

Confirmation of Propoxyphene and Norpropoxyphene in Urine by Laser Diode Thermal Desorption (LDTD)

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Introduction

Propoxyphene is a centrally acting narcotic analgesic agent. Detection and quantification of Propoxyphene, (PPX) and its metabolite Norpropoxyphene (NPPX) in urine is traditionally performed by LC/MS/MS or GC/MS analysis which are known to be time-consuming analytical techniques limiting the throughput capacity. We are proposing to use the rapid and direct sample introduction capability of a LDTD ionization source, coupled with a Triple Quadrupole MS, for the development of a robust high throughput confirmation method for Propoxyphene and Norpropoxyphene.

Goals

- Illustrate the efficiency of the LDTD-APCI source for a highly charged matrix such as urine.
- Develop a confirmation LDTD-APCI MS/MS method to detect and quantify Propoxyphene and Norpropoxyphene in less than 14 seconds per sample.

Instrumentation

- Phytronix Technologies LDTD ionization source (model S-960)
- AB SCIEX, API 4000™ System

LDTD ionization process

The LDTD source used an infrared laser to desorb samples that had been dried onto a stainless steel sample well 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.

Sample Preparation

A Solid Phase Extraction (SPE) was performed with elution using a basic solution to extract Propoxyphene and Norpropoxyphene from human urine. An

automated liquid handling system was used to place 2 μ L 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 25 to 12,800 ng/mL and both PPX and NPPX exhibited excellent linearity ($r^2 > 0.99$) as shown in Figures 1 and 2.

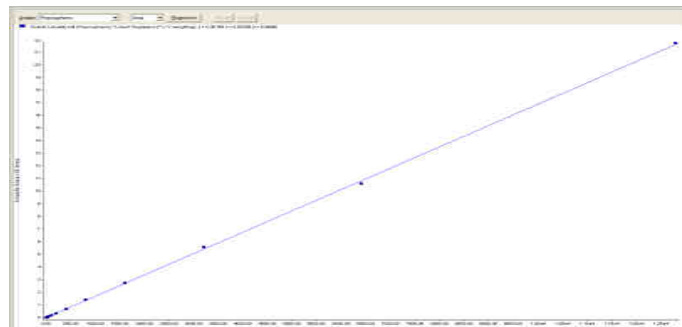


Figure 1. Calibration curve of Propoxyphene in human urine.

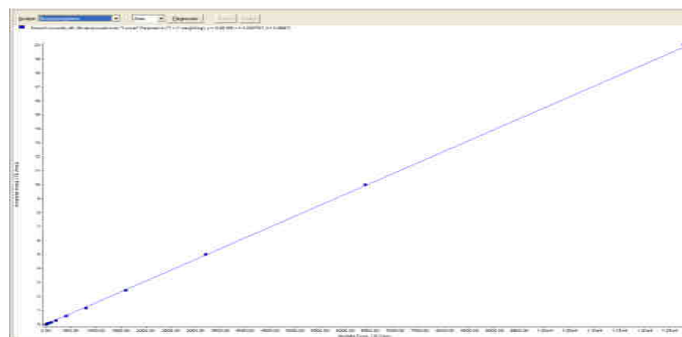


Figure 2. Calibration curve of Norpropoxyphene in human urine.

From the blank signal, the limits of detection and quantification were evaluated at 25 and 50 ng/mL respectively (same values for PPX and NPPX). The upper limit of linearity was established at 12,800 ng/mL and the carryover limit was evaluated over 1,000,000 ng/mL. The accuracy, evaluated from the back-calculated concentration was between 93.3 and 109 % for PPX and between 90.9 and 114 % for NPPX.

Within and Between-run Precision

The within-run was evaluated by running 15 replicates of 3 specimens (real samples) at low, middle and high concentrations. As shown in **Table 1**, the precision was excellent at all concentrations for both PPX and NPPX (CV ≤ 6.5 %).

Table 1 Specimen sample within-run precision

	PPX	NPPX	
Sample 1	Average Concentration (ng/mL)	182.9	1729.2
	SD (ng/mL)	11.9	33.7
	CV (%)	6.5	1.9
Sample 2	Average Concentration (ng/mL)	459.9	12,600
	SD (ng/mL)	12.8	233.5
	CV (%)	2.8	1.9
Sample 3	Average Concentration (ng/mL)	3395.8	21,733.3
	SD (ng/mL)	71	465.8
	CV (%)	2.1	2.1

The between-run precision was evaluated by running 40 specimen samples on 3 different batches. The calculated CVs were below 6.3 % for PPX and 3.2 % for NPPX.

Method Accuracy

To establish accuracy, 40 patient specimens were sent to a reference laboratory for GC/MS analysis, tested in-house by LDTD and the results compared. The reference laboratory had a reportable range of 100

ng/mL to 4000 ng/mL as compared to the 50 ng/mL to 12,800 ng/mL range established by the LDTD. All samples within the reportable range correlated quantitatively within ±20 %. All negative samples correlated qualitatively. Moreover, samples provided for proficiency testing by the College of American Pathologists (CAP) were also analyzed by LDTD to further establish accuracy. Results were all within the acceptable CAP published range.

Sample Stability, Carryover and Interferences

The wet stability was evaluated to within 4 days and the LazWell plate (dry sample) illustrated stability at 3 days. 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 Propoxyphene and Norpropoxyphene in urine. Moreover, the LDTD-MS/MS analysis time is 12 seconds sample to sample compared to standard GC/MS time 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:

- 12 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 2% to 6%
- Reliability in clinical production since 2009

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