Evaluation of PaperSpray Ionization Source for Screening of Drugs of Abuse in Urine Coupled to HRAM Mass Spectrometer

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Overview

Purpose: To evaluate a paper spray ionization source coupled to an Orbitrap-based HR-MS/MS for rapid analysis of drugs of abuse. To compare results to those obtained with a conventional HPLC-HR-MS/MS method.

Methods: Urine samples spotted directly to a paper cartridge, dried and automatically processed for electrospraying from paper. Thermo Scientific HR-MS and MS/MS analyzed from inclusion list.

Results: Screening of drug panels, four groups of 10 drugs per sample cartridge, were rapidly analyzed by PaperSpray® technology.

Introduction

Forensic toxicologists are always looking for quicker and easier analytical tools to generate fast and accurate results. Immunoassays are fairly quick and easy, but lack selectivity within drug classes. Liquid chromatography mass spectrometry (LCMS) offers greater selectivity, but can be more cumbersome and time consuming both in sample preparation and analysis time. Paper spray is a direct ionization technique that can provide results within one minute per sample and does not require sample preparation. Since it is a direct ionization source, coupling it to a high-resolution tandem mass spectrometer (HR-MS/MS) improves method selectivity.

Methods

Sample Preparation

• Urine samples were fortified with EDDP-d3 internal standard and 6 μL of urine was spotted directly onto a Velox Sample Cartridge (Prosolia, IN).

• The samples were dried at room temperature for 20 min.

• Limits of detection samples were analyzed in 4 panels, composed of 10 analytes per panel plus one IS (Table 1).

Note: Only one deuterated analog was used as internal standard since screening of DoA was the intent of this study.

Mass Spectrometry

• Cartridges were loaded onto a Velox 360™ PaperSpray source (Prosolia, IN) for sample introduction into a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer.

• PaperSpray solvent used for analyte extraction from dry urine in paper was 90/10/0.1 acetonitrile/H_2O/acetic acid.

• The Q Exactive mass spectrometer was operated in full-scan data-dependent MS^2 mode. In this mode, high-resolution, full-scan data at resolution of 70k were collected and then MS^2 spectra at a resolution of 17.5k were triggered for compounds entered in the inclusion list.

Methods (cont.)

Data Processing

• Data were acquired with Thermo Scientific™ TraceFinder™ software, version 3.2 and analyzed with Thermo Scientific™ ToxFinder™ software, version 1.0.

• ToxFinder software identified compounds based on exact mass of precursor, isotopic pattern and MS^2 spectra. Semi-quantitation can be performed either by using a single point calibrator or by using internal standard ratio.

• Additionally quantitative capabilities of PaperSpray source were demonstrated by calculating calibration curve for EDDP using EDDP chronographic peak reconstructed with mass accuracy of 5 ppm (full scan data).

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Method performance evaluation

Pooled donor urine was spiked with 40 compounds in groups of 10 (Table 1) at concentrations of 1, 5, 10, 50, 100 and 500 ng/mL. Two limits of detection were evaluated:

- Limit of detection based on chronogram peak area above specified threshold
- Limit of detection based on chronogram peak area above specified threshold, isotopic pattern confirmation and MS/MS spectra confirmation.

Table1. Drugs of Abuse (DoA) screen panels analyzed in this study and the class of drug.

Analyte	Screen Panel	Class of Drug	Analyte	Screen Panel	Type of Drug
Amphetamine	1	Stim/Amph*	Codeine	3	Opiates
Butylone	1	Stim/Amph	EDDP	3	Opiates
Cathinone	1	Stim/Amph	Fentanyl	3	Opiates
Cotinine	1	Stim/Amph	Methadone	3	Opiates
MDPV	1	Stim/Amph	Morphine	3	Opiates
Methamphetamine	1	Stim/Amph	Naloxone	3	Opiates
Methedrone	1	Stim/Amph	Naltrexol	3	Opiates
Methylone	1	Stim/Amph	Norfentanyl	3	Opiates
Nicotine	1	Stim/Amph	Oxycodone	3	Opiates
Pseudoephedrine	1	Stim/Amph	Oxymorphone	3	Opiates
Alprazolam	2	Benzos**	Amitriptyline	4	TCAs***
Diazepam	2	Benzos	Clomipramine	4	TCAs
α-OH-alprazolam	2	Benzos	Desipramine	4	TCAs
Nordiazepam	2	Benzos	Desmethyl clomipramine	4	TCAs
Oxazepam	2	Benzos	Desmethyl doxepin	4	TCAs
Temazepam	2	Benzos	Dothiepin	4	TCAs
Tramadol	2	Benzos	Doxepin	4	TCAs
Zaleplon	2	Benzos	Imipramine	4	TCAs
Zolpidem	2	Benzos	Nortriptyline	4	TCAs
Zoplicone	2	Benzos	Trimipramine	4	TCAs

* Stimulants/amphetamines/cathinones
** Benzodiazepines
*** Tricyclic Antidepressants

Results

Limits of Detection (LOD) based on presence of chronogram peak ranged from 1-100 ng/mL and were in the range of 1-5 ng/mL for most of the compounds analyzed (Table2). Figure 1 shows methamphetamine chronogram peaks at concentrations from 1 to 500 ng/mL in pooled urine.
Limits of Detection Confirmed (LODC) with isotopic pattern or MS/MS spectra ranged from 5 to 500 ng/mL (Table 2).
An example of analyte identification in a urine sample using ToxFinder software is presented at Figure 2.
Calibration curve calculated for EDDP by isotope dilution ranged from 1-500 (Figure 3).

Table 2. Limits of detection with and without confirmation for analyzed compounds in pooled donor urine.

Analyte	LOD	LODC	Analyte	LOD	LODC
Amphetamine	1	50	Codeine	50	100
Butylone	1	10	EDDP	1	5
Cathinone	1	50	Fentanyl	1	10
Cotinine	1	50	Methadone	1	5
MDPV	5	10	Morphine	100	500
Methamphetamine	1	50	Naloxone	50	100
Methedrone	1	10	Naltrexol	50	100
Methylone	1	50	Norfentanyl	10	10
Nicotine	1	50	Oxycodone	50	500
Pseudoephedrine	1	50	Oxymorphone	100	500
Alprazolam	5	10	Amitriptyline	5	50
Diazepam	5	5	Clomipramine	10	50
α-OH-alprazolam	5	50	Desipramine	5	50
Nordiazepam	5	5	Desmethyl clomipramine	10	10
Oxazepam	5	5	Desmethyl doxepin	5	10
Temazepam	5	50	Dothiepin	10	50
Tramadol	1	5	Doxepin	5	50
Zaleplon	5	500	Imipramine	50	50
Zolpidem	1	5	Nortriptyline	5	5
Zoplicone	100	500	Trimipramine	5	5

Figure 1. Chronograms of methamphetamine in pooled donor urine.

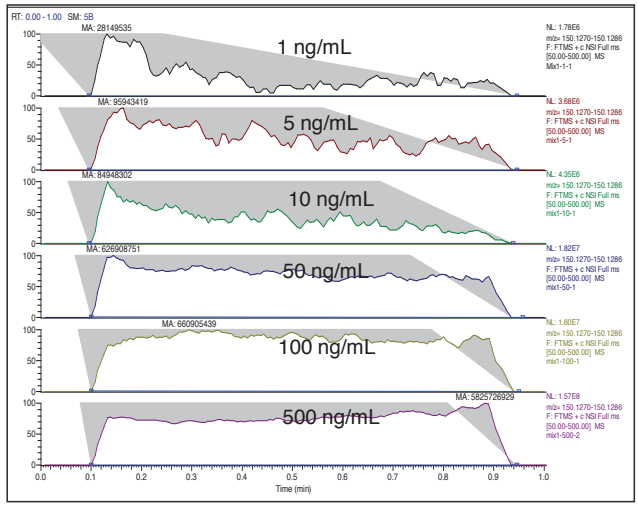


Figure 2. Diazepam at 50 ng/mL in pooled donor urine identified and confirmed with ToxFinder software (screen capture from data review page). Confirmation was based on isotopic distribution and spectral library matching of fragmentation data.

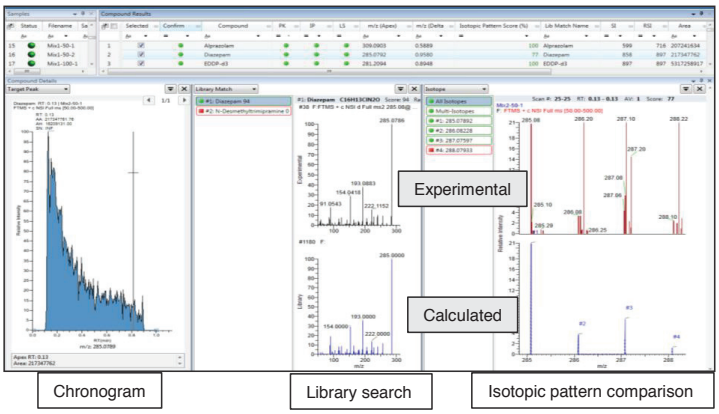
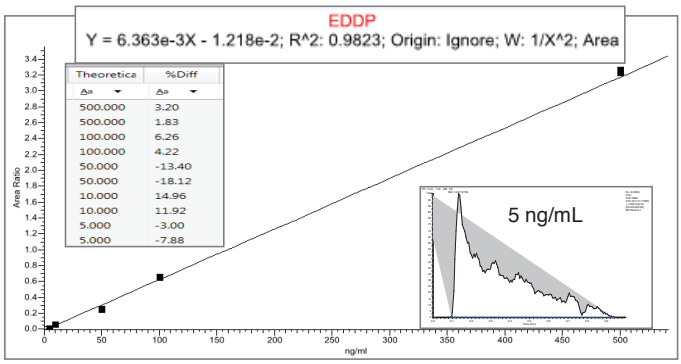


Figure 3. Calibration curve for EDDP in pooled donor urine.



Conclusion

- We have shown an easy to use technique (no sample preparation, no chromatography) that shows extraordinary potential for identifying drugs of abuse in urine samples.
- The current paper spray technology is a good screening tool for amphetamines, benzodiazepines, and tricyclic antidepressants. These drugs ionize efficiently with the cellulose-based paper substrate and used solvent. The technique is less suitable for opiates analysis under the stated conditions.
- The calibration curve calculated for EDDP demonstrated semi-quantitative capabilities of PaperSpray source.

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