased from Sera Labs International (Sussex, UK).

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 2.*)

Columns: ISOLUTE® SLE+ 400 µL capacity 'B' columns; 820-0055-B and 1 mL capacity 'C' columns; 820-0140-C.

Matrix Pre-treatment:

0.5 mL of whole blood was diluted with 0.5 mL water.

Sample Application:

0.3 mL was applied to the ISOLUTE SLE+ B column, or 0.8 mL was applied to the ISOLUTE SLE+ C column.

Analyte Extraction:

B columns: 2 x 1 mL aliquots of MTBE.
C columns: 2 x 2.5 mL aliquots of MTBE.

Each aliquot was allowed to flow under gravity for 5 minutes before applying a pulse of vacuum for 10-20 seconds to completely remove the final aliquot.

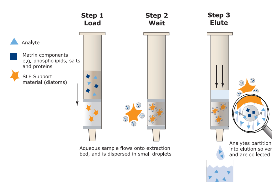

Figure 2. Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.

Post Extraction:

10 µL 0.5% tetrabutylammonium hydroxide in methanol was added to each tube. The extracts were evaporated to dryness without heat. Extracts were reconstituted in 100 µL heptane.

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.).
Column: Phenomenex Zebron ZB-Semivolatiles, 30 m x 0.25 mm **ID x 0.25 µm.**
Carrier Gas: Helium 1.2 mL/min (constant flow).
Inlet: Splitless, purge flow at 50 mL/min at 1 min. Temperature: 275 °C;
Injection volume: **2 µL**
Oven conditions: Initial 60 °C, hold for 1 minute, ramp 20 °C/min to 190 °C, ramp 120 °C/min to 330 °C.
Backflush: 3 void volumes (2.4 mins).
Transfer Line: 280 °C.
MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C.
Quadrupole Temperature: 150 °C.
Monitored Ions: Ionization was performed using EI. Signals were acquired using selected ion monitoring (SIM), as shown in ***Table 1.***

Table 1. *MS acquisition parameters*

SIM Group	Analyte	Target (Quantification) Ion	Qualifier Ion
1	Propofol-D17	195	177
1	Propofol	178	163

Results

It was observed during initial development that Propofol and its deuterated internal standard were suffering extensive losses during evaporation, even at ambient temperatures. This was overcome with the addition of tetrabutylammonium hydroxide (TBAH) to the elution solvent prior to evaporation. Experimental results for this are shown in ***Figure 3***. It is speculated that the basic additive prevents ionization of the –OH group, and therefore losses, in the blowdown stage.

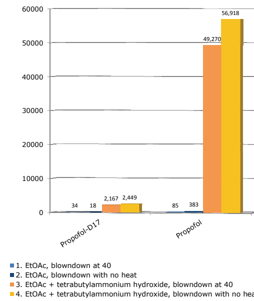

Figure 3. *Peak areas of Propofol and its internal standard following evaporation with and without TBAH at ambient and at 40°C.*

Various concentrations were evaluated and 0.5% TBAH (10 **µL**) was selected to help prevent the analyte losses. Greater concentrations did not improve method performance.

The optimization of analyte recovery from whole blood involved a single pre-treatment, and various water-immiscible elution solvents; heptane, MTBE and ethyl acetate. This was performed on the ISOLUTE SLE+ 400 µL capacity 'B' columns.

Figure 4. shows that although heptane is the preferred reconstitution choice prior to GC injection, it is a poor choice to extract Propofol from the supported liquid extraction columns. MTBE and ethyl acetate are close in terms of recoveries (greater than 94%) and RSDs (below 6%). However, MTBE is the cleaner of the two in terms of post-evaporation extract cleanliness.

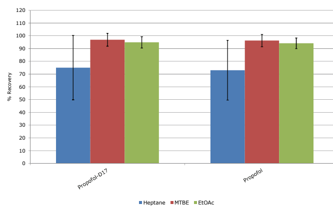

Figure 4. *Recoveries of Propofol and its ISTD. using various elution solvents, with water as a pre-treatment.*

This optimized extraction protocol with MTBE was scaled to a larger sample loading volume on the ISOLUTE SLE+ 1 mL C column. The analyte recovery is 104% and 95% for Propofol-D17 and Propofol respectively (n=7). RSDs are below 2% as shown in **Figure 5.**

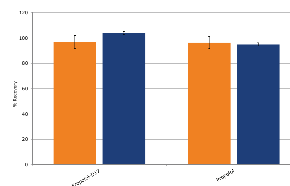

Figure 5. *Recoveries of Propofol and its ISTD. using the optimized method on ISOLUTE SLE+ 400 µL B and 1 mL C columns.*

The GC/MS chromatography of Propofol-D17 and Propofol following extraction from whole blood is shown in **Figure 6.** The spike concentrations are 100 ng/mL and 25 ng/mL respectively.

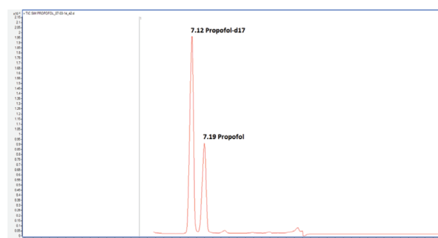

Figure 6. *Propofol chromatography following extraction from whole blood.*

Calibration curves were constructed at Propofol concentrations of 2.5, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/mL with Propofol-D17 fixed at 100 ng/mL on 400 µL capacity 'B' columns. The calibration experiment was performed with MTBE and ethyl acetate as an alternative solvent. ***Figure 7.*** shows the curve from the optimized MTBE method and demonstrates an r^2 value of 0.9998. ***Figure 8.*** shows the curve from an alternative ethyl acetate approach and demonstrates an r^2 value of 0.9984. The ethyl acetate approach allows quantification to 5 ng/mL. The cleaner solvent MTBE, allows quantification down to 2.5 ng/mL.

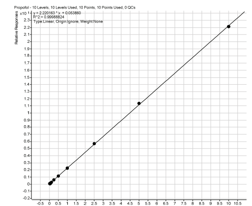

Figure 7. *Calibration curves for Propofol extracted from whole blood with MTBE. Concentration range is of 2.5-1000 ng/mL with a fixed Propofol-D17 concentration of 100 ng/mL.*

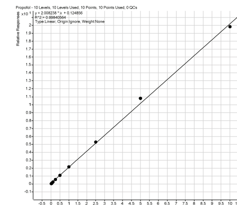

Figure 8. *Calibration curves for Propofol extracted from whole blood with ethyl acetate. Concentration range is of 5-1000 ng/mL with a fixed Propofol-D17 concentration of 100 ng/mL.*

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 ***(Figure 9.)*** 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.

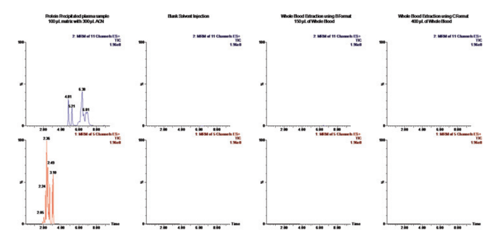

Figure 9. *Total Ion Chromatograms representing residual phospholipid presence in various methods. From left to right (i) Offline plasma precipitation with acetonitrile, (ii) Blank solvent, (iii) Optimized Propofol Whole Blood method on B column and (iv) Optimized Propofol Whole Blood method on C column.*

Conclusion

- This poster demonstrates the suitability of ISOLUTE® SLE+ for the rapid and reliable extraction of Propofol from whole blood, prior to GC/MS analysis.
- The addition of tetrabutylammonium hydroxide following extraction and prior to evaporation was critical to prevent evaporative losses of Propofol.
- MTBE and ethyl acetate both offer high percentage recovery of Propofol. However MTBE offers cleaner extracts and chromatographic baselines which allow for a lower limit of quantitation.
- Analysis of phospholipids using LC-MS/MS, demonstrates the optimized methods on ISOLUTE® SLE+ do not allow breakthrough of these whole blood components into the extract and is applicable and transferrable to LC as an end point.

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