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Drugs of Abuse Screening and Quantification Directly from Urine Using Paper Spray Technology for Clinical Research

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ABSTRACT

Purpose: Rapid drugs of abuse screening and quantification directly from dried urine spots using paper spray technology.

Methods: A new high-throughput and automated paper spray system was used to generate data for 19 drugs of abuse for screening, and two drugs/metabolites for quantification.

Results: Screening cutoffs were met for all 19 compounds, and quantification of cocaine and benzoylecgonine produced linear, precise, and accurate results with minimal sample preparation.

INTRODUCTION

Paper spray technology is a rapid analysis technology specifically suitable for clinical research 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 the 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 clinical research workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 1, left). The VeriSpray plate loader magazine holds up to 10 VeriSpray sample plates (Figure 1, right). Each VeriSpray sample plate contains 24 paper strips (12 on each side, A and B). Through Thermo Scientific™ Xcalibur™ software sequence setup, the full magazine can be run in an automated way.

Figure 1. VeriSpray ion source with VeriSpray plate loader (left) and VeriSpray sample plate (right).

Screening methods often require a large number of compounds to be run in a short amount of time. In LC/MS, peak widths are often on the order of a few seconds, which can make it difficult to achieve a sufficient number of scans across the peak per compound when running large panels. Using PaperSpray, the acquisition time is typically 1-2 minutes. During this time, all compounds elute off of the paper at the same time. This provides an increased time window to scan and detect compounds.

Here, we demonstrate a method for screening 19 drugs of abuse of different compound classes directly from dried urine spots, and subsequent quantification of cocaine and its metabolite, benzoylecgonine.

MATERIALS AND METHODS

Sample preparation

For the screening method, a total of 19 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. For the quantification, cocaine and its metabolite benzoylecgonine were spiked into urine to prepare calibrator samples covering the concentration range of 5-1,000 ng/mL. QC samples were prepared at three additional concentrations. Eight microliters of each respective urine sample was spotted onto VeriSpray sample plates for analysis.

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

The spray solvent consisted of 90% acetonitrile, 10% water, and 0.01 % acetic acid. Data was acquired for one minute per sample, and each concentration level was measured 5 times for screening and 3 times for quantification. Compounds were detected on a Thermo Scientific™ TSQ Quantis™ mass spectrometer. Three transitions were monitored per compound, with a cycle time of 1.5 seconds (screening assay) or 1 second (quantification) and a collision gas pressure of 2 mTorr. The spray voltage was 3.8 kV, applied from 0.1 to 0.9 min, the inlet capillary temperature was 325 ° C, and the distance from paper tip to inlet was approximately 5 mm. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

RESULTS

Screening Assay

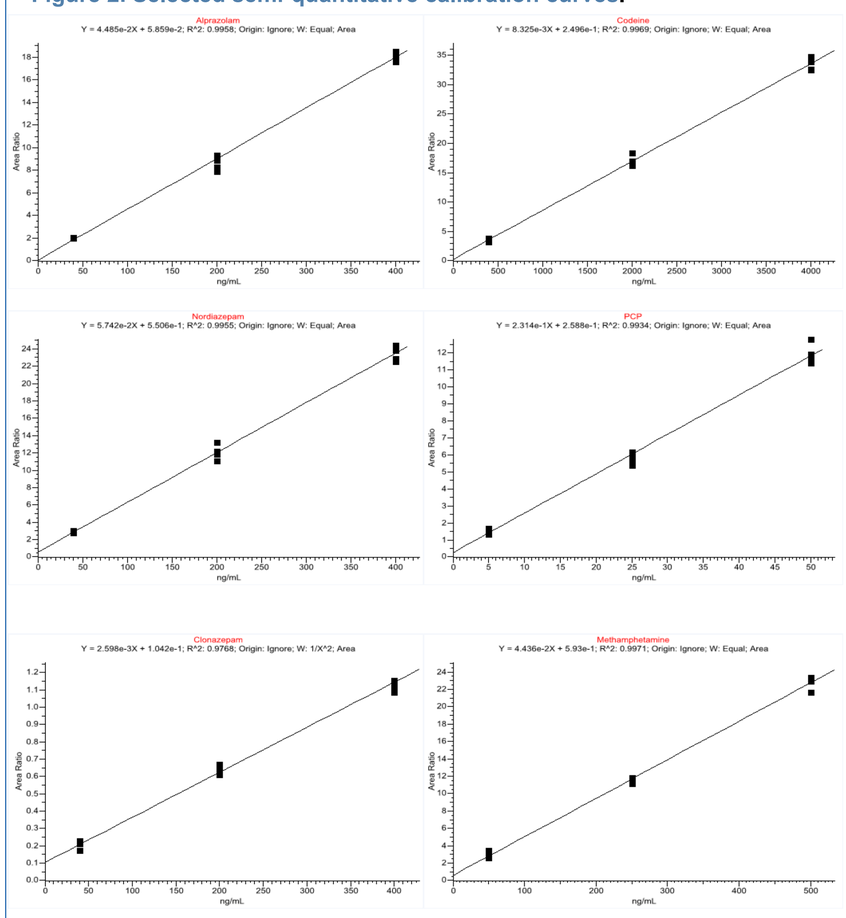
The criteria for whether or not the cutoff level of the respective compound is met, is as follows: Area under the curve (AUC) response at the cutoff level must be at least 4 times that of the matrix blank. Table 2 shows the S/N (signal-to-noise) ratios of each compound at the cutoff level. All 19 compounds met the screening criteria.

Table 2. AUC counts and S/N ratios at the respective cutoff level.

Compound	AUC at Cutoff	AUC Matrix Blank	S/N Ratio
Oxycodone	1.86E+05	2.12E+04	8.7
Clonazepam	4.87E+05	8.68E+04	5.6
Methadone	8.01E+07	1.57E+05	510.3
Alprazolam	5.59E+06	2.59E+04	215.8
Cocaine	2.15E+07	5.22E+04	411.6
Oxymorphone	2.46E+05	2.79E+04	8.8
Codeine	7.71E+06	2.75E+04	280.1
Hydrocodone	3.23E+06	2.87E+04	108.9
Benzoylecgonine	3.74E+06	3.23E+04	115.6
Oxazepam	6.91E+05	1.84E+04	37.7
Morphine	2.50E+06	1.82E+04	137.4
Diazepam	5.73E+06	5.47E+04	104.8
Nordiazepam	1.92E+06	2.94E+04	65.2
PCP	4.38E+06	1.70E+05	25.8
MDMA	1.42E+07	3.68E+04	386.1
MDA	1.01E+06	1.66E+05	6.1
Methamphetamine	1.86E+05	2.12E+04	42.2
Amphetamine	4.87E+05	8.68E+04	4.9
Hydromorphone	8.01E+07	1.57E+05	33.8

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.



Quantification Assay

A quantitative assay was run for cocaine and its metabolite, benzoylecgonine. Calibrator samples were run in triplicate in the concentration range of 5 – 1,000 ng/mL. As internal standard, cocaine-d3 and benoylecgonine-d8 were used. Figures 3 and 4 show the resulting calibration curves for both compounds.

Table 3. Compounds, cutoffs and LOQs for quantitation.

Analyte	Cutoff in Urine (ng/mL)	VeriSpray/ TSQ Quantis MS LOQ in Urine (ng/mL)
Benzodiazepines		
Alprazolam	10	2.3
Nordiazepam	20	2.3
Zolpidem Phenyl-4-carbo acid	10	2.3
Opiates		
Codeine	20	4.6
Hydrocodone	20	4.6
Hydromorphone	20	9.2
Morphine	20	18.6
Oxycodone	20	2.3
Oxymorphone	20	18.6
Cocaine		
Cocaine	20	18.6
Sedatives		
Diphenhydramine HCl	10	0.93
(+)-Norketamine HCl	10	4.6
PCP (Phencyclidine)	10	2.3
Amphetamines		
(+)-Methamphetamine	50	18.6
(+)-MDMA	50	18.6
Opioids		
EDDP	25	0.46
Methadone	20	9.2
Tramadol	20	9.2
Fentanyl	1	2.3

Figure 3. Calibration curve for Cocaine.

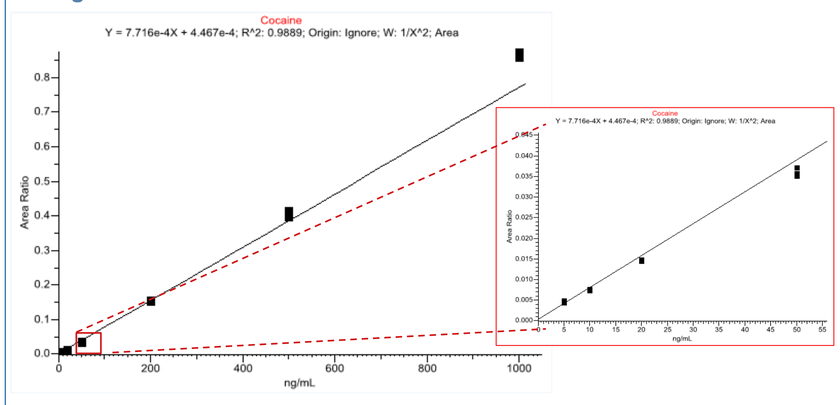
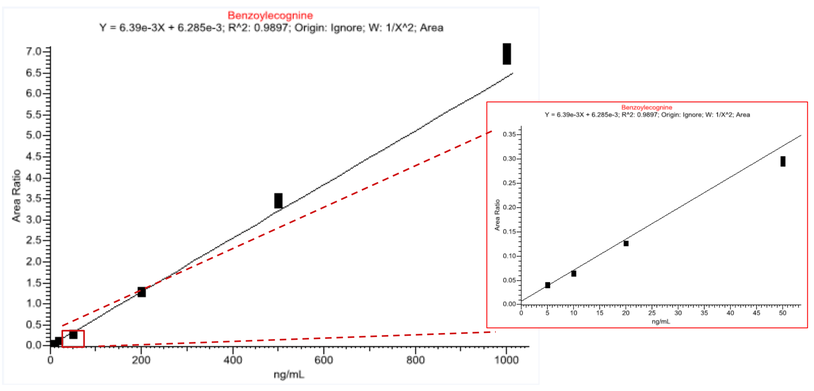


Figure 4. Calibration curve for Benzoylecgonine.



The LOQ for both compounds was determined to be **5 ng/mL**, based on the obtained precision and accuracy values for the calibrator samples, shown in Table 3.

Table 4. Precision and accuracy of calibrator samples.

Concentration (ng/mL)	% RSD		% Difference	
	Cocaine	Benzoylecgonine	Cocaine	Benzoylecgonine
5	5.6	2.9	< 14.4	< 10.2
10	2.1	1.5	< 11.0	< 10.9
20	1.1	0.8	< 9.2	< 6.9
50	2.8	2.1	< 10.2	< 11.6
200	1.4	2.0	< 1.7	< 2.7
500	2.5	2.4	< 7.9	< 11.2
1,000	0.8	2.2	< 13.0	< 11.1

QC samples were run for both compounds, at concentrations of 15, 100, and 800 ng/mL. The following table (Table 4) shows precision and accuracy of the QC samples.

Table 5. Precision and accuracy of QC samples.

Concentration (ng/mL)	% RSD		% Difference	
	Cocaine	Benzoylecgonine	Cocaine	Benzoylecgonine
15	2.5	3.2	0.0	- 6.3
			- 1.0	- 6.0
			- 3.4	- 0.4
100	1.8	0.7	+ 1.6	- 2.6
			- 0.5	- 1.4
			- 2.1	- 1.5
800	2.0	1.4	+ 11.7	+ 5.6
			+ 9.3	+ 8.4
			+ 7.5	+ 6.0

CONCLUSIONS

- PaperSpray technology is well suitable for fast drugs of abuse screening, due to short analysis times and the ability to analyze many compounds within a one-minute window.
- Quantification of cocaine and benzoylecgonine is easily achievable in the required measurement range, with the necessary precision and accuracy.
- The new VeriSpray ion source makes PaperSpray analysis easy, fast, and more automated than previous systems.

TRADEMARKS/LICENSING

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PO73232-EN 0919S

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