Confirmation of Methadone and EDDP in Urine by Laser Diode Thermal Desorption (LDTD)

Patrice Tremblay[1], Pierre Picard[1], Serge Auger[1], William Murphy[2], Stefany DeOliviera[2] and Claire Collins[2],
1.Phytronix Technologies, Québec, CANADA, 2. Calloway Labs, Woburn, MA, USA

P004

OVERVIEW

Purpose
– Methadone and EDDP confirmation in human urine samples
– Laser Diode Thermal Desorption technology (LDTD) tandem mass spectrometry

Method
– Drugs spiked into human urine
– Solid Phase Extraction
– Nominal calibration range : 15 to 9 600 ng/ml
– LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer

Results
• Excellent linearity over the calibration range ($R^2 > 0.998$)
• LOD and LOQ set at 15 and 30 ng/mL respectively
• Excellent accuracy within ± 20 % as compared to in house method.
• Excellent precision (within and between-run) ≤ 13.5 % for Methadone and ≤ 19.1 % for EDDP
• No carryover below 1 mg/mL
• No interference observed with common medication.

INTRODUCTION

The limitations of traditional GC-MS analysis of Methadone and its metabolite EDDP in urine include lengthy run-time, risk of carryover, and costs associated with derivatization and column replacement. By installing a LDTD ionization source on a Triple Quadrupole MS system (LDTD-MS/MS) an ultra fast instrumental system was developed that avoids these limitations and provides additional advantages. We propose to validate this method for the confirmation of Methadone and EDDP in urine. To demonstrate accuracy, real patient samples as well as proficiency test samples from the College of American Pathologists were tested using the LDTD-MS/MS and compared with the results obtained from multiple laboratories utilizing GC/MS analysis.

LDTD (Figure 1)
– Plug-and-play ionization source interface to Agilent Technologies 6410 QQQ
– Thermal desorption induced by a laser diode
– The sample is carried by a carrier gas to a corona discharge region for APCI
– Loader capacity up to 10 LazWell™ plates

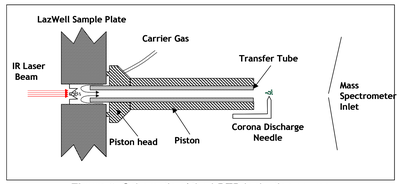


Figure 1 Schematic of the LDTD ionization source.

LazWell™ Plate (Figure 2)
– Standard 96-well plate format
– Low volume delivery (from 1 to 10 µL of sample per well)
– No carryover
– No enhancement matrix needed
– No sample desalting needed
– No liquid mobile phase needed
– Sample dried at room temperature

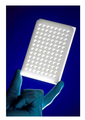


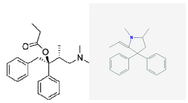
Figure 2 LazWell™ sample plate

METHOD

Instrumentation
– LDTD model S-960, Phytronix Technologies
– Agilent Texhnologies 6410 QQQ

MS Parameters

Analyte	Q1 → Q3	Dwell time (msec)	Fragmentor	CE (volt)
Methadone	310.3 → 265.1	20	135	10
Methadone-d_9	319.3 → 105.1	20	135	25
EDDP	279.2 → 234.1	20	135	30
EDDP-d_3	281.3 → 234.1	20	135	30



A) Methadone **B)** EDDP

Figure 3 Chemical structure

LDTD Parameters
– Laser power patterrn :
  – Increase laser power to 60 % in 2.0 sec.
  – Hold at 60 % for 1.5 sec.
  – Shut down laser power to
– Carrier gas flow : 3mL/min(Air)
– Deposited sample volume: 2µL

Sample Preparation
– Calibration curve, quality control and patient specimens are spiked with internal standards (Methadone-D_9 and EDDP-D_3).
– Solid Phase Extraction (SPE) was performed on urine samples using a basic solution during the elution step.
– An automated liquid handling system was used to spot the 2µL of eluate from the SPE directly into the individual wells of the LazWell plates.
– The solvent is evaporated at room temperature .

RESULTS

Calibration Curves
The calibration curves were evaluated over a nominal range of 15 to 9600 ng/ml (**Figure 4**) and representative LDTD desprotion is presented in **Figure 5**.

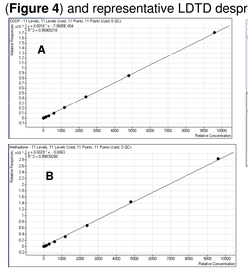


Figure 4 Calibration curve sof A) Methadone and B) EDDP

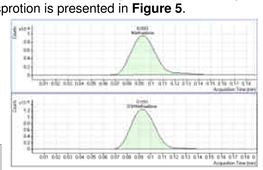


Figure 5 Representative LDTD desorption signal (Methadone and Methadone-d_9 traces).

Calibration curve (continuation)
The accuracy and reproducibility (CV) were evaluated over the calibration range (**Table 1**). The carryover was evaluated running 1 mg/mL standard solution followed by running two 100 ng/mL calibration point standard set as quality control. The 1 mg/mL solution is well in excess of any possible physiological level in human urine. No carryover was observed at the tested concentration.

Table 1 Calibration curve for the calibration curve of Methadone and EDDP

Methadone*				EDDP*			
Sample Conc. (ng/mL)	Cal. Conc. (ng/mL)	Accuracy (%)	CV (%)	Sample Conc. (ng/mL)	Cal. Conc. (ng/mL)	Accuracy (%)	CV (%)
15**	20.1	134	6.1	15**	17.6	117	11.9
30***	33.1	110	3.5	30***	30.4	101	6.3
75	63.3	84.4	1.9	75	71.0	94.7	1.5
150	130	86.7	4.3	150	139	92.7	2.3
300	266	88.7	3.7	300	283	94.3	2.4
600	551	91.8	0.8	600	583	97.2	2.5
1200	1152	96.0	3.0	1200	1174	97.8	2.8
2400	2339	97.5	0.9	2400	2419	101	4.1
4800	4797	99.9	2.7	4800	4912	102	4.2
9600	9882	103	1.9	9600	9637	100	2.0

* Data based on 6 replicates ** LOD *** LOQ

Within and Between run Precision
The within run was evaluated by extracting a known sample, spotting it 24 times and analyzed on the LDTD. The average standard deviation (SD), and the CV (%) for both Methadone and EDDP were within acceptable limits for the analysis performed (**Table 2**).

The between run precision was evaluated by running 24 samples of varied concentrations and run in triplicate (n=3). The CV (%) were calculated and the values were between 0.7 % and 13.5 % for Methadone and between 2.7 % and 19.1 % for EDDP. These CVs are for both analyte within acceptable limits.

Table 2 Within run precision on a patient specimens replicates (n=24) for Methadone and EDDP.

	Methadone	EDDP
Target Concentration (ng/mL)	2132	2956
Average Concentration (ng/mL)	2247	2735
SD (ng/mL)	66	130
CV (%)	3.0	5.0

Patient Specimens LDTD accuracy
To establish accuracy, 40 patient specimens were analyzed in GC-MS accordingly to Calloway's reference method and were also tested in-house by LDTD-MS/MS. The same calibration curve was also tested by both methods.

All the 40 patient specimens tested within the reportable range show LDTD-MS/MS and GC-MS Methadone and EDDP concentrations within ± 20 % from each other. The differences between both techniques were ranging from 0.5 % to 19.5 % for Methadone and from 0.4 % to 19.0 % for EDDP.

Moreover, two (2) samples provided for proficiency testing by the College of American Pathologists (CAP) were also analyzed by LDTD to further establish accuracy. Results show accurate results within one standard deviation of the relevant CAP UDC proficiency survey results.

Sample Stability
Extracted samples were tested over a period of 4 days to determine the stability of the solid phase extraction eluated solution (wet stability). The sample tubes were stored at 2-4ºC. We have also tested the samples' stability following their spotting on the LazWell plates and the solvent evaporation (autosampler-dry stability). The plates were stored at room temperature.

Over the tested period and under the storage conditions, the solid phase extract shows stable results. The dry samples on the LazWell plate show stable results over 3 days.

Interferences Evaluation
Various drugs were added one at a time, at a concentration of 50,000 ng/mL, to samples prepared with a Methadone/EDDP concentration of 600 ng/mL. A single extraction per drug was performed. No interference was noted for any of the following drugs: Phenylpanolamine, Pseudo-ephedrine, Ibuprofen, Lidocaine, Procaine, Ephedrine, Caffeine, Acetaminophen.

CONCLUSIONS

LDTD technology provides unique advantages in developing an ultra fast method for analysis of Methadone and EDDP in urine by the LDTD without the need of chemical derivatization. This method has demonstrated, both during validation and in production, the following characteristics:

• Fast sample-to-sample analysis: **8 seconds per sample (as compared to 15 minutes in GC-MS) .**

• Without a derivatization step, we are reducing costs associated to chemicals and hazardous materials disposal.

• The ultra fast analysis combined with the fast sample preparation **greatly improved sample throughput.**

• The results obtained in LDTD-MS/MS show excellent linearity, accuracy and reproducibility.

• No carryover