New High-Throughput analysis method for volatile amines by LDTD-MS/MS

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OVERVIEW

Purpose
- Quantification and method optimization of amine drugs of abuse.

Method
- Liquid-Liquid extraction with pH optimization
- Different HCl concentration are tested to reduce volatility of the amine by salt formation

Results
- Concentration of 0.001N HCl are enough to reduce amine volatility
- Excellent linearity over the calibration range ($R^2 > 0.99$)
- Accuracy ranging from 92.9 and 111.6% using area ratio value
- Precision ranging from 1.7 and 15.0 % using area ratio value
- **All these samples are analyzed with a run time of 9.6 seconds using LDTD-MS/MS technique**

INTRODUCTION

The Laser Diode Thermal Desorption is a rapid analysis approach in which samples are thermally desorbed by a laser diode. Molecules are channeled, using a carrier gas, to a corona discharge region for ionization prior to detection via a mass spectrometer. Samples deposited in a 96-wells plate and allowed to dry before analysis via the laser induced desorption process. In presence of a volatile compound, modification to the sample solution must be performed in order to avoid compound evaporation before the desorption process.

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 with strong resistance to ionic suppression characterize this type of ionization, and are 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.

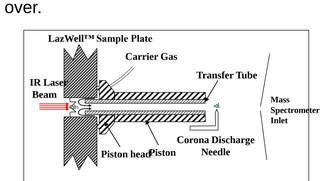

Figure 1 Schematic of the LDTD ionization source.

Figure 2 LDTD-Xevo-TQMS system

Instrumentation
- Phytronix Technologies LDTD ion source (model WX-960);
- Xevo-TQMS system, Waters

METHOD

Extraction procedure (PCP)
- 100 µL Urine
- 20µL Internal standard
- 100 µL NaOH (0.1N) in water
  - Vortex 30 seconds
- 600 µL 1-Chlorobutane
  - Vortex 30 seconds
  - Centrifuge 3500 rpm / 5 minutes
- Transfer 400 µL upper phase
  - Add 20 µL HCl (0.4N in Methanol)
- Evaporate to dryness
  - Reconstitute with 40 µL Methanol/Water (75/25)
- Spot 2 µL in LazWell plate.

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 45 % in 3.0 s
  - ➢Stay 2 seconds at 45% laser power.
  - ➢Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

Extraction procedure (Amphetamine/Metamphetamine)
- 100 µL Urine
- 10 µL Internal standard in Methanol
- 100 µL NaOH(0.1N) in water
  - Vortex 30 seconds
- 600 µL Ethyl Acetate
  - Vortex 30 seconds
  - Centrifuge 14000 rpm / 2 minutes
- Transfer 400 µL upper phase
  - Add 20 µL HCl (0.4N in Methanol)
- Evaporate to dryness
  - Reconstitute with 40 µL Methanol/Water (75/25) with HCl (0.1N)
- Spot 2 µL in LazWell plate.

MS Parameters
- APCI (+)

	Q1		Q3	Cone (V)	Collision (V)
Amphetamine	136	->	119	20	10
Amphetamine-d5	141	->	124	20	10
Metamphetamine	150	->	119	20	10
Metamphetamine-d9	159	->	125	20	10
PCP	244	->	159	15	15
PCP-d5	249	->	164	15	15

RESULTS:

HCl Salt formation effect

The goal of this experiment is to determine the ideal HCl concentration to avoid the amine evaporation before the Laser desorption process. In all manipulations, a HCl concentration greater than 0.001N is enough to achieve a stable salt formation (Drug concentration: 10 µg/ml).

Figure 3, 4 and 5 shows the effect of the presence of HCl. Baseline increases in wells not treated with HCl.

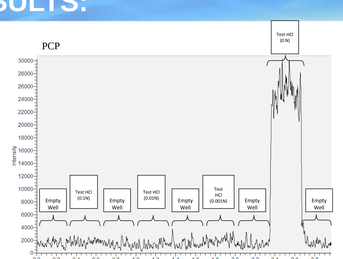

Figure 3 Optimisation HCl concentration for PCP salt formation

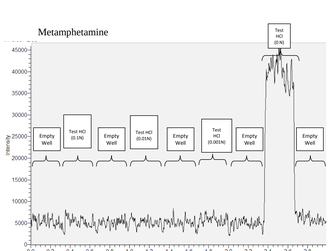

Figure 4 Optimisation HCl concentration for Metamphetamine salt formation

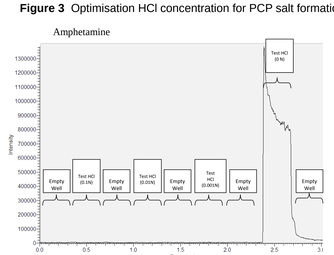

Figure 5 Optimisation HCl concentration for Amphetamine salt formation

Quantification results

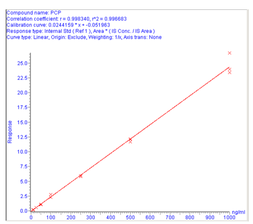

Figure 6 Standard curve

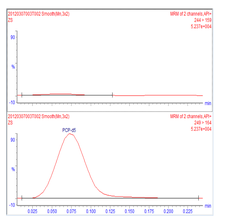

Figure 7 Blank desorption peak

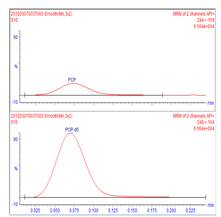

Figure 8 LLOQ desorption peak

	r^2	Slope	origin
PCP	0.9967	0.0244	-0.0520
Amphetamine	0.9956	0.0238	0.9811
Metamphetamine	0.9982	0.0335	0.4399

Table 1 Standard curve parameter

	QC-Low	QC-Med	QC-High
Nominal conc. (ng/ml)	25	100	500
N	3	3	3
Mean (ng/ml)	24.9	103.1	493.5
RSD (%)	1.7	11.5	2.1
%Nom. conc.	99.5	103.1	98.7

Table 2 Accuracy and precision results of PCP

	QC-Low	QC-Med	QC-High
Nominal conc. (ng/ml)	50	500	1000
N	3	3	3
Mean (ng/ml)	55.8	512.3	959.3
RSD (%)	3.8	9.7	11.7
%Nom. conc.	111.6	102.5	95.9

Table 3 Accuracy and precision results of Metamphetamine

	QC-Low	QC-Med	QC-High
Nominal conc. (ng/ml)	50	500	1000
N	3	3	3
Mean (ng/ml)	50.0	479.0	929.0
RSD (%)	15.0	6.2	6.8
%Nom. conc.	100.1	95.8	92.9

Table 4 Accuracy and precision results of Amphetamine

CONCLUSIONS

- A concentration of 0.001N of HCl is enough for the salt formation to avoid drug evaporation before analysis
- Good linearity, accuracy and precision for the method quantification of PCP, Amphetamine and Metamphetamine
- LDTD provides the High-Throughput analysis of sample extract in **9.6 seconds sample-to-sample**