High-Throughput Targeted Screening and Definitive Method for Barbiturate Drugs in Urine Using LDTD-MS/MS for Ultra-Fast Analysis

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OVERVIEW

Purpose

Ultra High-Throughput quantification of 6 Barbiturate drugs in urine.

Method

A Liquid-Liquid extraction was used for the Barbiturate analysis.

Quantification:

Linearity: $r^2 > 0.995$ over the calibration range (50 to 2000 ng/mL)

Samples were analyzed with a run time of 9 seconds sample-to-sample using LDTD-MS/MS system

INTRODUCTION

Screening methods are used by Toxicology laboratories to obtain a fast response and then confirm positive sample results using a more specific confirmation method. Analysis of barbiturate drugs can be challenging when a good separation between the isobaric drugs is required. Amobarbital and pentobarbital differ in the position of a methyl group. Therefore, a long chromatography is generally required to obtain sufficient resolution to separate these 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 **(Figure 1)** 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 a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 6 seconds sample-to-sample analysis time, without carry over.

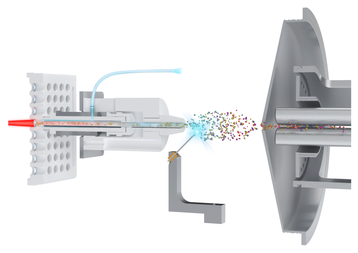

Figure 1 Schematic of the LDTD ionization source

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 orthogonally shaped DMS for separation of isobaric analytes based on sectional chemical properties as opposed to mass-to-charge (m/z).

METHOD

Liquid-Liquid extraction procedure:

5 µL urine sample (or standard)
55 µL Internal standard (18 ng/mL Phenobarbital-d5 in Methanol) in Sodium Phosphate (0.1 M, pH 4.5)
Vortex
100 µL Ethyl Acetate/Hexanes 75:25
Vortex
Wait 30 seconds for phase separation
Transfer 4 µL of organic upper layer in LazWell™ plate
Dry prior to analysis

METHOD

Instrumentation

- LDTD model: S-960
- MS: Sciex 5500 QTrap®

Figure 2 LDTD system on Sciex 5500 QTrap®

LDTD Parameters

Laser power pattern :

- Increase laser power to 55 % in 6.0 sec
- Decrease laser power to 0 %
- Carrier gas flow (Air) : 3 L/min

MS Parameters

- APCI (-)
- Dwell: 15 msec
- Corona discharge: 3 µA
- DP: -80 V
- MRM modes (see **Table 2**)

LC method parameters:

Mass spectrometer: TSQ® Vantage, Thermo Fisher Scientific
Column: SilliaChrom®SB-C18 (4.6X200 mm)
Flow rate: 0.4 mL/min
Mobile Phase A: H_2O/MeOH (90/10)+5 mM ammonium formate
Mobile Phase B: H_2O/MeOH (10/90)+ 5 mM ammonium formate
Volume injection: 5 µL
Ionization mode: Electrospray Ionization (ESI)

Compound	Screening method			Definitive method			
	Q1	Q3	CE (V)	Q1	Q3	CE (V)	COV (V)
Amobarbital*	225.2	42.0	-45	225.2	42.0	-45	6.8
	225.2	182.2	-15				
Pentobarbital*	225.2	42.0	-45	225.2	42.0	-45	5.8
	225.2	182.2	-15				
Secobarbital	237.3	42.1	-45	237.3	42.1	-45	5.0
	237.3	194.1	-15				
Butalbital	223.1	42.0	-45	223.1	42.0	-45	4.8
	223.1	180.0	-15				
Butabarbital	211.0	42.0	-45	211.0	42.0	-45	5.2
	211.0	168.0	-15				
Phenobarbital	231.0	42.0	-45	231.0	42.0	-45	4.8
	231.0	188.0	-15				
Phenobarbital-d5	236.0	42.2	-45	236.0	42.0	-45	5.2

* Amobarbital and Pentobarbital are not separated in the screening method

Table 1 MRM methods transitions for LDTD system

RESULT

Isomers separation:

Differential Ion mobility spectrometer is tuned to separate Amobarbital from the Pentobarbital with compensation voltage characteristics. **Figure 3** and **Figure 4** show the COV optimization for the Pentobarbital and the Amobarbital. Selected values of 5.8 V for Phenobarbital and 6.8 V for Amobarbital ensure less than 1% cross interference. Essentially, the ion mobility parameters are similar to other MRM transition variables operating at the same speed during data acquisition.

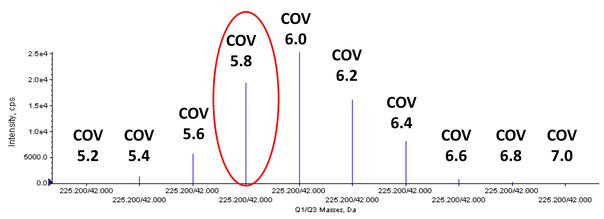

Figure 3 COV optimization for Phenobarbital

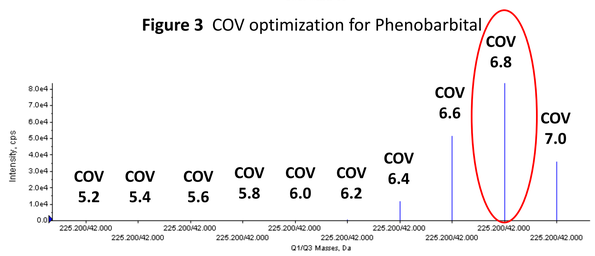

Figure 4 COV optimization for Amobarbital

RESULT

Quality control results:

The intra-run accuracy and precision across the calibration curves were between 95.4 and 104.1% and 2.8 and 15.6% for all compounds, respectively.

Wet stability:

Following the extraction procedure, all samples were stored at 39°F 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 12 hours was obtained with an accuracy between 87.4 and 98.8% and a precision between 6.0 and 14.1% for concentration equivalent to the LLOQ.

Matrix effect:

To verify the matrix effect, base concentrations of barbiturates were evaluated in 10 real samples and then fortified with a mixture of barbiturates at a known concentration. Fortified samples were within 20% of nominal value.

Linearity:

A standard calibration curve (with all 6 drugs) ranging from 50 to 2000 ng/mL has been prepared in blank urine matrix and analyzed in triplicate. All curves have ≥ 0.995 coefficients or better. **Figure 5** presents a typical calibration curve for Phenobarbital with LDTD.

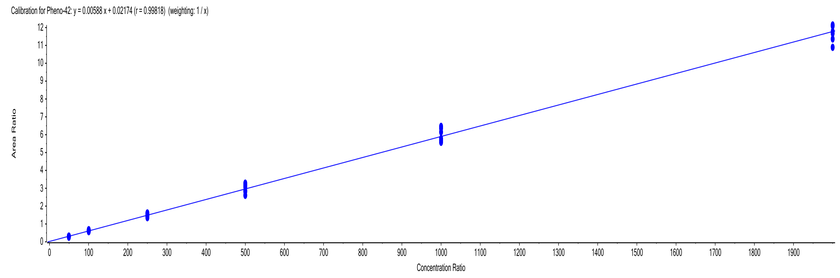

Figure 5 Phenobarbital calibration curve with LDTD

Screening results:

The screening MRM method does not separate Amobarbital from Pentobarbital – the positive value is attributed to both unless a definitive quantitation is performed. The most important aspect in a screening method is to provide a Positive flag for all samples that contain targeted drugs. Using a 100 ng/mL cutoff, no false negative reports were observed in 21 real samples tested using LDTD-MS/MS compared to definitive value from LC-MS/MS. During the assay, false positive results were observed on 2 particular samples for the same drug. A closer look at the integration shows a reduced signal for all internal standards under the acceptance level. Suppression effects are too significant to have an adequate quantitation. Further analysis shows that those 2 samples contain extreme concentrations of opiates (hundreds of µg/mL) causing this effect. The use of deuterated internal standards corrects the quantitation for all the other samples. A threshold level of IS area is used for the identification of overdosed urine samples.

CONCLUSION

- **Screening of 6 barbiturates in urine is performed in 6 seconds per sample giving a throughput capability of 400 samples per hour**
- **SelexION™ technology allows separation of two isomeric molecules by varying the COV parameter**
- **Use of ion mobility allows definitive quantitation of 6 Barbiturates in urine in 9 seconds sample-to-sample by LDTD-MS/MS**
- **Good precisions and accuracies are obtained**
- **No carryover was observed**

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