Ultra-fast screening method for Drugs of Abuse in urine using simple sample preparation combined to Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS)

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OVERVIEW

Purpose
- Simultaneous screening for various drugs of abuse in urine using a single MRM method.
- Minimal sample treatment for development of a cost effective assay.

Method
- Real patient samples were analyzed in LDTD®-MS/MS using 4 different sample preparation methods to determine optimal operation mode.

Results
- Efficient identification of positive and negative drug samples was obtained within a few seconds.
- Excellent linearity over the calibration range ($R^2 > 0.99$).
- Good accuracy and precision.
- **All samples are 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 drug samples. Some screening techniques are fast but less specific and generate by far too many false positive results. Confirmation of those additional false positive samples is both time and cost consuming.

Laser Diode Thermal Desorption Mass Spectrometry (LDTD®-MS/MS) offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a fast and simple extraction method using urine sample evaporation followed by organic/aqueous dilution. 31 drugs of abuse from different classes (opiates, benzodiazepines, amphetamines, etc.) are analyzed simultaneously, with quantitative screening results obtained in less than 9 seconds per 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. The sample gets dried and vaporized prior being carried by a gas in a corona discharge region. This type of ionization is characterized by a strong resistance to ionic suppression because of the absence of solvent. LDTD® ionization reduces sample-to-sample analysis time to 9 seconds and allows high throughput capabilities without carry over.

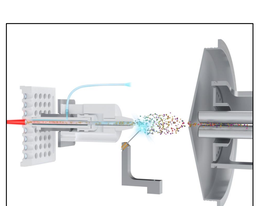

Figure 1 Schematic of the LDTD® ionization source.

METHOD

Sample Preparation

- Test 1: Solubilization
- Test 2: Solubilization + Basic LL
- Test 3: Hydrolysis + Solubilization
- Test 4: Hydrolysis + Solubilization + Basic LL Urine with/without β-Glucuronidase enzyme reaction (1h/60ºC)

Step 1
- 5 µL of urine, hydrolyzed or not
- 50 µL 0.1 % TFA in ACN containing the internal standard
- Vortex and evaporate to dryness

Step 2
- 100 µL of 0.1% TFA in hexane/EtAc 1:1 solubilization
  - Vortex
  - Option 2 and 4: add 40 µL Carbonate buffer (0.5 M, pH 10)
  - Vortex
  - Transfer 4 µL in LazWell™ plate*
  - Analyze after complete solvent evaporation

* EDTA coated LazWell™ plates were used

Instrumentation
- LDTD® model S-960, Phytronix Technologies
- QTRAP® 5500 Systems, AB Sciex

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 65 % in 6.0 s
  - ➢ Maintain at 65 % for 1 second
  - ➢ Decrease laser power to 0 %
- Carrier gas flow:3 L/min (Air)

MS Parameters
- APCI (+) positive
- Scan time : 5 msec
- DP: 100
- EP: 10
- CXP: 13

Total run time: 9 seconds
36 MRM transitions in a single method

Table 1&2 MRM transition of drugs

Compound	Q1	Q3	CE (V)
Metamphetamine	150	119	15
Amphetamine	136	119	10
PCP	244	159	20
Imipramine	281	86	20
MDA	180	133	20
MDMA	194	163	15
MDEA	208	163	15
Benzoylecgonine	290	168	20
6-Acetylmorphine	328	165	40
Codeine	300	215	35
Morphine	286	201	40
Oxycodone	316	187	35
Oxymorphone	302	227	35
2-OH-ethylflurazepam	333	211	46
7-aminoclonazepam	286	222	30
Alprazolam	311	274	40
Diazepam	285	154	32
Estazolam	295	205	48
OH-alprazolam	325	205	54
OH-triazolam	359	331	36
Lorazepam	321	275	26
Nordiazepam	271	140	32
Oxazepam	287	241	32
Temazepam	301	255	25
OH-midazolam	342	203	35
7-aminoflunitrazepam	284	236	36
Chlordiazepoxide	300	227	35
Clonazepam	316	214	50
Flunitrazepam	314	240	40
6-Acetylmorphine-d6	334	165	40
Oxycodone-d6	322	248	35
Benzoylecgonine-d8	298	171	20
PCP-d5	249	164	20
Oxazepam-d5	292	246	32
Temazepam-d5	306	260	47
OH-Triazolam-d4	363	335	47

RESULTS

Validation and Quantification Results

Each drug (31 total from different groups) of interest was spiked in blank human urine to prepare a calibration curve (concentration range from 50 to 1000 ng/mL). All compounds gave a linear response from 50 or 100 ng/mL to 1000 ng/mL. Individual spike tests were performed to verify interference. No interference between the drugs was obtained (except for morphine with hydromorphone and codeine with hydrocodone, which have the same elemental compositions and the same fragmentation patterns).

Precision and accuracy values are reported in Table 3. Accuracy ranging from 88.2 to 111.3 % using area ratio value and precision ranging from 2.1 to 13.8 % using area ratio values were obtained for Benzoylecgonine (BZE). Similar results were obtained for the other drugs (results not reported). Typical desorption peak and calibration curve are shown in Figure 2 and 3

Method Cross Validation

Drug concentrations in real patient samples were evaluated using Test 3. The sample preparation used for LC analysis reconstitutes the dried down sample in the mobile phase. The LC-MS/MS analytical method uses a sufficiently long gradient to limit the matrix effect and ionic suppression associated with the reduced sample cleaning.

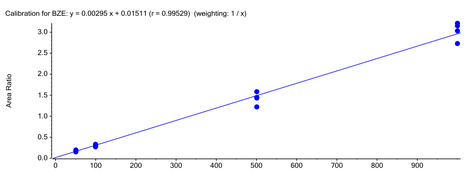

Figure 2 Typical calibration (BZE curve)

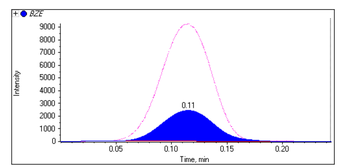

Figure 3 Typical Desorption peak: BZE (blue) / BZE-d8 (pink)

Table 3 Precision and accuracy results (Benzoylecgonine)

	S50	S100	S500	S1000
Conc. (ng/mL)	50	100	500	1000
N	4	4	4	4
Mean (mg/mL)	55.6	100.6	441.0	1008.5
%RSD	2.1	5.9	11.0	13.8
%Nom	111.3	100.6	88.2	100.8

Evaluation of False Positives

- All false positives, shown in Table 3, are related to very high concentrations (10000 to 50000 ng/mL) of another target drug found in the sample.
- No false positives were observed for totally drug free urine samples.
- QCs of mixed drugs at ULOQ, 1000 ng/mL, show no interferences between each tested drug. At very high concentrations, the chemical noise generated at many MRM transition causes a few false positives.

Table 3 Evaluation of false positive results of 38 real samples

Drug	LC-MS/MS	LDTD-MS/MS	
ID	Negative sample (N)	Method 3 False pos. (N)	Method 4 False pos. (N)
Metamphetamine	36	1	1
Amphetamine	32	NA**	0
PCP	33	0	0
Imipramine	38	0	0
MDA	35	8	2
MDMA	34	0	0
MDEA	38	0	0
Benzoylecgonine	31	7	NA**
6-Acetylmorphine	35	0	0
Codeine	23	0	1
Morphine	26	3	12
Oxycodone	29	1	0
Oxymorphone	30	11	12
2-OH-ethylflurazepam	38	2	0
7-aminoclonazepam	26	4	4
Alprazolam	23	0	0
Diazepam	37	5	1
Estazolam	38	0	1
OH-alprazolam	23	2	2
OH-triazolam	38	0	0
Lorazepam	33	0	0
Nordiazepam	31	0	0
Oxazepam	27	0	0
Temazepam	31	1	1
OH-midazolam	38	1	1
7-aminoflunitrazepam	38	12	10
Chlordiazepoxide	38	1	1
Clonazepam	36	6	5
Flunitrazepam	38	8	7

** NA: Not applicable: No curve obtained in these conditions.

Evaluation of False Negatives
- The most important aspect in a screening method is to provide a Positive flag for all samples that contain targeted drugs.
- Using Test 3 sample preparation, **no false negative reports were observed** using LDTD®-MS/MS with the 38 tested samples.
- Benzodiazepine requires glucuronide hydrolysis to be detected.
- Amphetamine required Test 4 sample preparation to remove interfering signal at the monitored transition.

DISCUSSION

- Proposed sample treatment reduces reagent quantity to minimal value in a single plate where all the sample preparations take place.
- Hydrolysis of glucuronide is necessary for adequate drug detection.
- Similar screening using Immunoassays would require 7 different drug class reagents with separate analyses of 10 seconds each.
- False positives for certain drugs are only observed in conjunction with a very high positive sample - several magnitudes higher in concentration than the ULOQ.

CONCLUSIONS

- Extraction method Test 3 and 4 are best suited to screen positive and negative drug samples.
- The LDTD® technology combined with a mass spectrometer system allows ultra-fast and specific drug screening in urine samples with minimal sample preparation.
- **One** MRM method and **One** well used to screen 31 drugs in **9 seconds per sample.**

1) Salary/Consultant Fees: Phytronix