Meconium Targeted Drug Screening in 9 Seconds per Sample Using Laser Diode Thermal Desorption Mass Spectrometry (LDTD®-MS/MS)

Pierre Picard[1], Stephanie J. Marin[2], Gwendolyn A. McMillin[2,3], Alex Birsan[1], Serge Auger[1] and Jean Lacoursière[1]

1) Phytronix Technologies Inc., Québec, CANADA, 2) ARUP Institute for Clinical and Experimental Pathology, Utah, USA, 3) University of Utah, Department of Pathology, Utah, USA

OVERVIEW

Purpose

- High-throughput screening of 26 drugs of abuse in meconium matrix

Method

- Meconium matrix preparation followed by either an acidic or basic oriented Liquid-Liquid extraction
- Validation:
  - Good results obtained for the 2 standard deviation (2 SD) approach
- **Samples were analyzed with a run time of 9 seconds using LDTD-MS/MS system**

INTRODUCTION

Drug abuse during pregnancy is a major medical issue associated with significant maternal and infant complications. Meconium is a common specimen used to identify and characterize drug-exposed infants. The proposed mechanism for drug presence in meconium is that the fetus excretes the drug into bile and amniotic fluid. Drug accumulates in the meconium either by direct deposit from bile or through swallowing of the amniotic fluid. ARUP Laboratories uses immunoassay to screen for the presence of different drug families. To reduce the number of screening assays and reduce the quantity of meconium required for testing, a Laser Diode Thermal Desorption Mass Spectrometry (LDTD®-MS/MS) method was developed.

Laser Diode Thermal Desorption Mass Spectrometry (LDTD®-MS/MS) offers specificity combined with an ultra-fast analysis for an unrivaled screening method. A fast and simple extraction procedure is described, with the following calibration range: 20 to 200 ng/g of meconium for amphetamines/cocaine/opiate/oxycodone/PCP/methadone classes and 50 to 500 ng/g of meconium for Barbiturates/Benzodiazepines classes. The lower limit of the calibration curves served as the cutoff for reporting results.

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, with no carryover.

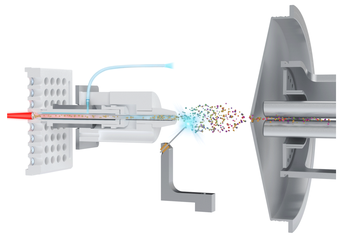

Figure 1 Schematic of the LDTD ionization source

METHOD

Sample Preparation

Meconium Solution Preparation Procedure:

- 0.1 g meconium in a 1.5 mL Eppendorf tube
- 1 mL Phosphate buffer (0.1 M, pH7)
- Vortex / sonicate 20 minutes
- Centrifuge 14,000 rpm for 10 minutes
- Filter solution using a 0.45 µm Nylon filter

Enzymatic Hydrolysis Mix:

- 110 µL meconium solution preparation in a 0.5 mL Eppendorf tube
- 5 µL Internal Standard (IS) solution
- 20 µL purified β-glucuronidase enzyme (IMCSzyme, >50 kU/mL)
- 25 µL rapid hydrolysis buffer (IMCSzyme)
- Vortex
- Incubate 15 minutes at 55°C

Extraction Procedure (Basic Drugs):

These were added to the Enzymatic Hydrolysis Mix:

- 100 µL Sodium Carbonate buffer (0.5 M, pH 10)
- Mix
- 200 µL Ethyl Acetate
- Mix
- Centrifuge 14,000 rpm for 2 minutes
- Transfer 4 µL of organic upper layer in a LazWell™ plate*
- Dry prior to analysis

METHOD

Extraction Procedure (Acidic Drugs):

These were added to the Enzymatic Hydrolysis Mix:

- 200 µL NaCl (saturated solution in water)
- Mix
- 400 µL Acetonitrile
- Centrifuge 14,000 rpm for 2 minutes
- Transfer 4 µL of organic upper layer in a LazWell plate*
- Dry prior to analysis

* LazWell™ coating: 96-well plates are pre-coated with 5µL of an EDTA solution (100 µg/mL) in MeOH/H_2O/NH_4OH (75/20/5) which is dried before sample deposition

LDTD Parameters

- Laser power pattern :
  - ➢ Increase laser power to 65 % in 6.0 sec
  - ➢ Decrease laser power to 0 %
- Carrier gas flow (Air) : 3 L/min

MS Parameters

- APCI
- Dwell: 5 msec
- Corona discharge: 3 µA
- DP: 100 V
- MRM mode (see **Table 1, 2 & 3**)

Instrumentation

- LDTD model: S-960 (**Figure 2**)
- MS: Sciex 5500 QTrap®

Figure 2 LDTD system on Sciex 5500 QTrap®

Table 1 MRM method transitions for basic drugs

Compound	Q1	Q3	CE (V)
Alprazolam	311	274	40
Diazepam	285	154	32
α-OH-Alprazolam	325	205	54
Oxazepam	287	241	30
Temazepam	301	255	25
D5-Oxazepam	292	246	32
D5-Temazepam	306	260	25
MDA	180	133	20
MDEA	208	163	12
MDMA	194	163	12
Amphetamine	136	119	15
Methamphetamine	150	119	15
D9-Methamphetamine	159	125	15
PCP	244	159	20
D5-PCP	249	164	20
Methadone	310	265	20
EDDP	278	234	30
MOR-HYM	286	165	50
Oxycodone	316	241	40
Oxymorphone	302	227	40
COD-HYC	300	215	35
D9-Methadone	319	268	20

Acidic drugs are analyzed using positive and negative ionization using two different experiments in the same MS method (showed in **Figure 3**):

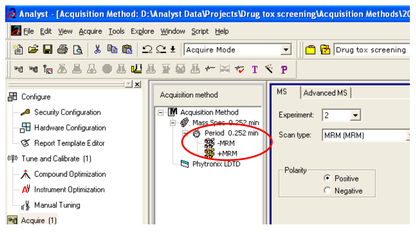

Figure 3 Positive/Negative MRM method for LDTD® -MS/MS analysis.

Table 2 MRM method transitions for acid drugs

Compound	Q1	Q3	CE (V)	Ionization mode
BZE	290	168	25	Pos
D8-BZE	298	171	25	Pos
Amobarbital /Pentobarbital	225	182	-15	Neg
Phenobarbital	231	42	-45	Neg
Secobarbital	237	42	-45	Neg
Butalbital	223	42	-45	Neg
Butabarbital	211	42	-45	Neg
D5-Phenobarbital	236	42	-45	Neg

RESULTS

Accuracy and precision results:

For each drug, peak area against internal standard signal ratio was used for signal normalization. The precision tests at the decision point were used to evaluate the analytical performance. The curves for each concentration showing the mean plus or minus two times the standard deviation (±2SD) for each sample must not overlap for the decision point to be valid. All drug curves were valid.

According to ±2SD rule for the precision test, overlay graphs were drawn. In **Figure 4**, the curves for Methadone are showed.

RESULTS

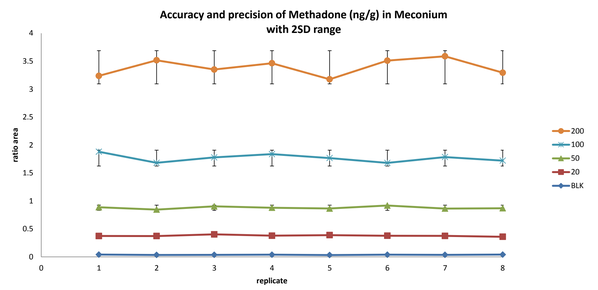

Figure 4 ±2SD curve test for Methadone in Meconium

Patient Specimen Comparison LC-MS/MS vs LDTD-MS/MS

30 residual meconium patient specimens de-identified according to a University of Utah Institutional Review Board (IRB) protocol were extracted and analyzed by LDTD-MS/MS, and results compared to LC-MS/MS or GC-MS results generated at ARUP Laboratories. The LC-MS/MS or GC-MS methods used a sufficiently long gradient to minimize ion suppression and matrix effects.

The most important aspect in a screening method is to provide a positive result for all samples that contain targeted drugs and **no false negative** results.

A summary of results are shown in **Table 3** below.

Table 3 Authentic Patient Specimen Comparison LC-MS/MS vs LDTD-MS/MS

Drug	LC-MS/MS		LDTD-MS/MS		False positive / negative	
	POS	NEG	POS	NEG	FALSE POS	False neg.
Amphetamine	4	26	4	26	0	0
Methamphetamine	5	25	5	25	0	0
MDA	0	30	0	30	0	0
MDEA	0	30	0	30	0	0
MDMA	0	30	0	30	0	0
Butalbital	1	29	1	29	0	0
Pentobarbital/ Amobarbital	0	30	0	30	0	0
Phenobarbital	0	30	0	30	0	0
Secobarbital	0	30	0	30	0	0
Butabarbital	0	30	2	28	2	0
Oxazepam	4	26	6	24	2	0
Temazepam	1	29	2	28	1	0
Alprazolam	0	30	0	30	0	0
Diazepam	0	30	0	30	0	0
α-OH-Alprazolam	0	30	0	30	0	0
BZE	1	29	1	29	0	0
Methadone	4	26	4	26	0	0
EDDP	4	26	4	26	0	0
PCP	0	30	9	21	9	0
Morphine/ Hydromorphone	14	16	16	14	2	0
Codeine/ Hydrocodone	7	23	9	21	2*	0
Oxymorphone	4	26	5	25	1*	0
Oxycodone	4	26	5	25	1*	0

* Additional false positives were observed for this transition, but these samples were also positive for other opiates.

Enzymatic Efficiency :

Meconium extracts were spiked with Oxazepam at 87.2 µM and another sample spiked with Oxazepam-Glucuronide at 87.2 µM. Both samples were hydrolyzed, extracted and analyzed. Oxazepam transition was used to monitor the signal of each sample. The enzymatic efficiencies were evaluated using area ratio of Oxazepam-Glucuronide sample against the Oxazepam sample signal. Complete hydrolysis of Oxazepam-Glucuronide was obtained (Results shown in **Table 4**)

Table 4 Enzymatic Efficiency

Sample ID	Mean ratio area
Oxazepam-Glu (87.2 nM)	3.61
Oxazepam (87.2 nM)	3.35
Enzymatic Hydrolysis efficency	107.5%

CONCLUSION

- The LDTD® technology combined with a mass spectrometer system allows ultra-fast and specific drug screening in meconium samples in **9 seconds per sample**
- Full drug detection was achieved with a single MS/MS analysis method
- Good enzymatic hydrolysis was obtained
- No false negatives were observed

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