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Applikationsbericht

Quantitative Analysis of Barbiturates in Urine Using UPLC-MS/MS

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For forensic toxicology use only.

Abstract

Analyzing barbiturates by UPLC-MS/MS, the use of a very simple sample dilution step eliminates both the liquid-liquid extraction and post-extraction derivatization steps that are required for GC-MS analysis. The elimination of the extraction step would reduce the time taken to prepare a typical batch of samples by more than 50%.

Benefits

- Elimination of extraction step
- Elimination of derivatization step prior to analysis
- Improved sample throughput

Introduction

Barbiturates act as central nervous system depressants producing effects ranging from mild sedation to general anesthesia. They have largely been replaced by benzodiazepines as prescription medicines, owing to their relatively low therapeutic index and their high potential for dependence. However, it is known that the use of barbiturates is still common in certain regions of Eastern Europe;[1] consequently, their analysis is still of key importance in both forensic analysis and workplace drug testing.

Barbiturates have traditionally been measured by GC.[2,3] The arrival of newer technologies into the modern laboratory, such as UPLC-MS/MS, often leads to an overall requirement to consolidate analytical methods and transfer existing methodologies to the newer platforms. Furthermore, UPLC-MS/MS permits the development of more sensitive techniques.

We report a quantitative method based on simple dilution and UPLC-MS/MS. The method has been verified, and its performance evaluated using authentic samples. Data were compared to results obtained with a traditional method that used liquid-liquid extraction followed by derivatization and analysis by GC-MS.

Experimental

Sample description

Phenobarbital and thiopental were purchased from Sigma Aldrich (Dorset, UK) and dissolved in methanol to 1 mg/mL. All other barbiturates (1 mg/mL) and deuterated internal standards (ISTDs) at 0.1 mg/mL were obtained as certified standard solutions from LGC Standards (Teddington, UK). Deuterated internal standards were not available for all of the barbiturates.

Quality control reference urine samples (Bio-Rad Liquichek Urine Toxicology Control: C2, C3, C4, S1, and S2) were obtained from Bio-Rad Laboratories Ltd (Hemel Hempstead, UK).

Urine samples for method development were obtained from donors at Waters Corporation.

Nineteen samples containing pre-analyzed barbiturates were obtained from Concateno, London, UK.

Sample preparation

Urine, either sample or calibrator, was centrifuged at 13,000 rpm for 5 min, then 50 µL was transferred to a Waters maximum recovery vial and diluted with 950 µL water containing 25 ng of each available ISTD.

UPLC Conditions

System:	ACQUITY UPLC
Column:	ACQUITY BEH C_{18} 2.1 x 100 mm with BEH C_{18} 2.1 x 5 mm Vanguard pre-column
Column temp.:	50 °C
Sample temp.:	5 °C
Injection volume:	15 µL (PLNO)
Strong wash:	0.001% formic acid in acetonitrile
Weak wash:	0.001% formic acid in water

Flow rate:	400 µL/min
Mobile phase A:	0.001% formic acid in water
Mobile phase B:	0.001% formic acid in acetonitrile
Gradient:	Hold at 5% B for 0.5 min, then switch to 27.5% B, hold until 4 min, then switch to 35% B, hold until 5.25 min, then switch to 90% B, hold until 6.25 min, then switch to 5% B.

MS conditions

Mass Spectrometer:	Xevo TQ
Ionization mode:	ESI negative
Capillary voltage:	2.75 kV
Cone voltage:	25 V
Collision energy:	12 eV
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/h
Cone gas:	25 L/h
Acquisition mode:	Multiple reaction monitoring (MRM), as shown in Table 1.

Data management

MassLynx v4.1 incorporating TargetLynx Application Manager

Barbiturate	Precursor ion (*m/z*)	Product ion (*m/z*)	ISTD
Amobarbital	225.1	182.0	Pentobarbital-d5
Butabarbital	211.1	168.0	Phenobarbital-d5
Butalbital	223.0	180.0	Butalbital-d5
Pentobarbital	225.1	182.0	Pentobarbital-d5
Phenobarbital	231.1	188.0	Phenobarbital-d5
Secobarbital	237.1	194.1	Secobarbital-d5
Thiopental	241.1	57.9	Secobarbital-d5
Butalbital-d5	228.0	185.0	
Pentobarbital-d5	230.1	186.9	
Phenobarbital-d5	236.1	193.0	
Secobarbital-d5	242.1	199.1	

Table 1. MRM transitions for analytes and ISTDs.

Results and Discussion

Method verification

The MRM transitions for all of the barbiturates and ISTDs are shown in Table 1. All were monitored using a single transition. Figure 1 shows a chromatogram of a 500 ng/mL barbiturate-spiked urine.

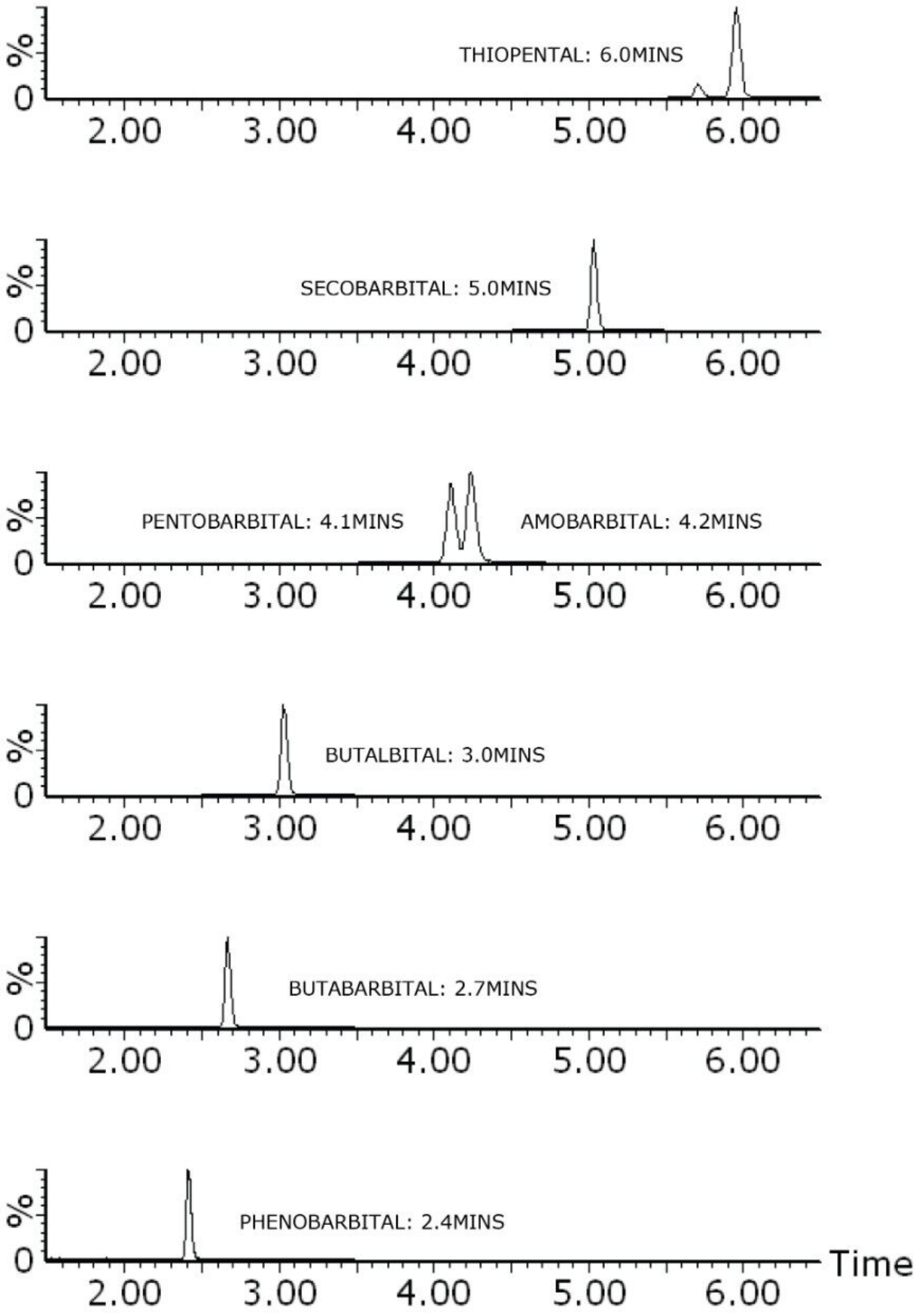

Figure 1. Chromatogram showing barbiturates spiked into urine at 500 ng/mL.

To investigate linearity for all barbiturates, spiked urine calibrators were prepared at 0, 25, 50, 100, 250, 500,

Quantification was performed by integrating the area under the peak for each analyte MRM trace, and refer

2. Pocci R *et al. J Anal Toxicol*. 1992; 16(1): 45-72.

3. Johnsonn LL and Garg U. *Methods Mol Biol.* 2010; 603: 65-74.

Acknowledgments

Concateno plc, London, UK for supplying the anonymized authentic urine samples.

A full validation by the user would be necessary prior to adoption in a laboratory.

Featured Products

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TargetLynx <https://www.waters.com/513791>

MassLynx MS Software <https://www.waters.com/513662>

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