Determination of Propofol in Biological Samples

Application Note

Forensic Toxicology

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Abstract

A method has been developed on the Agilent 220 Quadrupole Ion Trap using EI-MS/MS for the identification and quantification of Propofol in biological samples. A working range of 0.1-2.0 µg/mL shows the method linearity of Propofol. In the analysis of Propofol, the benefits of using GC Quadrupole Ion Trap MS\MS cannot be underestimated, in terms of reducing sample matrix interference, improving signal-to-noise and coupling its high selectivity and sensitivity.

Introduction

Propofol is an anesthetic agent frequently used in the induction of general anesthesia. A typical dose to induce anesthesia is approximately 2–2.5 mg/kg and maintenance of anesthesia requires an infusion of approximately 0.2 mg/kg/hr.

This application note describes an analytical method for the analysis of serum, whole blood, vitreous fluid, urine, or tissue homogenates. A minimum of 1.0 mL of sample is required for analysis.

Propofol is isolated from the biological samples after they are alkalinized to a basic pH followed by extraction with heptane.

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Experimental

Standards and reagents

Reagents - Heptane and Ammonium Hydroxide – Reagent Grade.

Standards - Propofol (P-076 1 mg/mL) and Propofol d-17 (P-077 100 mcg/mL) internal standard were purchased from Cerilliant.

Propofol Stock QC Standard - (1 g - MP Biomedical) was used to prepare a working QC stock standard of 1 mg/mL in methanol.

Sodium Carbonate/Bicarbonate Buffer - pH = 9.8 (Mix 100 g Na_2CO_3 and 50 g $NaHCO_3$ in 1,000 mL de-ionized water. Adjust pH to 9.8 by drop wise addition of 5 N NaOH or 10% phosphoric acid).

Working standards were then made:

Propofol- 10 µg/mL (dilute 0.1 mL of the Cerilliant stock to 10 mL with methanol in a volumetric flask).

Propofol d-17 -10 µg/mL (dilute 1.0 mL of the Cerilliant stock to 10 mL with methanol in a volumetric flask).

Propofol working QC standard -10 µg/mL (dilute 0.1 mL of the MP Biomedical working QC stock to 10 mL with methanol in a volumetric flask).

Store all standards at 2–8 °C, stable for 2 years.

Controls and Calibration Standards

Negative Control - Drug free whole blood obtained from American Red Cross, dilute 1:2 with normal saline (0.9%) store at –20 °C, stable for 1 year.

Low Control - (0.25 µg/mL) 25 µL of working Propofol QC Standard (10 µg/mL) to 975 µL blank blood in a 16 × 100 mm screw cap culture tube.

High Control 1 - (0.75 µg/mL) 75 µL of working Propofol QC Standard (10 µg/mL) to 925 µL blank blood in a 16 × 100 mm screw cap culture tube.

High Control 2 - (1.5 µg/mL) 150 µL of working Propofol QC Standard (10 µg/mL) to 850 µL blank blood in a 16 × 100 mm screw cap culture tube.

Sample Preparation

1. Prepare a calibration curve using the working standard and drug free blood in 16 × 100 mm culture tubes as follows:
   - 0.1 µg/mL-10 µL std. and 990 µL blood
   - 0.2 µg/mL-20 µL std. and 980 µL blood
   - 0.5 µg/mL-50 µL std. and 950 µL blood
   - 1.0 µg/mL-100 µL std. and 900 µL blood
   - 2.0 µg/mL-200 µL std. and 800 µL blood
2. Pipet 1 mL of samples, negative, and positive controls into a labeled 16 × 100 mm screw cap culture tube.
3. Add 100 µL of working internal standard to each tube.
4. Add 2 mL of pH 11.0 buffer. Add 0.5 mL of heptane.
5. Cap and vortex (approximately 15 seconds).
6. Rotate for 15 minutes and centrifuge at 3,000 rpm for a minimum of 10 minutes.
7. Transfer approximately 200 mcl of heptane layer to ALS vials with 300 mcl inserts.
8. Crimp cap and transfer to GC/MS for analysis.

GC/MS Ion Trap Analysis

Column	Agilent DB-5ms Ultra Inert or equivalent 25 m × 200 mm, 0.33 µm
Injection volume	2 µL
Injection mode	Splitless
Inlet temperature	250 °C
Carrier gas	Helium
Column flow	1.3 mL/min
Oven program	70 °C, 1 minute hold 25 °C/min to 310 °C, 4.4 minute hold

Quadrupole Ion Trap MS Conditions

Tune	Auto-tune
Acquisition	EI-MS/MSScan 60–180 da
Solvent delay	5.0 minutes
MS temperatures	Trap 210 °C, manifold 50 °C, transfer line 310 °C

Compound	Rt (min)	Precursor	Quant ion	Qualifiers	Excit volt	Filament	Multiplier	Target
Propofol	5.944	163	121	107/135	0.31 V	50 µA	+50 V	3,000
Propofol d-17	6.007	177	129	113/145	0.37 V	50 µA	+50 V	3,000

Results and Discussion

The following criteria are used to determine the presence and amount of Propofol:

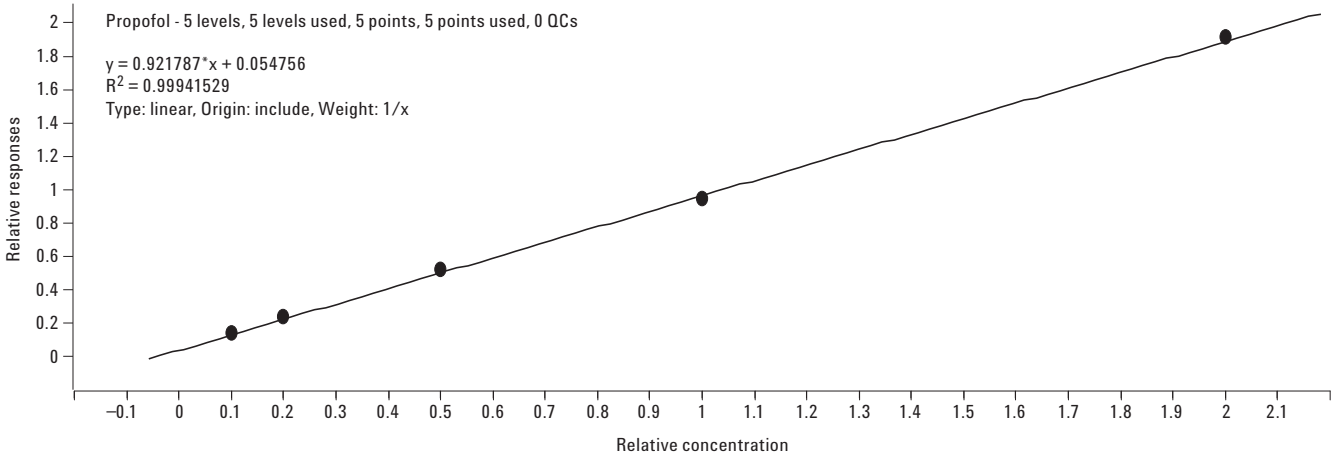
- The chromatography is acceptable (peak resolution, peak symmetry, absence of carryover).
- The selected ions for quantitation and qualification are present.
- Ion ratios are within 20% of the target values determined from the calibration.
- The retention times of the presumed Propofol from the test specimen is within ± 2% of the retention times for the latest calibration.

Method Limits

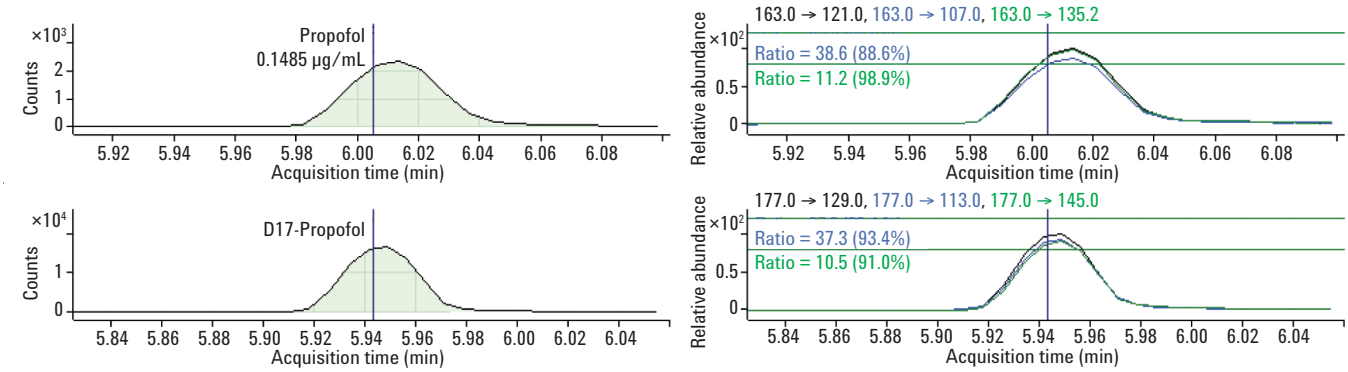
The area of the Propofol and the internal standard quantitative ions are used for quantitative analysis. Quantitation is accomplished by comparison of the relative response of unknowns and controls against a calibration curve produced from the relative responses for each calibrator concentration. The positive controls must be within their target ranges and Propofol must be absent in the negative control.

Linearity	0.1–2.0 µg/mL
Limit of Detection (LOD)	0.05 µg/mL
Limit of Quantitation (LOQ)	0.10 µg/mL
Carryover	No carryover noted after measured concentrations of 2.0 µg/mL
Interferences	None known

Propofol Calibration



Low Standard 0.1 µg/mL



Batch results

Sample							Propofol ...	Propofol Results							Qualifier...		Qualifier...		D17-Propofol (I...		Qualifier...		Qualifier...	
!		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		.10STD	.10STD 2-10-2012 1-24-12 PM.SMS.D	Cal	1	2/10/2012 11:24 AM	0.1000	6.014	5033		0.1485	0.1485	148.5	38.6		11.2		5.949	34101	37.3		10.5	
		.20STD	.20STD 2-10-2012 1-46-03 PM.SMS.D	Cal	2	2/10/2012 11:46 AM	0.2000	6.013	6423		0.2396	0.2396	119.8	43.4		12.4		5.941	26970	38.5		11.1	
		.50STD	.50STD 2-10-2012 2-07-51 PM.SMS.D	Cal	3	2/10/2012 12:07 PM	0.5000	6.012	13168		0.5274	0.5274	105.5	46.8		11.1		5.941	25124	39.7		11.0	
		1.0STD	1.0STD 2-10-2012 2-29-51 PM.SMS.D	Cal	4	2/10/2012 12:29 PM	1.0000	6.005	24141		0.9533	0.9533	95.3	45.0		11.7		5.941	25480	40.0		11.8	
		2.0STD	2.0STD 2-10-2012 2-51-46 PM.SMS.D	Cal	5	2/10/2012 12:51 PM	2.0000	6.005	45371		1.9312	1.9312	96.6	42.5		10.5		5.941	23640	44.2		13.5	
!		NEG	NEG 2-10-2012 3-13-40 PM.SMS.D	Sample		2/10/2012 1:13 PM												5.941	21533	45.7		12.8	
		LOWQC	LOWQC 2-10-2012 3-35-34 PM.SMS.D	Sample		2/10/2012 1:35 PM		6.006	6433		0.2861	0.2861		48.5		12.4		5.941	22624	37.5		11.5	
		HIGH1QC	HIGH1QC 2-10-2012 3-57-28 PM.SMS.D	Sample		2/10/2012 1:57 PM		6.005	18310		0.8419	0.8419		42.7		11.2		5.941	21884	38.2		13.3	
		HIGH2QC	HIGH2QC 2-10-2012 4-19-26 PM.SMS.D	Sample		2/10/2012 2:19 PM		6.004	41553		1.7810	1.7810		42.3		10.4		5.941	23476	37.6		12.0	
!		BLANK	BLANK 2-10-2012 4-41-22 PM.SMS.D	Sample		2/10/2012 2:41 PM																	
		663AM	663AM 2-10-2012 5-03-22 PM.SMS.D	Sample		2/10/2012 3:03 PM		6.014	1460		0.0594	0.0594		46.8		11.3		5.941	24752	33.1		10.4	
		663PM	663PM 2-10-2012 5-25-20 PM.SMS.D	Sample		2/10/2012 3:25 PM		6.004	74428		3.3371	3.3371		42.8		11.6		5.941	22442	38.8		12.4	
!		BLANK	BLANK 2-10-2012 6-09-23 PM.SMS.D	Sample		2/10/2012 4:09 PM																	
		0.05STD	0.05STD 2-10-2012 6-31-19 PM.SMS.D	Sample		2/10/2012 4:31 PM		6.006	1455		0.0721	0.0721		40.7		13.3		5.941	20290	38.2		13.0	

Note tags for outliers and below calibration.

Conclusions

This application note presents a sensitive, selective, and robust analytical method to determine Propofol in biological samples using Propofol d-17 as an internal standard. For the analysis of Propofol, the benefits of GC Quadrupole Ion Trap MS\MS cannot be underestimated. In terms of reducing sample matrix interference, improving signal-to-noise and coupling its high selectivity and sensitivity the GC Quadrupole Ion Trap MS\MS provides a more confidence driven solution for the analysis of Propofol. GC Quadrupole Ion Trap MS\MS analysis has the potential to reduce false positive and negatives as well as providing an additional degree of confidence in the results obtained. Using the optimized method listed above, a fast, targeted GC/MS/MS method can be used to solve the current Propofol analysis problem facing forensic laboratories today. Three positive controls were used in conjunction with a negative control to assure accurate quantification and rule out false negatives in the unknown biological samples. Low µg/mL detection limits were observed in various sample matrices.

References

1. Baselt, R.C., Cravey, RH, Disposition of Toxic Dugs and Chemicals in MAN, 7th Edition, pages 949-951.
2. Hikiji, W., Kudo, K., Usumoto, Y., Tsuji, A., Ikeda, N., A Simple and Sensitive Method for the Determination of Propofol in Human Solid Tissues by Gas Chromatography-Mass Spectrometry, Journal of Analytical Toxicology, Vol. 34, September **2010**.

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For More Information

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