Application Note 1104

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

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## 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 derivitization and column replacement. By installing an LDTD ionization source and coupling it with a Triple Quadrupole MS/MS, an ultra fast LDTD-MS method was developed that avoids these limitations and provides additional advantages.

## Goals

- Illustrate the efficiency of the LDTD-APCI source for highly charged matrix such as urine.
- Develop a confirmation LDTD-APCI MS/MS method to detect and quantify Methadone and EDDP without chemical derivitization.

## Instrumentation

- Phytronix Technologies LDTD ionization source (model S-960)
- Agilent Technologies, 6410 Triple Quadrupole MS

## LDTD ionization process

The LDTD source used an infrared laser to desorb samples that have been dried onto stainless steel sample wells in a 96-well plate. The desorbed gas phase molecules were carried by a carrier gas into the corona discharge region for APCI and then transferred directly into the mass spectrometer.

## Samples Preparation

In this method, internal standards (Methadone-$D_9$ and EDDP-$D_3$) were added to specimens, which were buffered and extracted from the urine matrix by solid phase extraction (SPE) with elution performed using a basic solution to extract Methadone and EDDP. An automated liquid handling system was used to place 2 µL of elution solvent in each LDTD well plate.

The liquid was allowed to dry at room temperature before being introduced into the LDTD-MS/MS system for analysis.

## Results and Discussion

### *Linearity, LOD and LOQ*

The calibration curve was evaluated from 15 to 9600 ng/mL and both Methadone and EDDP displayed excellent linearity ($r^2$ > 0.998) as shown in Figures 1 and 2.

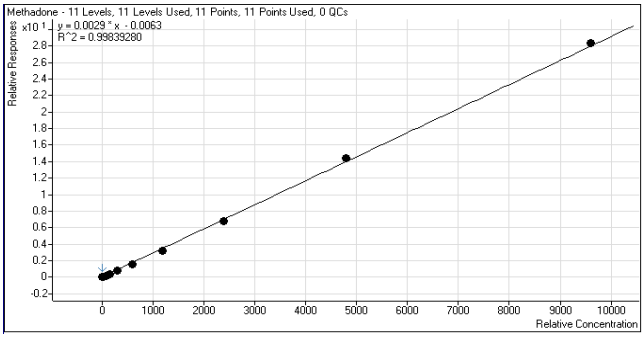


**Figure 1**. Calibration curve of Methadone in human urine.

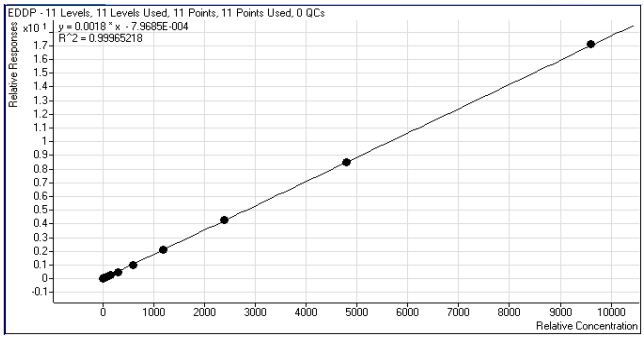


**Figure 2**. Calibration curve of EDDP in human urine.

 

From the blank signal the limits of detection and quantification were evaluated to be 15 and 30 ng/mL respectively (same values for Methadone and EDDP). The accuracy, evaluated from the back-calculated concentration, was between 84 and 134 % for Methadone and between 92 and 117 % for EDDP.

### *Within and Between-run*

The within run precision was tested by extracting a known sample, spotting it 24 times and analyzing on the LDTD. The average, standard deviation (SD) and CV for both Methadone and EDDP were within acceptable limits for the analysis performed (**Table 1**).

**Table 1** Within-run precision for specimen samples.

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

Twenty four samples of varied concentrations were tested on three different runs on the LDTD. The maximum CV for Methadone was 13.5 % and the minimum 0.7 %. The maximum CV for EDDP was 19.1 % and the minimum 2.7 %. The coefficient of variation for both Methadone and EDDP was within acceptable limits.

### *Method Accuracy*

To establish accuracy, 40 patient specimens were run in GC/MS analysis and tested in-house by LDTD-MS/MS with comparable results. Representative specimens were chosen to include both positive and negative specimens covering the range of concentration observed. All samples correlated .The maximum difference for Methadone was 19.5 % and the minimum 0.5 %. The maximum difference for EDDP was 19.0 % and the minimum 0.4 %. Moreover, two samples provided for proficiency testing by the College of American Pathologists (CAP) were also analyzed by LDTD to further establish accuracy. The samples tested using the LDTD-MS/MS method were within 1 standard deviation of the relevant CAP UDC proficiency survey results.

### *Samples Stability*

Extracted samples were tested over a period of 4 days to determine the stability of the solid phase extraction eluate and also spotted on a LazWell plate. Methadone and EDDP solid phase extraction eluate was stable for 4 days when stored in tubes and kept at 2–4° C. Stability of the sample when spotted and dried on LazWell plate is generally 3 days.

### *Carryover and Interferences*

Finally, no sample to sample carryover and no interferences from commonly available medications (Acetaminophen, Caffeine, Ibuprofen, Ephedrine, Lidocaine, Phenylpropanolamine, Procaine, and Pseudo-Ephedrine) were observed.

## Conclusion

LDTD technology provides unique advantages in developing an ultra fast method for analysis of Methadone and EDDP in urine. Moreover, The LDTD-MS/MS analysis time is 8 seconds sample to sample compared to standard GC/MS of up to 15 minutes per sample. The sample preparation is kept simple as no chemical derivitization is needed for the LDTD-MS/MS analysis, which reduces costs and hazardous materials handling. This method has demonstrated, both during validation and in clinical laboratory production since 2009, the following characteristics:

- 8 second sample to sample run time
- No carry over from sample to sample
- Extracted sample is stable for 4 days
- Excellent linearity over the calibration range
- Excellent method selectivity
- Excellent precision ranging from 3% to 5%
- Reliability in clinical production since 2009



 