High-Throughput Analysis of Opiates in Hair Samples using Laser Diode Thermal Desorption (LDTD®) coupled to Mass Spectrometry

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OVERVIEW

Purpose
• Ultra-Fast analysis of Opiates in hair samples using LDTD®-MS/MS

Method
• Standards, QC and samples preparation using hair matrix
• Solid-Phase Extraction (SPE)
• Deposit of a small volume of the organic phase in LazWell™ plate
• Fast Analysis using LDTD®-MS/MS system
• Cross validation LC vs LDTD®

Results
• Excellent linearity over the calibration range ($R^2 \geq 0.9967$)
• Accuracy ranging from 93,1 to111.1%
• Precision ranging from 0.7 to13.8 %
• Good sample stability (Wet and Dry in LazWell™)
• **All samples are analyzed with a run time of 9 seconds using LDTD®-MS/MS system.**

INTRODUCTION

Since the hair root is vascularized during its growth, illicit drugs present in the blood stream may enter the hair shaft via the root where they will be sequestered. Therefore, the use of illicit drugs can be revealed by analyzing a small hair sample. To increase the analysis throughput of hair samples, the laser diode thermal desorption (LDTD®) coupled to tandem mass spectrometry (MS/MS) was used for the identification and quantification of opiates.
The detection and quantification of drugs in hair samples is traditionally performed by LC-MS/MS or GC-MS analysis that require several minutes due to separation time. The LDTD®-MS/MS instrument significantly reduces analysis time and this increases the sample throughput with runtimes of 9 seconds sample-to-sample. In this study, the goal was to validate a quantitative method for the following opioid drugs in hair: 6-acetylmorphine (6-AM), codeine (COD), morphine (MOR), hydrocodone (HYC), hydromorphone (HYM), oxycodone OXC) and oxymorphone (OXM), using the LDTD® coupled to MS/MS.

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.

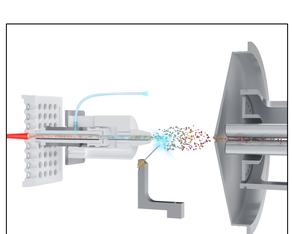

Figure 1 Schematic of the LDTD® ionization source.

METHOD

1) Pre-Wash
- 10 mg Negative Hair sample
- 2 mL Dichloromethane
- Sonicate 5 min
- Remove Dichloromethane
- 2 mL Ethanol
- Sonicate 5 minutes
- Remove Ethanol

2) Hair Digest process
- 10 mg Hair sample (pre-washed)
- 100 µL Working solution (for standards)
- 100 µL Internal standard (5 ng/mL)
- 1 mL Methanol/TFA (9:1)
- Sonicate 1 hour
- React 8 hours at room temperature
- Transfer Organic phase in glass tube and evaporate to dryness
- 100 µL MeOH
- 900 µL HCL 1N/H_2O (1:14)
- Vortex
- SPE

3) SPE (Sample Cleanup, 100 mg/1cc)

Activation
- 1 mL EtAc/IPA/NH_4OH (80:15:5)
- 1 mL MeOH
- 1 mL Water

Load
- 1 mL Sample

Wash
- 1 mL Water
- 1 mL CH_3COONa (100 mM, pH 4.5)
- 1 mL IPA

Elution
- 1.5 mL EtAC/IPA/NH_4OH (80:15:5)
- Evaporate to dryness
- 100 µL MeOH/H_2O (75:25)
- Spot 4 µL in LazWell™ plate
- Analyze after complete solvent evaporation

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

LDTD Parameters
• Laser power pattern :
  ➢ Increase laser power to 45 % in 6.0 s
  ➢ Hold for 2 seconds
  ➢ Decrease laser power to 0 %
• Carrier gas flow:3 L/min (Air)
• APCI (+) positive

LC Parameters
• ESI (+) positive
• Column: SilliaChrom SB-C18, 5µm (4.6x200)
• Flow rate: 0.5 ml/min
• MPA: Water/MeOH/FA (90/10/1)
• MPB: Water/MeOH/FA (10/90/1)
• Gradient:

Time	A	B
0	100	0
10	0	100
15	0	100
16	100	0
30	100	0

MS Parameters
• Scan time: 10 msec
• DP: 100

Table 1 MRM transitions of drugs

Compound	Q1	Q3	CE (V)
6-Acetylmorphine (Quan)	328	165	40
6-Acetylmorphine (Conf)	328	211	35
Codeine (Quan)	300	215	35
Codeine (Conf)	300	243	30
Morphine (Quan)	286	201	40
Morphine (Conf)	286	155	45
Hydromorphone (Quan)	315	272	25
Hydromorphone (Conf)	315	216	40
Hydrocodone (Quan)	329	185	40
Hydrocodone (Conf)	329	255	25
Oxycodone (Quan)	345	281	30
Oxycodone (Conf)	345	285	30
Oxymorphone (Quan)	331	267	35
Oxymorphone (Conf)	331	281	25
Codeine-D6	306	202	40
Hydrocodone-D6	335	188	40
Hydromorphone-D6	321	259	35
Oxycodone-D6	351	248	35
6-Acetylmorphine-D6	334	165	40
Morphine-D6	292	201	35
Oxymorphone-D3	334	284	25

RESULTS

Linearity results
For each drug, a calibration curve ranging from 5 to 100 ng/mL has been prepared in MeOH and spiked in negative hair samples. All curves are linear and show a good correlation coefficient, $R^2 \geq 0.9967$. **Figure 2** shows the calibration curve results for 6-AM using LDTD® (R=0.99768). **Table 2** shows the accuracy and precision results for 6-Acetylmorphine samples.

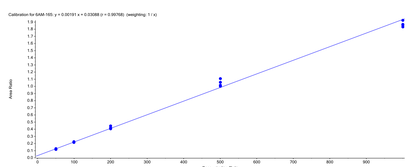

Figure 2 Calibration curve for 6-AM drug

Table 2 Precision and accuracy for 6-AM curve samples

Conc (ng/mL)	Conc (pg/mg)	Mean (pg/mg)	N	%RSD	%NOM
5	50	47.5	4	6.88	95.0
10	100	99.2	4	3.00	99.2
20	200	205.2	4	4.17	102.6
50	500	533.5	4	4.73	106.7
100	1000	964.6	4	2.16	96.5

Cross Validation LC vs LDTD®
Positive and negative real patient hair samples were analyzed both with LC–MS/MS and LDTD®-MS/MS to validate the efficiency of the LDTD® analysis method. No false positive results were obtained with the negative hair samples. Positive hair samples correlation results (pg/mg hair) are shown in Figure **3 to 8.** No positive samples (higher the LC-MS/MS cut off) are available for OXM.

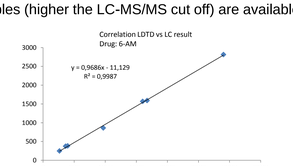

Figure 3 LC vs LDTD® cross validation results for 6-AM

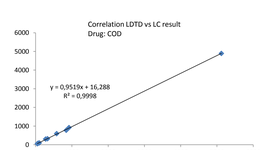

Figure 4 LC vs LDTD ® cross validation results for COD

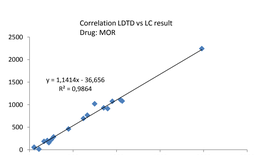

Figure 5 LC vs LDTD ® cross validation results for MOR

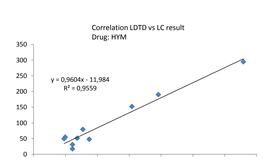

Figure 6 LC vs LDTD ® cross validation results for HYM

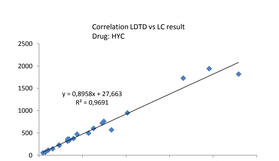

Figure 7 LC vs LDTD ® cross validation results for HYC

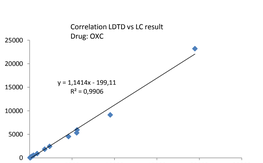

Figure 8 LC vs LDTD ® cross validation results for OXC

CONCLUSIONS

- LDTD® provides Ultra-Fast High-Throughput analysis of sample extract in **9 seconds sample-to-sample without any carry over.**
- LC-MS/MS and LDTD®-MS/MS values agree for cross validation of real patient samples.

A) Salary/Consultant Fees: Phytronix