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

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OVERVIEW

Purpose

– Propoxyphene and Norpropoxyphene 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 : 12.5 to 12 800 ng/ml.
– LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer.

Results

• Excellent linearity over the calibration range ($R^2 > 0.99$).
• LOD and LOQ set at 25 and 50 ng/mL respectively.
• Excellent accuracy within ± 20 % as compared to a reference laboratory method.
• Excellent precision (within and between-run) ≤ 6.3 %.
• No carryover below 50 000 ng/mL.
• No interference observed with common medication.

INTRODUCTION

Propoxyphene is a centrally acting narcotic analgesic agent. Detection and quantification of Propoxyphene and Norpropoxyphene (its metabolite) in urine is traditionally performed by LC/MS/MS or GC/MS analysis. The novel technology of the LDTD system initiates thermal desorption of analytes by use of an infrared laser, generating neutral molecules in the gas phase that have been deposited onto a specially designed 96-well plate. The desorbed sample is transported by a carrier gas (air) through a transfer tube to a corona discharge region to be ionized and then introduced into the mass spectrometer. We propose to validate a confirmation method for Propoxyphene and Norpropoxyphene in urine at a concentration ranging from 12.5 to 12800 ng/mL.

LDTD (Figure 1)

– Plug-and-play ionization source interface to AB Sciex API 4000 Triple quadrupole.
– 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.

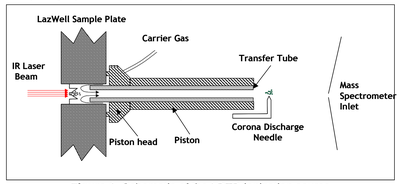


Figure 1 Schematic of the LDTD ionization source.

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.

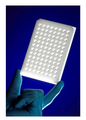


Figure 2 LazWell™ sample plate

METHOD

Instrumentation

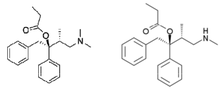
– LDTD model S-960, Phytronix Technologies
– AB Sciex API 4000

MS Parameters

Analyte	Q1 → Q3	Scan time (msec)	DP (volt)	CE (volt)
Propoxyphene	340.4 → 266.2	50	60	15
Norpropoxyphene	326.6 → 252.0	50	60	10
Propoxyphene-d_5	345.4 → 271.6	50	50	13
Norpropoxyphene-d_5	331.5 → 257.5	50	50	10

LDTD Parameters

– Laser power pattern :
  – Increase laser power to 35 % in 2.0 sec.
  – Hold at 35 % for 2.0 s
  – Shut down laser power to 0 %
– Carrier gas flow : 3 L/min (Air)
– Deposited sample volume: 2 µL



A) Propoxyphene **B)** Norpropoxyphene

Figure 3 Chemical structure

Sample Preparation

– Calibration curve, quality control and patient specimens are spiked with internal standards (Propoxyphene-D_5 and Norpropoxyphene-D_5).
– 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 12.5 to 12 800 ng/ml (**Figure 4**).

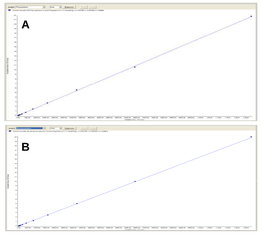


Figure 4 Calibration curves of A) Propoxyphene and B) Norpropoxyphene

Calibration curve (continuation)

The linearity and accuracy were excellent (**Table 1**) and the following calibration curve analytical parameters were obtained (**Table 2**). The carryover was evaluated running 1 mg/mL standard solution followed by 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 in LDTD-MS/MS under these conditions.

Table 1 Accuracy calculated for the calibration curve of Propoxyphene and Norpropoxyphene

Propoxyphene			Norpropoxyphene		
Sample Name	Cal Conc (ng/mL)	Accuracy (%)	Sample Name	Cal Conc (ng/mL)	Accuracy (%)
12.5	13.6	109	12.5	14.3	114
25	23.3	93.3	25	24.2	96.7
50	48.8	97.6	50	58.5	117
100	95	95.0	100	90.9	90.9
200	196	98.2	200	184	91.8
400	395	98.6	400	360	89.9
800	830	104	800	818	102
1600	1680	105	1600	1550	96.6
3200	3220	101	3200	3190	99.7
6400	6440	101	6400	6420	100
12,800	12,600	98.8	12,800	12,900	101

Table 2 Calibration curve parameters for Propoxyphene and Norpropoxyphene.

Analytical Parameters Established by LDTD				
Analyte	Upper Limit of Linearity*	LOQ*	LOD*	Carryover Limit*
Propoxyphene	12,800	50	25	>1,000,000
Norpropoxyphene	12,800	50	25	>1,000,000

*** Unit = ng/mL**

Within and Between run Precision

The within run was evaluated running 3 patient specimens 15-times. The precision was below 6.8 % for both analytes (**Table 3**). The between run precision was evaluated by running 40 patient specimens 3-times each. The average CV was 6.3 % for Propoxyphene and was 3.2 % for Norpropoxyphene.

Table 2 Within run precision on 3 patient specimens replicates for Propoxyphene and Norpropoxyphene.

		Propoxyphene	Norpropoxyphene
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

Patient Specimens LDTD accuracy

To establish accuracy, 40 patient specimens were sent to a reference laboratory for GC-MS analysis. The same samples were also tested in-house by LDTD. The same calibration curve was also tested by both methods to determine any quantitation bias and establish a correction factor if needed. A limiting factor in this correlation was the difference in reportable range: the reference laboratory has 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 on the LDTD.

All the 40 patient specimens tested within the reportable range show LDTD-MS/MS and GC-MS Propoxyphene and Norpropoxyphene concentrations within ± 20 % from each other. Moreover, all negative samples were reported as negative from both analytical techniques.

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

After SPE, eluate stored in capped glass test tubes at 2–4°C proved stable for a period of at least 4 days: 28 patient and control samples were removed from tubes and analyzed once a day for 4 days, yielding statistically similar results with CVs of less than 20%.

Eluate stored on a Phytronix LazWell plate (autosample stability test) at room temperature also proved stable over a period of 4 days: samples comprised of a full calibration curve were spotted on a new plate once a day for a period of 4 days. On day 4, all plates were analyzed and results were statistically similar to the original results with CVs of less than 20%.

Interferences Evaluation

Various drugs were added one at a time, at a concentration of 50,000 ng/mL, to samples prepared with a Propoxyphene/Norpropoxyphene concentration of 7500 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 Propoxophene and Norpropoxyphene in urine by the LDTD. This method has demonstrated, both during validation and in production, the following characteristics:

• Reproducibility comparable to reference laboratory GC/MS analysis
• Superior sensitivity and improved linear range compared to reference laboratory GC/MS analysis
• Excellent precision ranging from 2% to 6%
• Fast sample-to-sample analysis: **14 seconds per sample**
• No carryover