Quantitative Screening Method for Analysis of Drugs of Abuse in Urine Using LDTD with Benchtop Quadrupole-Orbitrap Mass Spectrometer in Forensic Toxicology

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Overview

Purpose: Evaluation of a laser diode thermal desorption (LDTD) ion source coupled to a high-resolution, accurate-mass (HRAM) mass spectrometer for ultrafast screening of drugs of abuse in urine, for forensic toxicology.

Methods: Samples were extracted by liquid-liquid extraction (LLE), plated onto a 96 well LazWell™ plate, and introduced into the HRAM MS using a Phytronix™ LDTD™ ion source.

Results: LLE with LDTD met cutoff requirements for drugs-of-abuse screening in urine for the representative compounds tested.

Introduction

Rapid screening is critical for many forensic toxicology laboratories. Laser diode thermal desorption coupled with high-resolution, accurate-mass mass spectrometry was evaluated to support this application. Sample analysis with LDTD is on the order of seconds as opposed minutes for LC-MS, allowing for rapid generation of results. Since chromatographic separation is eliminated by using LDTD, HRAM adds the selectivity required to analyze compounds all simultaneously introduced in to the MS by the LDTD source. It also adds selectivity to the full scan data collected to support a practically unlimited number of compounds in the method. The question investigated in this study was whether LDTD can offer limits of detection comparable to a traditional LC-MS approach and compatible with the required cutoffs for urine screening.

Methods

Sample Preparation

Samples were prepared by enzymatic hydrolysis followed by liquid-liquid extraction. Calibration standards (1-500 ng/mL) were prepared in negative urine. Briefly, 1 mL of urine (spiked calibrator) was spiked with internal standard, and incubated with 0.5 mL of 10,000 U/mL beta-glucuronidase enzyme in pH 5.5 buffer for 60 minutes at 60 °C. The resulting mixture was basified with 0.5 mL of 1M sodium carbonate and extracted with 6 mL of ethylacetate:hexane (1:1). The organic supernatant was evaporated to dryness under nitrogen at 37 °C. The residue was reconstituted in 100 µL of 50% methanol. A 96 well LazWell plate was prepared by depositing 5 µL of a 20 µg/mL EDTA solution in methanol:water:ammonium hydroxide (75:20:5) on to the plate and drying. Then 2 µL of processed urine was aliquoted onto the same plate and again dried. Samples were introduced in the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer by thermal desorption from the plate using a Phytronix LDTD source.

LDTD

The LDTD carrier gas flow was set at 3 L/min and the laser pattern was an energy ramp from 0 to 65% in 6 seconds followed by an immediate return to zero (Figure 1). The total acquisition time was ten seconds. Details of the LDTD process are shown in Figure 2.

Mass Spectrometry

The Q Exactive method consisted of full scans at a resolution of 35K. Analytes were quantitated using a mass tolerance of 5 ppm. Other Q Exactive source parameter settings are listed in Table 1.

Data Analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software version 3.2.

Calibration ranges, LODs and LOQs were evaluated based on concentration accuracy. Back-calculated concentrations had to be within 30% for these parameters.

FIGURE 1. LDTD energy pattern and gas flow.

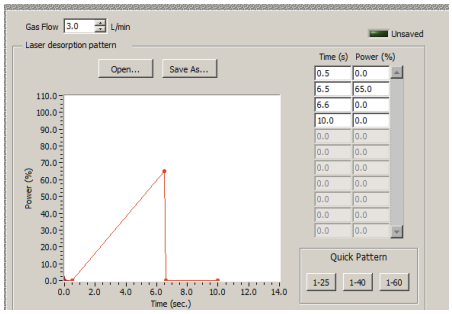


Table 1. Q Exactive source parameters.

Parameter	Value
Sheath Gas	0
Aux Gas	0
Sweep Gas	0
Discharge Current (µA)	3
Capillary temp. (°C)	300
S-lens RF level	50.0
Vaporizer temp. (°C)	0 (off)

FIGURE 2. Schematic showing the LDTD process.

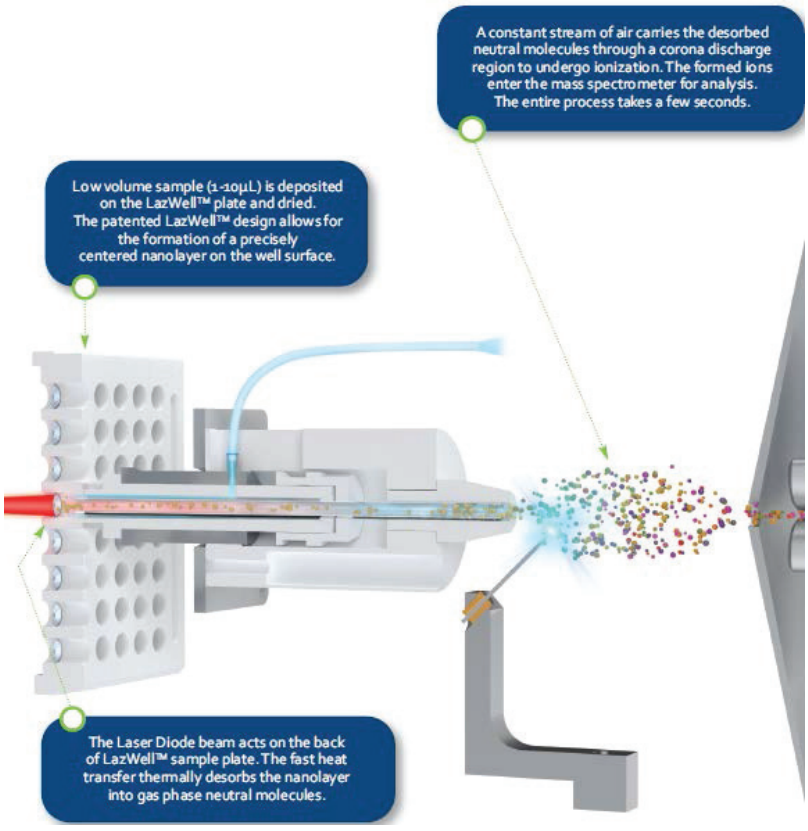
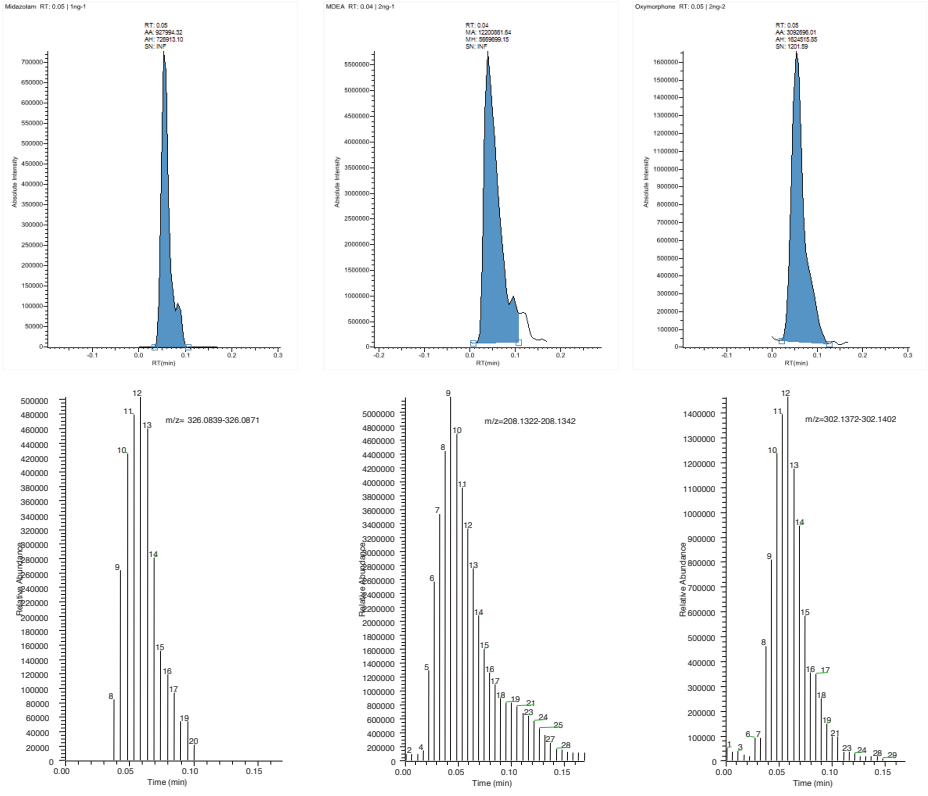


Table 2. Limits of quantitation obtained with LDTD source on Q Exactive mass spectrometer.

Compound	Cut-Off	LOQ	Compound	Cut-Off	LOQ
6-MAM	25	5	Methadone	25	25
7-aminoclonazepam	20	5	Methamphetamine	50	2*
Alprazolam	20	0.5	Midazolam	20	1
Amphetamine	50	5	Morphine	25	2*
Buprenorphine	25	2	Norbuprenorphine	25	10
Clonazepam	20	2	Nordiazepam	20	1
Clorazepate	20	1	Normeperidine	25	2
Codeine	25	2*	O-desmethyltramadol	25	1
Diazepam	20	2	Oxazepam	20	1
Dihydrocodeine	25	2	Oxycodone	25	10
EDDP	25	10	Oxymorphone	25	2
Fentanyl	25	1	Prazepam	20	1
Flurazepam	20	1	Ritalinic Acid	N/A	5
Lorazepam	20	5	Tapentadol	25	2
MDA	50	10	Temazepam	20	2
MDEA	50	5	Tramadol	25	2
MDMA	50	10	Triazolam	20	1
Meperidine	25	2			

FIGURE 3. LDTD extracted chronograms of selected compounds at LOQ in point and stick mode.



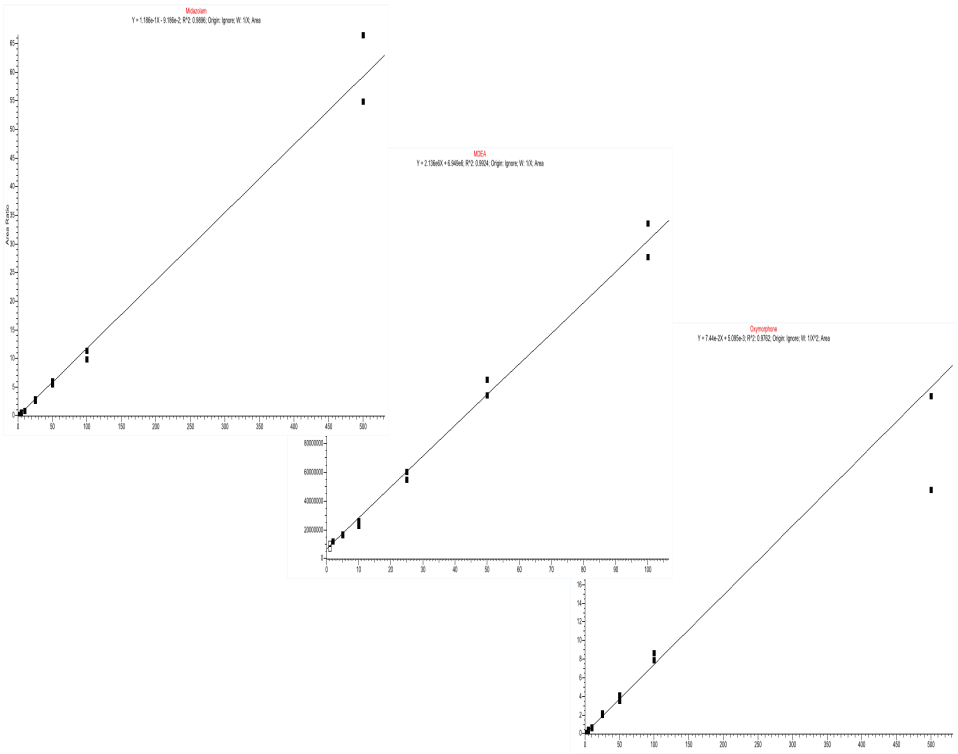
Results

Limits of quantitation, using parent compound full-scan data, as determined by back-calculated concentration difference of less than 30%, were all within standard cutoffs required for this method. The LOQs ranged from about 0.5 to 25 ng/mL for the various compounds (Table 2). Figure 3 shows representative chronograms extracted with a mass tolerance of 5 ppm for representative compounds at their respective LOQs. The chronograms are also shown in stick mode, showing at least 10 scans across the short acquisition window. Figure 4 shows calibration curves for the same compounds.

While the acquisition time was 10 seconds per sample, the total analysis time was 20 seconds per sample. This time can be decreased with available software that saves multiple samples in one data file as opposed to one data file per sample.

Since LDTD is a direct analysis technique and does not separate compounds chromatographically, the method would not be specific for isobaric compounds such as morphine/hydromorphone and codeine/hydrocodone.

Figure 4. Representative calibration curves for midazolam, MDEA and oxymorphone.



Conclusion

- LDTD coupled to HRAM-MS offer ultra-fast sample analysis that meets required cut-offs for forensic toxicology.
- HRAM allows for detection and retrospective data analysis of a virtually unlimited number of compounds.
- Future work includes optimization of sample processing methods and further validation.

Acknowledgements

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