ThP 204

Targeted Screening of Barbiturates in Urine Using High-Throughput LDTD-MS/MS

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OVERVIEW

Purpose
- High-throughput screening of 5 barbituric drugs in urine using LDTD-MS/MS

Method
- Liquid-Liquid extraction used in the sample preparation
- Organic phase directly spotted in LazWell plate and analyzed

Quantification
- Linearity: $r^2 > 0.995$ over the calibration range (50 to 2000 ng/mL)
- Inter-run Accuracy ranging from 95.4 to 104.1 %.
- Inter-run Precision ranging from 2.8 to 15.6 %
- **Samples analyzed with a run time of 9 seconds using LDTD-MS/MS system**

INTRODUCTION

Toxicology laboratories generally use screening methods to obtain a semi-quantitative response for suspected barbiturate drug samples. Some screening techniques offer speed, however they lack specificity and generate many false positives. Mass spectrometry combined with a ultra-fast high-throughput solution such as the LDTD ion source enhances specificity at equivalent or better speed when compared to immunoassays. Method assessment is achieved by cross validating the same sample extracts with LDTD against LC-MS/MS as the standard gold method. We developed an extraction method using LDTD-MS/MS for a fast targeted screening of amobarbital/pentobarbital, phenobarbital, secobarbital, butalbital and butabarbital in urine with analysis speed of 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 wells 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 9 seconds sample-to-sample analysis time, without carry over.

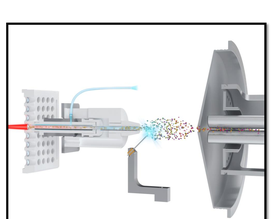

Figure 1 Schematic of the LDTD ionization source

METHOD

Extraction Procedure

5 µL patient urine sample (or STD or QC)
55 µL internal standard (18 ng/mL phenobarbital-d5 in Na_3PO_4 buffer (0.1 M pH 4.5))
Vortex
100 µL Hexane/Ethyl Acetate (25:75)
Vortex 30 seconds
Transfer 4 µL of the upper layer in LazWell™ plate
Analyze after complete solvent evaporation

LC method parameters:
- Column: SilliaChrom®SB-C18 (4.6X200 mm)
- Flow rate: 0.5 mL/min
- Mobile Phase A: H_2O/MeOH (90/10) + 1% Formic Acid
- Mobile Phase B: H_2O/MeOH (10/90) + 1% Formic Acid
- Injection volume: 20 µL*
- Ionization mode: Electrospray Ionization (ESI)

*Same extraction but concentration of 10x by: 800 µL of upper layer is transfer, evaporate to dryness and reconstitute with 80 µL of methanol.

MS Parameters
- APCI (-)
- DP: -80 V
- Dwell: 15 msec
- CE: -45 V
- MRM mode **(Table 1)**

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

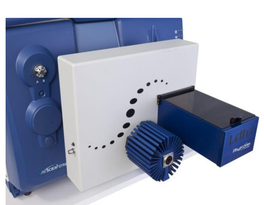

Figure 2 LDTD system on Sciex 5500 QTrap®

Compound	Q1	Q3
Amobarbital / Pentobarbital	225.2	42.0
Phenobarbital	231.2	42.0
Phenobarbital-d5	236.1	42.2
Secobarbital	237.3	42.0
Butalbital	223.1	42.0
Butabarbital	211.0	42.0

Table 1 MRM transitions for Barbituric drugs

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 55 % in 6.0 sec
  - ➢ Maintain power 2 sec
  - ➢ Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

RESULT

Linearity results:

A standard calibration curve (with all 5 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 3** presents typical calibration curves for Butalbital with LDTD.

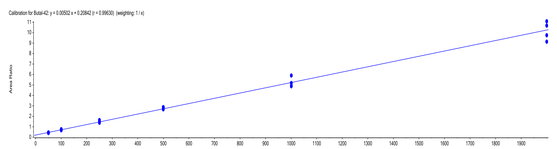

Figure 3 Butalbital calibration curve using LDTD-MS/MS system

Wet stability

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.

Inter-run

High, medium and low concentration QCs were added in the analysis. The inter-run accuracy and precision across the calibration curves were between 95.4 to 104.1% and 2.8 to 15.6% for all drugs, respectively. **Table 2** shows the Butalbital inter-run results.

	QC-Low	QC-Med	QC-High
Conc. (ng/ml)	100	500	1000
N	26	26	26
Mean (ng/ml)	101.01	519.91	1002.80
%RSD	5.7	7.6	8.3
%Nom	101.0	104.0	100.3

Table 2 Butalbital inter-run results

LC cross-validation

The most important aspect in a screening method is to provide a Positive flag for all samples that contain targeted drugs. All 21 patient urine samples were cross validated using LC-MS/MS system. **Table 3** shows the results for the 5 Barbituric drugs with a cut-off of 100 ng/mL.

Drug	Neg. samples	False positive
Butalbital	20	0
Secobarbital	21	0
Phenobarbital	20	0
Amobarbital/ Pentobarbital	21	0
Butabarbital	21	5

Table 3 Cross validation results

Matrix effect

To verify the matrix effect, basal barbiturate concentrations were evaluated in 10 real patient samples and then fortified with a mixture of barbiturates. Fortified samples were within 15% of nominal value for all drugs. **Table 4** shows the results for the matrix effect analysis for the Butalbital drug.

Matrix	Conc. (ng/mL)	Calc. conc. (ng/mL)	%NOM
M1	237.9	206.0	87
M2	237.9	257.8	108
M3	237.9	216.9	91
M4	237.9	233.9	98
M5	237.9	258.0	108
M6	237.9	252.0	106
M7	237.9	206.3	87
M8	237.9	251.2	106
M9	237.9	208.5	88
M10	237.9	238.7	100

Table 4 Matrix effects for Butalbital

CONCLUSION

- **Simultaneous screening of 5 Barbituric drugs in urine can be performed in 9 seconds using LDTD-MS/MS.**
- **Only 4.8% of the samples were false positives.**
- **Good precision and accuracy are obtained. No carryover was observed.**
- **Good linearity with coefficients higher than 0.995 for all curves was obtained.**
- **No matrix effect was observed.**