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Drugs of Abuse Screening in Urine and Quantification from Whole Blood using PaperSpray Technology for Forensic Use

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ABSTRACT

Purpose: Rapid drugs of abuse screening from dried urine spots and EDDP direct quantification from whole blood spots using PaperSpray technology.

Methods: A new high-throughput and automated PaperSpray™ system was used to generate data for 20 drugs of abuse for screening. For EDDP a one-minute quantitative method was developed and tested for whole blood quantitation.

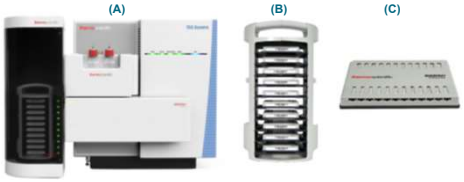
Results: Screening cutoffs were met for all 20 compounds, and quantification of EDDP in whole blood produced linear, precise, and accurate results with minimal sample preparation. The lower limits of quantification (LLOQ) for EDDP in both blood and urine were 3.5 ng/mL.

INTRODUCTION

PaperSpray technology is a rapid analysis technology specifically suitable for forensic samples. Quick sample turnaround times of 2 minutes or less make it very competitive compared to traditional LC/MS-based techniques. Minimal sample preparation is required for analysis of dried urine or blood spots from a piece of triangular-shaped paper. A rewet solvent is applied directly onto the dried sample spot to extract analytes. Next, a spray solvent is dispensed onto the paper, and a high voltage is applied to the paper to facilitate spray and ion formation.

The new Thermo Scientific™ VeriSpray™ system uses PaperSpray technology to make forensic workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. Using the new Thermo Scientific VeriSpray system, up to 240 samples can be analyzed in an unattended fashion. The VeriSpray Plate Loader and magazine holds up to 10 VeriSpray sample plates. Each VeriSpray sample plate is equipped with 24 paper strips. Figure 1 shows a picture of the VeriSpray system, magazine and VeriSpray sample plate.

Figure 1. (A) VeriSpray ion source and plate loader mounted to Thermo Scientific™ TSQ Quantis™ mass spectrometer (B) VeriSpray magazine and (C) VeriSpray sample plate



Here we present a method for rapid screening of 20 drugs of abuse directly from dried urine spots. We then developed a one-minute analytical method by paper spray-mass spectrometry that detects EDDP, a primary opioid metabolite of methadone, in whole blood and that eliminates the need for sample preparation and enables quick turnaround times needed for routine compliance testing.

MATERIALS AND METHODS

Sample Preparation

For the screening method, a total of 20 compounds from the following compound classes: opiates, amphetamines, cocaine and PCP, were spiked into human donor urine. Four concentration levels were prepared: at cutoff, 2 times higher than cutoff, 5 times lower than cutoff, and blank. Table 1 lists the compounds with their respective cutoff concentration, and the two additional concentrations prepared below and above cutoff. Isotopically labelled internal standards were added. Eight microliters of each respective urine sample was spotted onto VeriSpray sample plates for analysis.

For the quantitation method, whole blood samples were spiked with EDDP, and with internal standard EDDP-d3. Two sets of 240 samples of EDDP in blood (2 magazines, 480 samples total), consisting of calibrators, controls and robustness sample were spotted on to the sample plates (spotting volume is 5 µL for whole blood).

Sample plates were oven-dried at a temperature of 45 ° C for 5 mins and 30 mins for urine and whole blood, respectively.

Table 1. Compounds and concentrations prepared for screening

Compound	Low (ng/mL) 5x below cutoff	Cutoff (ng/mL) at cutoff	High (ng/mL) 2x above cutoff
Oxycodone	20	100	200
Clonazepam	40	200	400
Methadone	30	150	300
Alprazolam	40	200	400
Cocaine	20	100	200
Oxymorphone	20	100	200
Codeine	400	2,000	4,000
Hydrocodone	20	100	200
Benzoylecgonine	20	100	200
Oxazepam	40	200	400
Morphine	400	2,000	4,000
Diazepam	40	200	400
Nordiazepam	40	200	400
PCP	5	25	50
MDMA	50	250	500
MDA	50	250	500
Methamphetamine	50	250	500
Amphetamine	50	250	500
Hydromorphone	20	100	200
EDDP	14*	100	200

* Please note that for EDDP, the 'below cutoff' concentration was approx. 7 times below cutoff.

PaperSpray Conditions

For the screening method in urine, rewetting (20 µL) and spray (110 µL) solvents were 90/10 Acetonitrile/Water 0.01 % acetic acid, for the EDDP quantitation method in whole blood, rewetting (20 µL) and spray (110 µL) solvents were 95/5 Methanol/Water 0.01% acetic acid.

Mass Spectrometry

Both the screening and the quantitation methods were carried out on a Thermo Scientific™ TSQ Quantis™ mass spectrometer connected to the Thermo Scientific VeriSpray system. Three transitions were monitored per compound, with a cycle time of 1.5 seconds (screening assay) or 0.8 seconds (quantification) and a collision gas pressure of 2 mTorr. Table 2 presents the corresponding SRM transitions for EDDP. The spray voltage was 3.8 kV, applied from 0.1 to 0.9 min, the inlet capillary temperature was 325 ° C (screening assay) or 350 ° C (quantification). The paper tip to MS inlet distance was set to 5 mm for screening and 6.5 mm for the robustness study to maintain system robustness without compromising the system sensitivity. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

Table 2. Optimized MS transitions for EDDP on TSQ Quantis MS

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
EDDP	278.29	234.21	31.5	206
	278.29	158.21	44.9	206
	278.29	186.21	35.7	206
EDDP-d3	281.29	234.21	32.0	202
	281.29	157.18	52.3	202
	281.29	189.21	36.5	202

RESULTS

Screening Assay

The criteria for whether the cutoff level of the respective compound is met, are as follows:

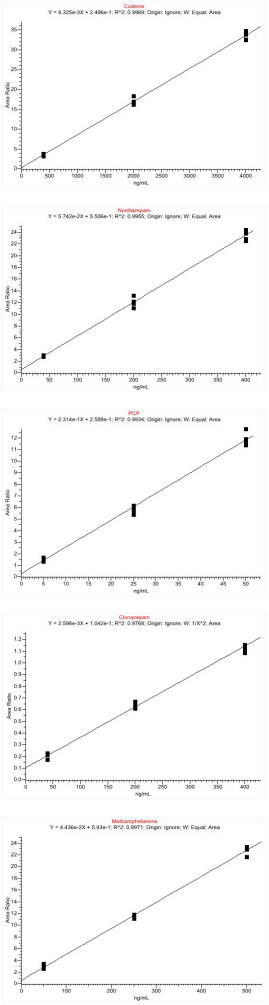
Area Under the Curve (AUC) response at the cutoff level must be at least 4 times that of the matrix blank. Table 3 shows the S/N (signal-to-noise) ratios of each compound at the cutoff level. All 20 compounds met the screening criteria.

Table 3. AUC counts and S/N ratios at the respective cutoff level

Compound	AUC at cutoff	AUC matrix blank	S/N ratio
Oxycodone	185,630	21,235	8.7
Clonazepam	487,321	86,841	5.6
Methadone	80,066,278	156,898	510.3
Alprazolam	5,593,279	25,924	215.8
Cocaine	21,482,707	52,199	411.6
Oxymorphone	245,704	27,850	8.8
Codeine	7,714,065	27,537	280.1
Hydrocodone	3,233,438	28,707	108.9
Benzoylecgonine	3,737,256	32,319	115.6
Oxazepam	691,260	18,350	37.7
Morphine	2,497,123	18,172	137.4
Diazepam	5,729,539	54,682	104.8
Nordiazepam	1,919,336	29,434	65.2
PCP	4,375,303	169,618	25.8
MDMA	14,237,904	36,872	386.1
MDA	1,014,502	166,613	6.1
Methamphetamine	27,860,611	660,915	42.2
Amphetamine	7,105,503	1,454,938	4.9
Hydromorphone	1,388,447	41,053	33.8
EDDP	19,589,305	89,437	219

Semi-quantitative calibration curves for selected compounds of different compound classes are shown in Figure 2. Deuterated internal standards were added for each compound.

Figure 2. Selected semi-quantitative calibration curves in urine



Quantification Assay: EDDP in human whole blood

We were able to achieve linear range of 3.5 – 500 ng/mL for EDDP in whole blood. The lower limit of quantification (LLOQ) on the TSQ Quantis mass spectrometer for EDDP was 3.5 ng/mL, as defined as the lowest calibration standard analyzed that yielded < 20% accuracy and < 15% CV for 4 replicate samples. The overlaid 4 calibration curves are shown in Figure 3. All four calibration curves yielded the same LLOQ for EDDP. Accuracy and CV for calibration points are presented in Table 4.

Figure 3. Overlaid 4 calibration curves of EDDP in human whole blood showing excellent reproducibility. All calibration curves gave the same LLOQ of 3.5 ng/mL for EDDP in whole human blood

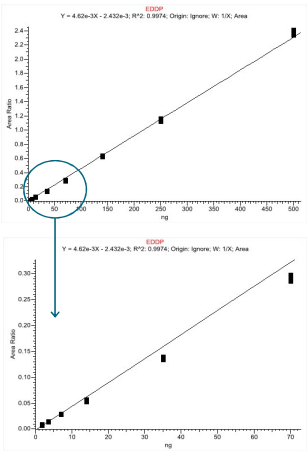
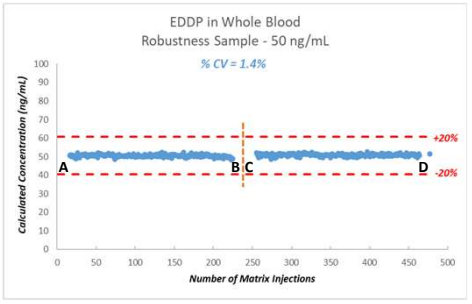


Table 4. Precision and accuracy of the EDDP calibrators in human whole blood

Theoretical Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy	%RSD
3.5	3.64	4.1	2.6
7	6.79	-3.0	2.2
14	12.3	-12.1	3.8
35	30.2	-13.6	1.7
70	63.3	-9.6	1.9
140	138	-1.8	1.2
250	249	-0.3	1.5
500	516	3.2	1.4

The precision and accuracy of the robustness sample, 50 ng/mL, met the method requirements and had an RSD of 1.4% for the calculated concentration throughout 480 samples. Wiping the ion transfer tube with a disposable wipe soaked with water and methanol was enough to remove all visible traces of blood and produced reproducible data between the first 240 injections (first full magazine) and latter 240 injections (second full magazine). Results of this study can be observed in Figure 3.

Figure 3. Precision of EDDP robustness sample in whole blood on TSQ Quantis MS over 480 samples. The orange line represents the end of first set of 10 sample plates, at which point the ion transfer tube was cleaned externally by wiping with a disposable wipe saturated with a mixture of water: methanol (1:1). The points A, B, C and D represent where in the sequence calibration curves were run.



CONCLUSIONS

- PaperSpray mass spectrometry is an alternative or complimentary method for many forensic applications.
- PaperSpray technology proved here to be suitable for fast drugs of abuse screening, because of short analysis times and the ability to analyze many compounds within a 1-minute window.
- This technology can also be further applied for the confirmation and quantification in whole blood.
- We have also demonstrated that this technology is robust and able to run for an extended period of time without the need for maintenance and with no significant loss in signal, both critical requirements for any routine analytical method.
- The new VeriSpray ion source makes PaperSpray analysis easy, fast, and more automated than previous systems.

TRADEMARKS/LICENSING

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