High-throughput Targeted Screening and Definitive method for Barbiturate drugs in Urine using LDTD-MS/MS with ultra-fast analysis at 9 seconds sample to sample

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Overview

Screening methods are used by Toxicology laboratories to obtain a fast response and then confirm positive samples results using a more specific confirmation method. Analysis of barbiturate drugs is challenging since a good separation between the isobaric drugs is necessary. Amobarbital and pentobarbital differ in the position of a methyl group; therefore, a long chromatography is generally required to obtain sufficient resolution to separate the two compounds on a LC-MS/MS system. Ultra-Fast LDTD technology combined with a mass spectrometer equipped with differential mobility spectrometry is evaluated to achieve an accurate, specific and reproducible analytical method.

Two different approaches are presented: one using LDTD-MS/MS method for a fast screening of barbiturates in urine and the second is a fast confirmation method using LDTD-MS/MS with ion mobility to separate and quantify amobarbital and pentobarbital in 9 seconds sample to sample.

LDTD Ionization Source

The LDTD uses a Laser Diode to produce and control heat on the sample support which is a 96 well plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in an APCI region. High efficiency protonation with strong resistance to ionic suppression characterizes the ionization due to the absence of solvent and mobile phase. The LDTD permits very high throughput capabilities with ultra-fast analysis in 9 seconds sample-to-sample, without any carry over.

Ion mobility technology:

The SelexION™ technology is a Differential Mobility Spectrometer (DMS) placed in front of the inlet of the mass spectrometer**.** The ionized molecules travel into the orthogonal geometry shaped DMS for separation of isobaric analytes based on sectional chemical properties and not mass-to-charge (m/z).

Sample preparation

Extraction Procedure

- 5 µL urine sample
- 55 µL Internal standard (Phenobarbital-d5) in Sodium phosphate (0.1M, pH 4.5)
- vortex
- 100 µL Ethyl Acetate/Hexanes 75:25
- Vortex
- Wait 0.5 minutes for phase separation
- Transfer 4 µL of organic layer in LazWell plate
- Dry prior to analysis

Instruments setting

1) Mass spectrometer (Screening method):

Mass spectrometer AB Sciex 5500 QTrap

MRM transitions used with negative mode with 3 µA corona discharge current, 15 msec dwell time, and a -80 V DP

Compound	Q1	Q3	CE(V)
Amobarbital / Pentobarbital	225.2	42.0	-45
	225.2	182.2	-15
Secobarbital	237.3	42.1	-45
	237.3	194.1	-15
Butalbital	223.1	42.0	-45
	223.1	180.0	-15
Butabarbital	211.0	42.0	-45
	211.0	168.0	-15
Phenobarbital	231.0	42.0	-45
	231.0	188.0	-15
Phenobarbital-d5	236.0	42.2	-45

2) Mass spectrometer (Definitive Confirmation method):

Mass spectrometer AB Sciex 5500 QTrap with SelexION™

MRM transitions used with negative mode with 3 µA corona discharge current, 15 msec dwell time, and a -80 V DP. Water is used as a modifier and 4300 V as separation voltage (SV).

Compound	Q1	Q3	CE(V)	COV(V)

Amobarbital	225.2	42.0	-45	6.8
Pentobarbital	225.2	42.0	-45	5.8
Secobarbital	237.3	42.1	-45	5.0
Butalbital	223.1	42.0	-45	4.8
Butabarbital	211.0	42.0	-45	5.2
Phenobarbital	231.0	42.0	-45	4.8
Phenobarbital-d5	236.0	42.2	-45	5.2

3) LDTD:

LDTD model S-960 operated with a gas flow rate of 3 L/min and a laser pattern ramp from 0 to 55% in 6 seconds.

Methodology

The standard curve calibration is made with stock solutions of each drug spiked in blank urine matrix. The concentrations of the standards are 50 to 2000 ng/mL. The internal standard is prepared with a stock solution in methanol for a final concentration of 18 ng/mL. Each real sample and calibration curve is extracted with an Ethyl Acetate: Hexane mixture and the upper layer is spotted in a LazWell plate.

Results

The linearity of a calibration curve is expressed by the correlation coefficient R presented in the following table. All curves have ≥ 0,995 coefficients or better for LDTD-MS/MS analysis.

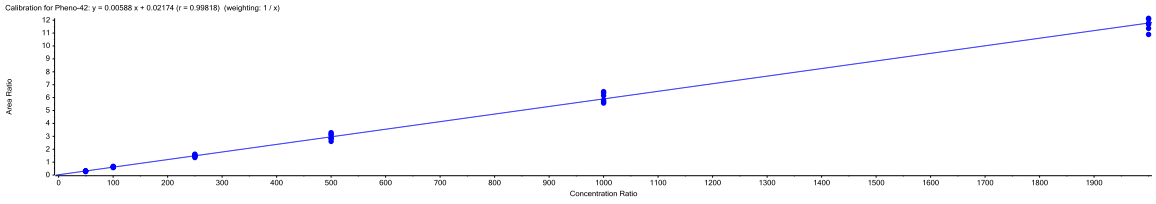

Figure 1: Typical LDTD-MS/MS calibration curve (Phenobarbital analysis)

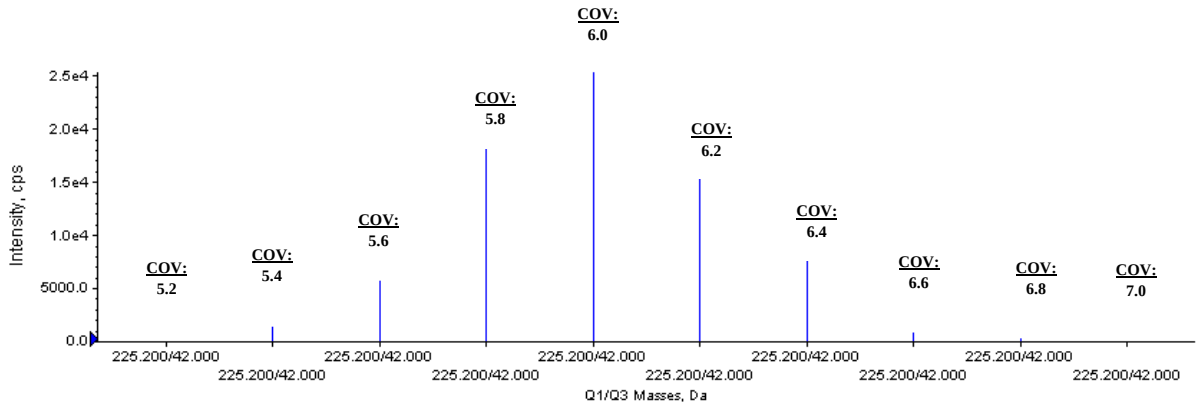

Figure 2: COV optimization for pentobarbital

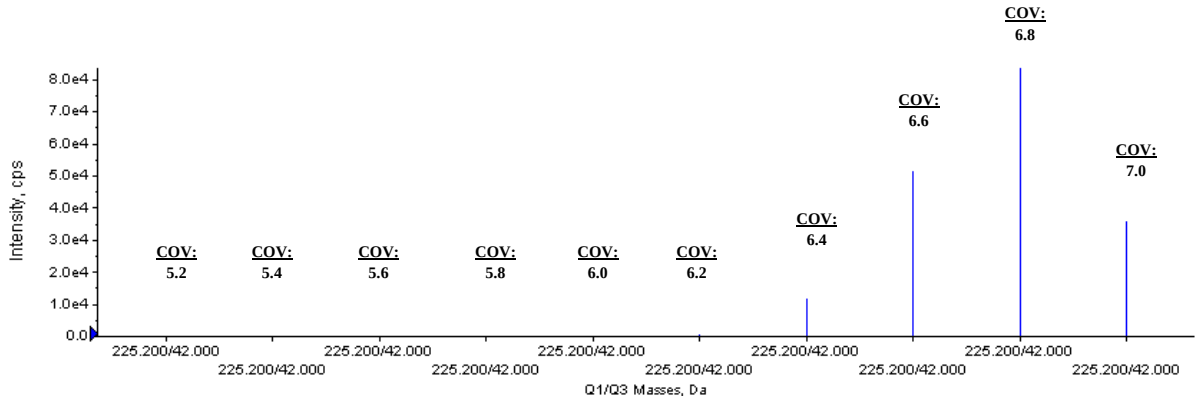

Figure 3: COV optimization for amobarbital

The intra-run accuracy and precision across the calibration curves were between 95.4 and 104.1% and 2.8 and 15.6% for both compounds, respectively. Following the extraction procedure, all samples were stored at 4°C to evaluate the drugs temporal stability in a wet state. After a waiting period, all samples were re-spotted and analyzed. A wet stability greater than 12h was obtained with accuracy between 87.4 and 98.8% and precision between 6.0 and 14.1% for concentrations equivalent to the LLOQ is observed.
To verify the matrix effect, base concentrations of barbiturates were evaluated in 10 real samples then fortified with a mixture of barbiturates at a known concentration. Fortified samples were within 20% of nominal value. A larger screening/confirmation study was performed and positive samples were cross validated with an LC-MS/MS method.

Conclusion:

The LDTD technology combined with a mass spectrometer system (with or without SelexION™ technology) allows either ultra-fast drug screening or definitive confirmation of Barbiturate drugs in urine samples with minimal sample preparation. **One** MRM method and **one** well were used to analyse 6 drugs in 9 seconds per sample.