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Quantitation of drugs of abuse and their metabolites in urine using PaperSpray tandem mass spectrometry for clinical research and forensic toxicology

Authors: Scott A. Borden[1,2], Armin Saatchi[1], Chris G. Gill[1,2], Neloni R. Wijeratne[3]

[1]Applied Environmental Research Laboratories Vancouver Island University, Nanaimo, BC

[2]University of Victoria, Victoria, BC

[3]Thermo Fisher Scientific, San Jose, CA

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TSQ Fortis™ mass spectrometer with the Thermo Scientific™ VeriSpray™ PaperSpray ion source.

Application benefits

- Developement of a robust, reliable quantitative assay for 41 drugs of abuse and their metabolites in urine in a single quantitative method
- No sample preparation, reduced cost per sample, and increased sample throughput

Goal

To develop a reliable quantitative MS-based urinalysis procedure for drugs of abuse and their metabolites for clinical research or forensic toxicology able to meet established cutoff values using the Thermo Scientific™

Introduction

Forensic and clinical laboratories routinely quantify drugs of abuse in biological matrices, urine being one of the most common due to ease of procurement. Most often, drugs are quantified using time-consuming chromatographic methods that require significant sample preparation. Therefore, an alternative method that provides comparable high-throughput results is of significant value to forensic and clinical laboratories. PS-MS is a viable alternative to chromatography for the rapid analysis of drugs of abuse in urine, demonstrating sufficient sensitivity, precision, and accuracy to be of clinical and forensic relevance.

Paper spray mass spectrometry (PS-MS) was first described in 2010[1] as a method for the direct analysis of complex samples without sample preparation. PS-MS uses small sampling volumes (<10 µL) directly deposited onto a strip of paper.[1] Directly in front of the inlet of the mass spectrometer, solvent is applied to the paper to extract analytes and high voltage is applied to ionize them. A chronogram of the ion current is collected for a short duration (usually <1 min) and is integrated to quantitate the analyte. Paper spray ionization coupled with triple-quadrupole mass spectrometry allows for rapid and sensitive (low ng/mL) quantitative measurements in a variety of complex samples, such as blood and urine. The VeriSpray PaperSpray system includes a VeriSpray plate loader and magazine that holds up to 10 VeriSpray cartridges, each containing 24 paper strips. This fully automated system allows for high-throughput analysis without sample carryover. Figure 1 shows the VeriSpray system mounted to the TSQ Fortis MS, as well as the removable magazine and VeriSpray cartridges.

In this study, the VeriSpray PaperSpray ion source coupled to a TSQ Fortis triple-quadrupole mass spectrometer was evaluated as a tool for the quantification of drugs of abuse and their metabolites in human urine samples for applications in clinical research and forensic toxicology. A suite of 41 different drugs and metabolites chosen to represent major drugs classes (*e.g.,* opiates and opioids, amphetamines, fentanyl and its analogs, and benzodiazepines) was quantified in pooled human urine samples, with method validation carried out using matrix-matched quality control samples.

Experimental

Sample preparation

A selection of 41 drugs and metabolites from various drug classes (including opioids, amphetamines, benzodiazepines, and novel psychoactive substances) were used to create calibration curve standards in pooled blank human urine samples from five sources. Nine-point calibration curves (n = 6 replicates) were prepared accordingly to meet established cutoff values and encompass the range of concentrations expected in urine samples. Working solutions of drugs and isotopically labeled internal standards were prepared in methanol and spiked into the pooled urine matrix. For analysis, 8 µL aliquots of spiked urine were spotted on VeriSpray cartridges and allowed to dry at ambient temperature for 30 minutes.

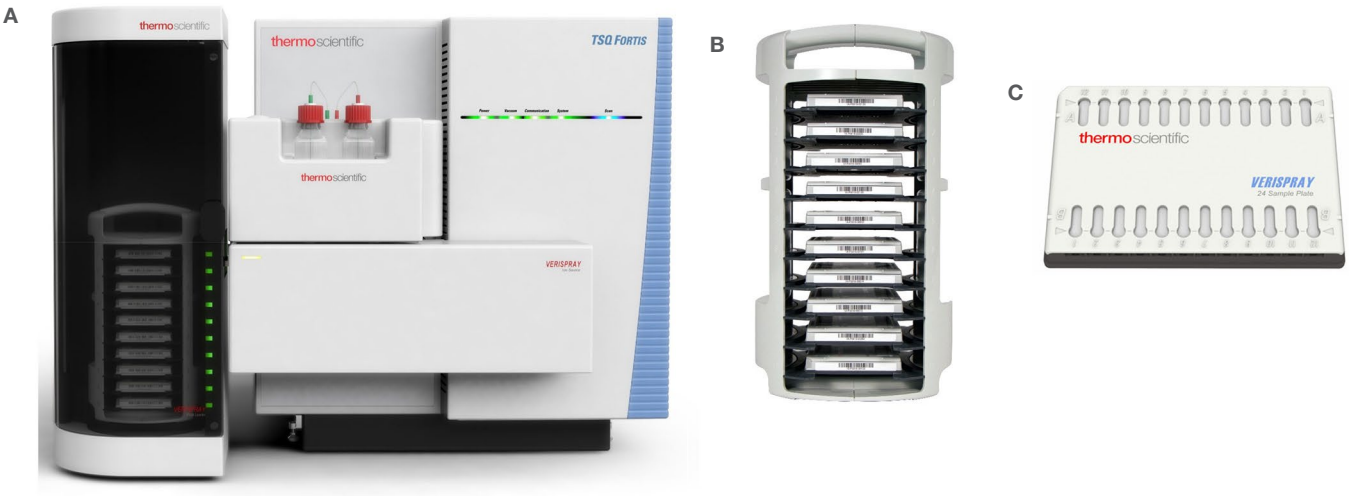

Figure 1. (A) VeriSpray PaperSpray system mounted to TSQ Fortis triple quadrupole MS, (B) magazine, and (C) VeriSpray cartridge

PaperSpray and MS conditions

The TSQ Fortis triple quadrupole mass spectrometer was used for all analyses and was coupled with the VeriSpray PaperSpray system for ionization. PaperSpray solvents used (sample rewet and spray solvents) were acetonitrile/water 90/10 with 0.1% acetic acid and applied according to the settings in Table 1. Mass spectrometry parameters were optimized using a spray voltage of +3.8 kV, a cycle time of 1.2 s, and Q1 and Q3 resolution at 0.7 Da FWHM. Source parameters are outlined in Table 2. The optimum RF lens settings and collision energies for the product ions were determined by direct infusion of standards into the mass spectrometer using the heated electrospray ionization source. Optimized SRM transitions for all target analytes and internal standards are listed in Table 3. The paper tip to MS inlet distance was set to 5.0 mm.

Table 1. VeriSpray solvent application parameters.
Each rewetting and solvent dispense is 10 µL.

Rewetting dispense	Delay (s)
1	1
Solvent dispense	**Delay (s)**
1	1
2	1
3	1
4	1
5	3
6	3
7	5
8	5
9	5
10	5
11	7
12	7
13	7
14	7
15	7

Table 2A. TSQ Fortis parameters

Ion source parameter	Value
Spray voltage	Time dependent
Positive ion	3800 V
Sweep gas	0 Arb
Ion transfer tube temperature	300 °C
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
CID gas	2 mTorr

Table 2B. Time-dependent spray voltage settings

Time (min)	Voltage (V)
0	0
0.1	3800
1.1	0

Method validation

Method validation was done according to the Scientific Working Group for Forensic Toxicology's (SWGTOX) standard practices for method validation in forensic toxicology.[2] Low, medium, and high QC samples were prepared such that the low QC sample was near the cutoff value, the high QC sample was at 80% of the highest calibrator, and the medium QC was at the midpoint of the high and low QC samples. The QC samples were analyzed daily (n = 5 replicates), over a period of five days to determine within-run and between-run precision (%CV) and accuracy (%Bias) values:

$$\text{Within-run \%CV} = \frac{\text{Standard deviation of a single day of samples}}{\text{Mean calculated value of a single day of samples}} \times 100 \quad (1)$$

$$\text{Between-run \%CV} = \frac{\text{Standard deviation of grand mean}}{\text{Grand mean}} \times 100 \quad (2)$$

$$\text{\%Bias} = \frac{\text{Grand mean of calculated concentration - nominal concentration}}{\text{Nominal concentration}} \times 100 \quad (3)$$

Data acquisition and analysis

Thermo Scientific™ TraceFinder™ software version 4.1 SP5 optimized for clinical research was used for automated data acquisition and processing. All linear regressions used a 1/× weighting. Lower limits of quantitation (LLOQs) were determined from the lowest calibration standard that met the following criteria: $R^2 \geq 0.98$, %CV ≤ 15, and %Bias ≤ ±20, and a S/N value > 4. Noise was defined as the average of 10 replicate measurements of pooled human urine.

Table 3. Optimized SRM transitions using positive polarity

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
N-phenyl-1-(2-phenylethyl)-4-piperidinamine (4-ANPP)	281.2	188.1	17	146
	281.2	105.1	31	146
4-ANPP-D_5	286.2	188.3	17	96
	286.2	105.0	32	96
Acetylfentanyl	323.2	188.2	23	97
	323.2	105.1	36	97
Acetylfentanyl-$^{13}C_6$	329.3	188.1	23	100
	329.3	105.1	36	100
Acetylmorphine	328.2	211.1	26	114
	328.2	165.1	38	114
Acetylmorphine-D_6	334.2	211.1	27	128
	334.2	165.1	38	128
Acrylfentanyl	335.2	188.2	22	104
	335.2	105.1	36	104
Acrylfentanyl-D_5	340.3	188.1	22	102
	340.3	105.1	37	102
Alprazolam	309.1	281.1	25	111
	309.1	205.1	41	111
Alprazolam-D_5	314.2	286.1	26	111
	314.2	210.1	42	111
Aminoclonazepam	286.1	222.1	25	113
	286.1	250.1	20	113
Aminoflunitrazepam	284.2	135.2	27	102
	284.2	227.1	25	102
Amphetamine	136.1	91.2	17	73
	136.1	65.1	36	73
Amphetamine-D_{11}	147.2	98.1	19	79
	147.2	70.1	38	79
Benzoylecgonine	290.1	168.2	19	97
	290.1	77.1	47	97
Benzoylecgonine-D_8	298.2	171.2	20	93
	298.2	82.2	48	93
Butyrylfentanyl	351.2	188.2	23	104
	351.2	105.1	38	104
Carfentanil	395.2	335.2	18	100
	395.2	363.2	13	100
Carfentanil-D_5	400.3	340.2	18	110
	400.3	368.3	14	110
Codeine	300.2	215.1	25	108
	300.2	165.1	41	108
Codeine-D_3	303.2	215.1	26	123
	303.2	165.1	41	123

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Cyclopropylfentanyl	349.2	188.2	23	98
	349.2	105.1	37	98
Desalkylflurazepam	289.1	140.1	30	119
	289.1	226.1	28	119
Desalkylflurazepam-D_4	293.1	140.0	30	123
	293.1	230.2	29	123
Diazepam	285.1	193.0	32	117
	285.1	154.1	28	117
Ecgonine methyl ester	200.1	82.1	26	80
	200.1	119.0	25	80
Ecgonine methyl ester-D_3	203.1	85.2	26	80
	203.1	119.0	25	80
2-Ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP)	278.2	234.2	31	95
	278.2	249.2	24	95
EDDP-D_3	281.2	234.1	31	98
	281.2	249.2	25	98
Ethylpentylone	250.2	202.1	18	81
	250.2	189.1	24	81
Ethylpentylone-D_5	255.2	207.1	19	82
	255.2	194.1	24	82
Fentanyl	337.2	188.2	23	101
	337.2	105.1	37	101
Fentanyl-D_5	342.3	188.2	23	100
	342.3	105.1	37	100
4-Fluoroisobutyryl-fentanyl (FIBF)	369.2	188.1	24	108
	369.2	105.1	39	108
Fluorofentanyl	355.2	188.1	23	107
	355.2	105.1	37	107
Fluorofentanyl-D_3	358.2	188.1	24	108
	358.2	105.1	38	108
Furanylfentanyl	375.2	188.2	22	106
	375.2	105.1	38	106
Furanylfentanyl-D_5	380.2	188.1	23	102
	380.2	105.1	38	102
HO-Alprazolam	325.1	216.1	39	117
	325.1	205.1	45	117
HO-Alprazolam-D_5	330.2	210.1	45	127
	330.2	221.1	40	127
Hydrocodone	300.2	171.1	39	114
	300.2	241.1	27	114
Hydrocodone-D_3	303.2	171.1	39	117
	303.2	241.1	27	117

Table 3 (continued). Optimized SRM transitions using positive polarity

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Hydromorphone	286.1	185.1	30	116
	286.1	157.1	41	116
Hydromorphone-D_3	289.2	185.1	30	134
	289.2	157.1	42	134
Isobutyrylfentanyl	351.2	188.2	24	101
	351.2	105.1	38	101
Ketamine	238.1	125.0	27	80
	238.1	207.0	14	80
3,4-Methylenedioxy-amphetamine (MDA)	180.1	105.1	22	83
	180.1	135.1	19	83
MDA-D_5	180.1	133.1	18	83
	185.1	110.1	23	102
3,4-Methylenedioxy-methamphetamine (MDMA)	185.1	138.1	19	102
	185.1	137.1	20	102
MDMA-D_5	194.1	163.1	13	75
	194.1	135.1	21	75
Meperidine	248.2	220.1	21	100
	248.2	174.2	21	100
Meperidine-D_4	252.2	224.2	22	101
	252.2	178.2	21	101
Methadone	310.2	265.2	15	72
	310.2	105.0	27	72
Methadone-D_9	319.3	268.2	16	77
	319.3	105.1	28	77
Methamphetamine	150.1	91.1	19	78
	150.1	119.1	11	78
Methamphetamine-D_{11}	161.2	97.1	19	80
	161.2	127.2	12	80
Morphine	286.1	152.1	55	109
	286.1	201.1	26	109
Morphine-D_6	292.2	153.1	43	134
	292.2	201.1	26	134

Compound	Precursor (m/z)	Product (m/z)	Collision energy (V)	RF lens (V)
Methamphetamine-D_{11}	161.2	97.1	19	80
	161.2	127.2	12	80
Morphine	286.1	152.1	55	109
	286.1	201.1	26	109
Morphine-D_6	292.2	153.1	43	134
	292.2	201.1	26	134
Normeperidine	234.2	160.1	16	99
	234.2	56.1	23	99
Normeperidine-D_4	238.2	164.2	16	93
	238.2	58.1	23	93
Ocfentanil	371.2	188.2	22	101
	371.2	105.1	38	101
Phenylcyclidine (PCP)	244.3	86.2	13	68
	244.3	91.1	31	68
Phenylcyclidine-D_5	249.2	86.2	13	70
	249.2	96.1	32	70
Sufentanil	387.2	238.2	19	92
	387.2	111.0	36	92
Sufentantil-D_5	392.2	238.2	20	97
	392.2	111.0	36	97
Temazepam	301.1	255.1	22	103
	301.1	177.0	38	103
Temazepam-D_5	306.1	260.2	24	112
	306.1	177.0	38	112
U-47700	329.2	284.1	17	87
	329.2	173.0	32	87
Valerylfentanyl	365.3	188.2	24	102
	365.3	105.1	39	102
Zolpidem	308.2	235.2	35	108
	308.2	263.2	26	108
Zolpidem-D_7	315.2	242.2	36	104
	315.2	270.2	27	104

Results and discussion

Calibrations with good linearity ($R^2 > 0.98$) were achieved for all 41 drugs and metabolites and used for the quantitation of quality control samples prepared in pooled blank urine. The calibration models, LLOQs, and cutoff values are outlined in Table 4. Method validation was carried out according to SWGTOX guidelines; acceptable results are indicated when precision (%CV) values do not exceed 20% and accuracy (%Bias) values do not exceed ±20%.

Typical results from representative compounds of the major drug classes are outlined in Table 5. Determined LLOQ values are below or near established cutoff values for forensic or clinical toxicology. Total analysis time for dried urine spots, including extraction (ca. 1.1 min) followed by mass spectrometric analysis (1.2 min), was approximately 2.3 minutes.

Table 4. Compound calibration models (9 levels, 6 replicates), R^2 values, lower limits of quantitation (LLOQ) and cutoff values

Compound	Internal standard	[Internal standard] (ng/mL)	Calibration range (ng/mL)	R^2	LLOQ (ng/mL)	Cutoff (ng/mL)
4-ANPP	4-ANPP-D_5	25	0.5 – 600	0.9956	1	2
Acetylfentanyl	Acetylfentanyl-$^{13}C_6$	25	0.5 – 600	0.9966	1	2
Acetylmorphine	Acetylmorphine-D_6	100	7.5 – 900	0.9880	7.5	10
Acrylfentanyl	Acrylfentanyl-D_5	25	0.5 – 600	0.9946	1	2
Alprazolam	Alprazolam-D_5	100	7.5 – 1800	0.9968	3	10
Aminoclonazepam	Alprazolam-D_5	100	7.5 – 1800	0.9803	30	10
Aminoflunitrazapem	Alprazolam-D_5	100	7.5 – 1800	0.9809	75	5
Amphetamine	Amphetamine-D_{11}	500	5 – 6000	0.9963	25	50
Benzoylecgonine	Benzoylecgonine-D_8	500	5 – 6000	0.9964	10	50
Butyrylfentanyl	Acrylfentanyl-D_5	25	0.5 – 600	0.9967	1	2
Carfentanil	Carfentanil-D_5	25	0.5 – 600	0.9970	1	2
Codeine	Codeine-D_3	500	5 – 6000	0.9937	250	20
Cyclopropylfentanyl	Furanylfentanyl-D_5	25	0.5 – 600	0.9913	1	2
Desalkylflurazepam	Desalkylflurazepam-D_4	100	7.5 – 1800	0.9906	7.5	25
Diazepam	Alprazolam-D_5	100	1.5 – 1800	0.9904	7.5	20
Ecgonine methyl ester	Ecgonine methyl ester-D_3	500	5 – 6000	0.9953	25	50
EDDP	EDDP-D_3	100	1.5 – 1800	0.9976	1.5	10
Ethylpentylone	Ethylpentylone-D_5	100	1.5 – 1800	0.9974	3	30
Fentanyl	Fentanyl-D_5	25	0.5 – 600	0.9956	1	2
FIBF	FIBF-D_3	25	0.5 – 600	0.9936	0.5	2
Fluorofentanyl	Fluorofentanyl-D_3	25	0.5 – 600	0.9983	0.5	2
Furanylfentanyl	Furanylfentanyl-D_5	25	0.5 – 600	0.9966	1	2
HO-Alprazolam	HO-Alprazolam-D_5	100	1.5 – 1800	0.9921	75	10
Hydrocodone	Hydrocodone-D_3	500	5 – 6000	0.9975	10	20
Hydromorphone	Hydromorphone-D_3	500	5 – 6000	0.9955	10	20
Isobutyrylfentanyl	Acrylfentanyl-D_5	25	0.5 – 600	0.9967	1	2
Ketamine	Ethylpentylone-D_5	100	1.5 – 1800	0.9967	1.5	30
MDA	MDA-D_5	500	5 – 6000	0.9971	25	100
MDMA	MDMA-D_5	500	5 – 6000	0.9971	5	100
Meperidine	Meperidine-D_4	100	1.5 – 1800	0.9973	1.5	10
Methadone	Methadone-D_9	100	1.5 – 1800	0.9970	1.5	10
Methamphetamine	Methamphetamine-D_{11}	500	5 – 6000	0.9979	10	50
Morphine	Morphine-D_6	500	25 – 3000	0.9800	250	20
Normeperidine	Normeperidine-D_4	100	1.5 – 1800	0.9930	1.5	10
Ocfentanil	Fluorofentanyl-D_3	25	0.5 – 600	0.9963	1	2
Phenylcyclidine	Phenylcyclidine-D_5	100	1.5 – 1800	0.9947	1.5	10
Sufentanil	Sufentanil-D_5	25	0.5 – 600	0.9952	1	2
Temazepam	Temazepam-D_5	100	1.5 – 1800	0.9907	7.5	20
U-47700	Fluorofentanyl-D_3	25	0.5 – 600	0.9932	2.5	2
Valerylfentanyl	Fluorofentanyl-D_3	25	0.5 – 600	0.9847	10	2
Zolpidem	Zolpidem-D_7	100	1.5 – 1800	0.9972	1.5	10

Table 5. Method validation results for representative compounds of each drug class tested at three different QC levels

Compound	Low QC				Medium QC				High QC			
	[QC] (ng/mL)	Within run (%CV)	Between run (%CV)	% Bias	[QC] (ng/mL)	Within run (%CV)	Between run (%CV)	% Bias	[QC] (ng/mL)	Within run (%CV)	Between run (%CV)	% Bias
Amphetamine	60	8.3	7.8	-0.1	2430	6.0	4.0	-4.8	4800	6.6	3.7	-0.7
Alprazolam	30	8.0	4.7	2.8	729	7.1	4.8	-2.2	1440	6.2	5.0	-0.4
Benzoylecgonine	100	5.0	4.1	9.4	2430	3.1	2.6	-4.9	4800	3.5	3.3	-0.4
Carfentanil	10	6.5	4.9	13.4	243	4.8	3.5	-5.2	480	6.0	4.2	0.8
EDDP	18	5.1	3.8	9.1	729	2.7	2.0	-4.5	1440	5.9	4.0	0.6
Ethylpentylone	30	4.3	3.4	10.3	729	4.1	2.8	-3.0	1440	4.4	3.5	1.7
Fluorofentanyl	6	3.5	3.3	16.5	243	4.4	3.2	-4.4	480	3.2	2.4	-1.4
Meperidine	18	11.6	6.9	15.0	729	6.3	3.8	-4.2	1440	3.5	2.4	0.9
Methadone	18	7.0	4.9	10.6	729	6.7	4.7	-3.8	1440	5.6	3.5	-0.1
Methamphetamine	60	4.2	2.9	4.2	2430	4.8	2.8	-4.9	4800	4.7	2.8	-0.4
Phencyclidine	18	10.8	6.0	12.7	729	5.0	3.8	-5.7	1440	6.2	3.6	0.0
Zolpidem	18	7.6	5.0	14.5	729	4.2	3.2	-4.9	1440	3.1	2.5	1.8

Conclusion

The VeriSpray system was successfully applied for the accurate and precise quantitation of various drugs of abuse and their metabolites in human urine samples for use in clinical research or forensic toxicology. A total analysis time of ~2 minutes along with removing the requirement of sample pre-treatment/preparation and separation enables every clinical research and/or forensic toxicology laboratory to address more samples with increased confidence, and in turn, address their business and scientific goals.

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