Determination of Pentobarbital in Biological Samples

Application Note

Forensic Toxicology

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Abstract

An analytical method has been developed on the Agilent 220 Quadrupole Ion Trap using CI-MS for the identification and quantification of Pentobarbital in biological samples. A working range of 2-20 µg/mL shows the method linearity of Pentobarbital. For the analysis of Pentobarbital, the benefits of GC Quadrupole Ion Trap MS cannot be underestimated, in terms of reducing sample matrix interference, improving signal to noise, and coupling its high selectivity and sensitivity.

Introduction

Pentobarbital is a barbiturate available for oral, intramuscular, or rectal administration as both a free acid and a sodium salt.

This application note describes a 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.

An internal standard, Pentobarbital d-5 was added to an aliquot of sample. The solution was extracted at pH 6.8 with chloroform/isopropanol. The solvent was concentrated and analyzed by CI-Quadrupole Ion Trap MS.

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Experimental

Standards and reagents

Reagents - Chloroform/Isopropanol (9:1 mixture), Methanol-HPLC grade. Sodium Phosphate dibasic (Na_2HPO_4), Potassium Phosphate, monobasic (KH_2PO_4), 100 mm Phosphate Buffer-pH 6.8 (Dissolve 7.1 g of Na_2HPO_4 and 3.4 g of KH_2PO_4 in 1 L of deionized water, pH to 6.8 with phosphoric acid)

Standard stocks - Pentobarbital (1 mg/mL) and Pentobarbital d-5 (1 mg/mL) were purchased from Cerilliant.

Quality control stock - Pentobarbital (1 mg/mL) was purchased from Alltech/Grace.

Working calibration standard - Pentobarbital – 50 µg/mL (0.5 mL of the 1 mg/mL Cerilliant std. diluted with 9.5 mL deionized water).

Working internal standard - Pentobarbital d-5 at 30 µg/mL (300 µL Cerilliant std. diluted with 9.7 mL deionized water).

Working control standard - Pentobarbital QC -50 µg/mL was prepared from the 1 mg/mL Alltech/Grace std. using 0.5 mL diluted with 9.5 mL deionized water.

Store at 4–8 °C, stable for 1 year.

Controls and Calibration Standards

Negative control - drug free whole blood was obtained from American Red Cross, diluted 1:1 with normal saline (0.9%) store at -20 °C, stable for 1 year.

Low control - (5 µg/mL) 50 µL of working Pentobarbital QC Standard to 450 µL blank blood in a 16 × 100 mm labeled extraction tube.

High control - (15 µg/mL) 150 µL of working Pentobarbital QC Standard to 350 µL blank blood in a 16 × 100 mm labeled extraction tube.

Prepare a calibration curve using the working standard and drug free blood in labeled 16 × 100 mm extraction tubes as follows:

2 µg/mL -20 µL std. and 480 µL drug free blood,
5 µg/mL -50 µL std. and 450 µL drug free blood,
10 µg/mL -100 µL std. and 400 µL drug free blood,
20 µg/mL -200 µL std. and 300 µL drug free blood.

Sample Preparation

1. Pipet 500 µL of each sample and the negative control into labeled 16 x 100 mm extraction tubes.
2. Add 100 µL of working internal standard to samples, and prepared calibrators and controls.
3. Add 500 µL of 100 mM phosphate buffer - pH 6.8, and 10 mL of chloroform:isopropanol (9:1) to each tube.
4. Cap.
5. Rotate mix for 10 minutes then centrifuge at 3,000 rpm for a minimum of 10 minutes.
6. Discard the upper aqueous layer and filter the chloroform (organic) layer into clean 16 x 100 mm disposable test tubes, dry with nitrogen at 40 °C.
7. Reconstitute each dried extract with 200 µL of methanol.
8. Transfer to autosampler vials with inserts, cap and transfer to GCMS for analysis.

GC/MS Ion Trap Analysis

Column	DB-5MS or equivalent 25 m x 200 mm, 0.33 µm
Injection volume	1 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	CI-Scan 40-450 da
CI reagent	Methanol
Solvent delay	4.0 minutes
MS temperatures	Trap 210 °C, Manifold 50 °C, Transfer Line 310 °C
Target	5,000
Filament current	10 µA

Compound	Rt(min)	Quant Ion	Qualifiers
Pentobarbital	7.99	227	157/228
Pentobarbital d-5	8.02	232	162/233

Results and Discussion

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

- 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 pentobarbital from the test specimen is within ±2% of the retention times for the latest calibration.
- The area of the pentobarbital 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 pentobarbital must be absent in the negative control.

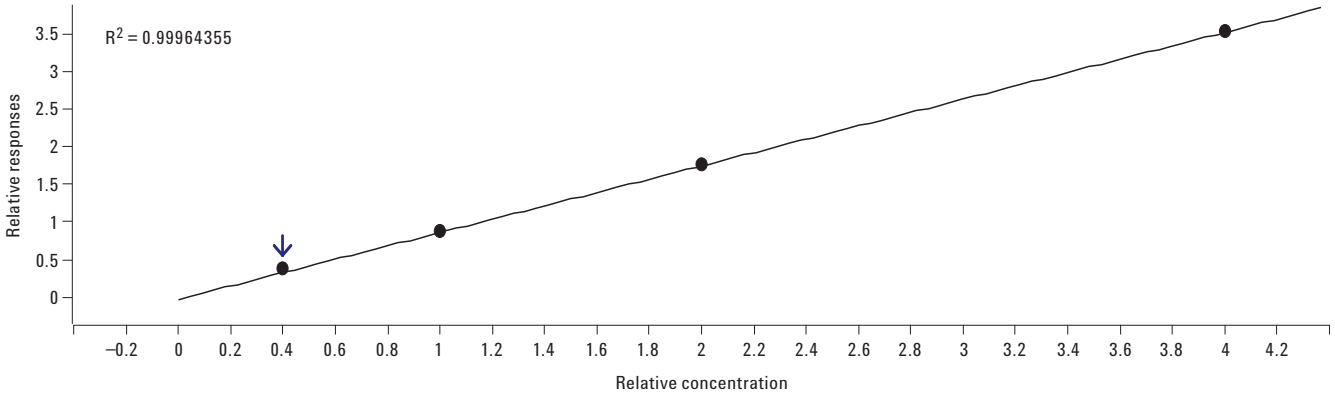
Method Limits

Linearity	2–20 µg/mL
Limit of Detection (LOD)	1 µg/mL
Limit of Quantitation (LOQ)	2 µg/mL

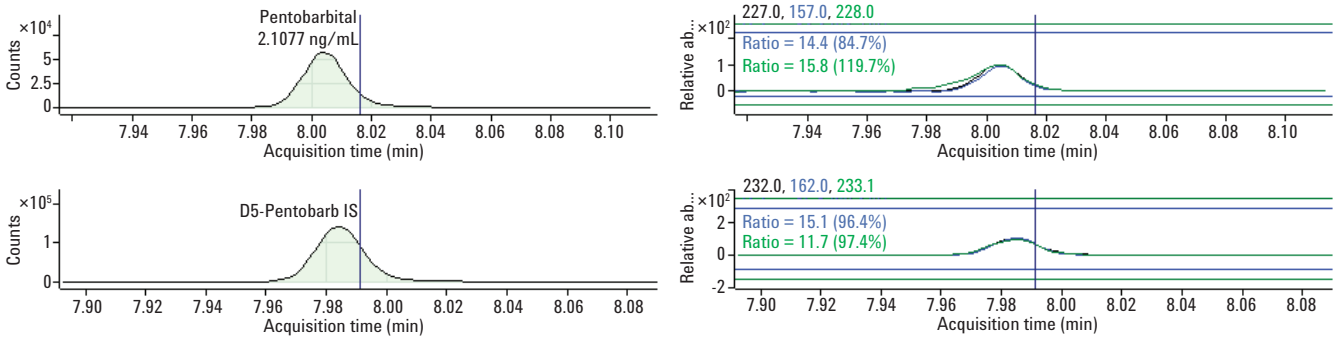
Sample results less than 1 µg/mL are to be reported negative. Results above 1 and less than 2 µg/mL will be reported as less than 2 µg/mL.

Interferences	None known

Pentobarbital Calibration



Low Standard 2.0 ng/mL



Batch results

Sample							PENTOBARBITAL Method	PENTOBARBITAL Results							Qualifier...		Qualifier...		D5-PENTOBA...		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
		2STD	2STD 3-12-2012 9-14-59 PM.SMS.D	Cal	1	3/12/2012 7:15 P...	2.0000	8.004	63421		2.1077	2.1077	105.4	14.4		15.8		7.984	169809	15.1		11.7	
		5STD	5STD 3-12-2012 9-36-48 PM.SMS.D	Cal	2	3/12/2012 7:36 P...	5.0000	8.007	172044		4.9543	4.9543	99.1	17.0		12.9		7.985	195971	15.4		11.7	
		10STD	10STD 3-12-2012 9-58-50 PM.SMS.D	Cal	3	3/12/2012 7:58 P...	10.0000	8.010	321674		9.9800	9.9800	99.8	18.5		12.1		7.988	181894	14.9		12.1	
		20STD	20STD 3-12-2012 10-20-37 PM.SMS.D	Cal	4	3/12/2012 8:20 P...	20.0000	8.016	609199		19.9580	19.9580	99.8	20.7		14.0		7.993	172257	15.8		12.6	
		FTC-04	FTC-04 3-12-2012 8-23-30 PM.SMS.D	Sample		3/12/2012 6:23 P...																		
		BLANK	BLANK 3-12-2012 10-42-37 PM.SMS.D	Sample		3/12/2012 8:42 P...																		
		NEG	NEG 3-12-2012 11-04-38 PM.SMS.D	Sample		3/12/2012 9:04 P...												7.982	176543	15.3		12.1	
		LOW	LOW 3-12-2012 11-26-34 PM.SMS.D	Sample		3/12/2012 9:26 P...		8.004	145762		4.6531	4.6531		15.8		13.2		7.982	176783	15.2		11.1	
		HIGH	HIGH 3-12-2012 11-48-21 PM.SMS.D	Sample		3/12/2012 9:48 P...		8.011	440390		13.6238	13.6238		18.7		12.4		7.988	182421	15.6		12.8	

Note tags for outliers and below calibration

Conclusions

This application note presents a sensitive, selective, and robust analytical method to determine Pentobarbital in biological sam-ples using Pentobarbital d-5 as an internal standard. For the analysis of Pentobarbital, the benefits of GC Quadrupole Ion Trap CI-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 CI-MS provides a more confidence driven solution for the analysis of Pentobarbital. GC Quadrupole Ion Trap CI-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/CI-MS method can be used to solve the current Pentobarbital analysis problem facing forensic laboratories today. Positive controls were used in conjunction with negative controls to assure accurate quantification and rule out false negatives in the unknown biological samples. Low µg/mL detection limits were observed for Pentobarbital in various sample matrices.

References

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For More Information

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