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<sup>1,2</sup>, Armin Saatchi<sup>1</sup>, Chris G. Gill<sup>1,2</sup>, Neloni R. Wijeratne<sup>3</sup>

<sup>1</sup>Applied Environmental Research Laboratories Vancouver Island University, Nanaimo, BC

<sup>2</sup>University of Victoria, Victoria, BC

<sup>3</sup>Thermo Fisher Scientific, San Jose, CA

Keywords: Illicit drugs, drugs of abuse, opiates, opioids, amphetamines, benzodiazepines, urine, PS-MS/MS, TSQ Fortis MS, VeriSpray PaperSpray ion source, TraceFinder, forensic toxicology, clinical research

### **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<sup>™</sup>



TSQ Fortis<sup>™</sup> mass spectrometer with the Thermo Scientific<sup>™</sup> VeriSpray<sup>™</sup> PaperSpray ion source.

#### 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<sup>1</sup> 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.<sup>1</sup> 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 matrixmatched 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  $\mu$ L aliquots of spiked urine were spotted on VeriSpray cartridges and allowed to dry at ambient temperature for 30 minutes.

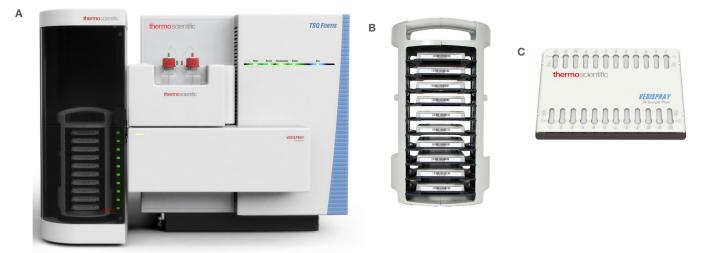


Figure 1. (A) VeriSpray PaperSpray system mounted to TSQ Fortis triple quadrupole MS, (B) magazine, and (C) VeriSpray cartridge

#### 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 $10 \ \mu$ L.

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.<sup>2</sup> 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:

#### Data acquisition and analysis

Thermo Scientific<sup>™</sup> TraceFinder<sup>™</sup> 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<sup>2</sup> ≥ 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

<table-container>       Nephysical      Participant (Constraint)      Participant)      Pa</table-container>	Compound	Precursor ( <i>m/z</i> )	Product ( <i>m/z</i> )	Collision energy (V)	RF lens (V)	Compound	Precursor ( <i>m/z</i> )	Product ( <i>m/z</i> )	Collision energy (V)	RF lens (V)
4-piperfunctionationationationationationationationa		281.2	188.1	17	146	Cucleorepulfentenul	349.2	188.2	23	98
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	4-piperidinamine	281.2	105.1	31	146	Cyclopropylientanyl	349.2	105.1	37	98
adviction of the sector of	(4-ANPP)						289.1	140.1	30	119
According      No.9	4-ANPP-D <sub>5</sub>					Desalkylflurazepam	289.1	226.1	28	119
Activitation32.310.510.61010.020.120.110.010.020.120.120.020.120.0 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>293.1</td> <td>140.0</td> <td>30</td> <td>123</td>							293.1	140.0	30	123
Actyleritaryl-PQ Actyleritaryl-PQ Actyleritaryl-PQ Actyleritaryl-PQ B28.218.118.11918.11918.11918.11918.119	Acetylfentanyl					Desalkylflurazepam-D <sub>4</sub>	293.1	230.2	29	123
Activitation of the set of							285.1	193.0	32	117
Action or phase      28.2      21.1      26      14      Page intermeting entry intermetintery intermeting entry intermetintery intermeting entr	Acetylfentanyl-13C	329.3	188.1	23	100	Diazepam	285.1	154.1	28	117
Acetymorphine      38.2      165.1      3      14      France      200.1      10.3      25      80        Actymorphine-De      34.2      211.3      27      128      Eggenmently      30.3      80.2      28.4      80.4      80.2      28.4      60.5		329.3	105.1	36	100		200.1	82.1	26	80
328.2      16.1      38      14        Aeetylenorphine-D      33.4.2      21.1      27      18        Astrylentanyl      33.2.2      161.0      36      164        Arrylentanyl      33.2.2      162.0      22      104      24.110.0      25.2      24.0      36.0        Arrylentanyl-O      33.2.3      168.1      22      102      24.1      31.0      36.1	Acetvlmorphine	328.2	211.1	26	114	Ecgonine methyl ester	200.1	119.0	25	80
Activitymerphine-D <sub>0</sub> 33.4.2      21.1.1      27      128      Explain a monthly apprecision of the physical strain s		328.2	165.1	38	114	<b>_</b>	203.1		26	80
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Acetylmorphine-D	334.2	211.1	27	128					
Arylenanyl Arylenanyl-D 362.0362.0162.022164 104dimetry-13diphenyl- (PDP) (PDP)278.2249.2249.2249Arylenanyl-D 303.0105.137102 301.020		334.2	165.1	38	128	2-Ethylidene-1,5- 278.				
interm      35.2      10.51      36      104      (EDDP)      20.2      20.4      24.4      30        Arrylentaryl-D      340.3      188.1      22      102 $20.102$ 281.2      29.1      31      36      36        Aprazolam      340.3      0.51      41      111 $20.12$ 29.2      18.8      26      111        Aprazolam-D      314.2      20.1      42      111 $20.22$ 18.2      20.1      18      21      22      18      20.1	Acrultontonul	335.2	188.2	22	104	dimethyl-3,3-diphenyl-				
Arryferitanyl-b, Aprazolam      103.1      105.1      97      102      EDDP-b, Hypentylone      281.2      249.2      28      98        Aprazolam      309.1      261.1      261.1      262.2      189.1      24      81        Aprazolam-D, Manodonazepam      14.2      261.1      262.1      189.1      24      81        Amodonazepam      286.2      201.1      26      111      255.2      194.1      24      82        Amodonazepam      286.2      201.1      26      133      255.2      194.1      24      23      102      26      102      265.2      194.1      24      23      102 </td <td>Acrynentanyi</td> <td>335.2</td> <td>105.1</td> <td>36</td> <td>104</td> <td></td> <td>278.2</td> <td>249.2</td> <td>24</td> <td>95</td>	Acrynentanyi	335.2	105.1	36	104		278.2	249.2	24	95
Image: state sta		340.3	188.1	22	102		281.2	234.1	31	98
Alreadam39.129.1411Ethylentylone20.219.12481Alreadam-Da14.226.126.126.1212610.126.227.110.12426.1Annodonazepan26.120.12010.3Penanyl37.210.12710.1Annodonazepan26.120.12710.2Penanyl37.210.13710.1Annodonazepan26.227.12610.2Penanyl37.210.13710.1Annohumano26.227.127.02610.210.13710.110.1Annohumano10.491.21773Penanyl-D36.218.12410.1Annohumano10.161.13670Penanyl-D36.218.12410.1Annohumano10.161.1367010.136.218.12410.1Annohumano10.171.173Penanyl-Di36.218.12410.1Annohumano10.171.173Penanyl-Di36.218.12410.1Annohumano10.171.173Penanyl-Di36.218.12410.1Annohumano10.171.17173Penanyl-Di36.218.12410.1Annohumano10.271.171717171717171717171<	Acrylfentanyl-D <sub>5</sub>	340.3	105.1	37	102	EDDP-D <sub>3</sub>	281.2	249.2	25	98
Name  309.1  205.1  41  111  250.2  189.1  24  81    Aprazolam-D <sub>a</sub> 314.2  286.1  26  111  255.2  194.1  24  82    Aminocionazepam  286.1  222.1  25  113  265.2  194.1  24  82    Aminocionazepam  286.1  222.1  25  113  37.2  188.2  23  101    Aminocionazepam  284.2  135.2  27  102  147.2  105.1  37  101    Aminocionazepam  284.2  227.1  25  102  102  107  103  106.1  37  101    Amphetamine  136.1  91.2  17  73  4.Fluoroisobutyryl-  686.2  105.1  37  100    Amphetamine-D <sub>n</sub> 147.2  96.1  19  79  4.Fluoroisobutyryl-  655.2  105.1  37  107    Bencoyleogonine-D <sub>n</sub> 290.1  168.2  19  97  4.Pluoroientanyl-D <sub>0</sub> 355.2  186.1  24  108    Bencoyleogonine-D <sub>n</sub> 291.2  171.2  20  93  4.Pluoroientanyl-D <sub>0</sub> 356.2  105.1  38  108    Bencoyleogonine-		309.1	281.1	25	111		250.2	202.1	18	81
Alprazolam-b, Aninoclonacepam      11.4.2      10.1      42      11.4      210.1      42      11.4      210.1      42      11.4      210.1      25.1      19.1      24.0      22.1      25.0      10.1      37.2      18.2      23.0      10.1        Aminoclonacepam      284.2      25.0      20      102	Alprazolam	309.1	205.1	41	111	Ethylpentylone	250.2	189.1	24	81
Name      Number of the sector of th		314.2	286.1	26	111		255.2	207.1	19	82
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Alprazolam-D <sub>5</sub>	D <sub>5</sub>	Ethylpentylone-D <sub>5</sub>	255.2	194.1	24	82			
14.266.1250.12011314.337.2105.137101 $A_{mholunitazepam}$ 284.2135.227102 $P_{entanyl-D_{a}}$ 42.3105.137100 $A_{mholamine}$ 165.126102 $P_{entanyl-D_{a}}$ 69.2188.124108 $A_{mholamine-D_{a}}$ 165.13673 $P_{entanyl (FBF)}$ 69.2105.139108 $A_{mholamine-D_{a}}$ 147.298.11979 $P_{entanyl (FBF)}$ 65.2105.137107 $P_{ancoleconine}$ 20.1168.21997 $P_{entanyl (FBF)}$ 65.2105.137107 $P_{ancoleconine-D_{a}}$ 168.270 $P_{entanyl (FBF)}$ 65.2105.137107 $P_{ancoleconine-D_{a}}$ 20.1168.21997 $P_{entanyl (FBF)}$ 65.2105.138108 $P_{ancoleconine-D_{a}}$ 20.177.14797100.2105.138108108 $P_{ancoleconine-D_{a}}$ 20.2171.22093 $P_{entanyl-D_{a}}$ 65.2105.138108108 $P_{ancoleconine-D_{a}}$ 35.2186.223106 $P_{entanyl-D_{a}}$ 360.2165.136102 $P_{ancoleconine-D_{a}}$ 35.2363.218100 $P_{anaylennyl-D_{a}}$ 360.2105.138102 $P_{ancoleconine-D_{a}}$ 36.2363.314100 $P_{anaylenn-D_{a}}$ <		286.1	222.1	25	113		337.2	188.2	23	101
Aninofluitinizacepanic      284.2      27.1      25      102      Fertanyl-D <sub>g</sub> 342.3      105.1      37      100        Amphetamine      136.1      91.2      17      73      4.Fluoroisobutyryl-fentanyl (FIBF)      369.2      188.1      24      108        Amphetamine-D <sub>11</sub> 147.2      96.1      19      70      4      Fluoroisobutyryl-fentanyl (FIBF)      369.2      188.1      24      108        Amphetamine-D <sub>11</sub> 147.2      70.1      38      79      4      100 of the fentanyl (FIBF)      369.2      188.1      23      107        Barzoylecgonine      290.1      168.2      19      97      4      100      368.2      188.1      24      108        Barzoylecgonine-D <sub>2</sub> 298.2      171.2      20      93      4      100      368.2      108.1      38      108      108      368      22      106      38      108      368      108      368      108      368      108      108      108      108      108      108      108      108	Aminoclonazepam	286.1	250.1	20	113	Fentanyl	337.2	105.1	37	101
284.2    227.1    25    102    342.3    105.1    37    100      Amphetamine    136.1    91.2    17    73    36.2    188.1    24    108      Amphetamine    136.1    65.1    36    73    69.2    105.1    39    108      Amphetamine-D,    147.2    98.1    19    79    1000000000000000000000000000000000000		284.2	135.2	27	102		342.3	188.2	23	100
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Aminoflunitrazepam	284.2	227.1	25	102	Fentanyl-D <sub>5</sub>	342.3	105.1	37	100
Amphetamine    136.1    65.1    36    73    74100 05000000000000000000000000000000000									24	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Amphetamine									
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $										
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Amphetamine-D <sub>11</sub>					Fluorofentanyl				
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $										
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Benzoylecgonine					Fluorofentanyl-D <sub>3</sub>				
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $										
Butyrylfentanyl351.2188.223104 $\mu_{unnylfentanyl-D_{g}}$ 380.2188.123102351.2105.138104 $\mu_{unnylfentanyl-D_{g}}$ 380.2188.123102 $\alpha_{arfentanil}$ 395.2335.218100 $\mu_{O-Alprazolam}$ 325.1216.139117 $\alpha_{arfentanil}$ 300.2340.218100 $\mu_{O-Alprazolam-D_{g}}$ 302.2210.145127 $\alpha_{arfentanil}$ 300.2215.125108 $\mu_{O-Alprazolam-D_{g}}$ 300.2211.140127 $\alpha_{odeline-D_{g}}$ 300.2215.125108 $\mu_{odeline-D_{g}}$ 300.2171.139114 $\alpha_{odeline-D_{g}}$ 303.2215.126123 $\mu_{odeline-D_{g}}$ 303.2171.139117	Benzoylecgonine-D <sub>8</sub>					Furanylfentanyl				
Butyrylfentanyl      351.2      105.1      38      104      Furanylfentanyl-D <sub>5</sub> 380.2      105.1      38      102 $Carfentanil      395.2      335.2      18      100      HO-Alprazolam      325.1      216.1      39      117        Carfentanil-D_5      395.2      363.2      13      100      HO-Alprazolam      325.1      216.1      39      117        Carfentanil-D_5      400.3      340.2      18      100      HO-Alprazolam      300.2      210.1      45      127        Carfentanil-D_5      400.3      368.3      14      100      HO-Alprazolam-D_5      330.2      210.1      45      127        Codeine      300.2      215.1      25      108      HO-Alprazolam-D_5      300.2      171.1      39      114        Codeine - D_5      300.2      215.1      26      123      Hydrocodone - D_5      303.2      171.1      39      114        Codeine - D_5      303.2      171.1      29      114      114      114      114      114 $										
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Butyrylfentanyl					Furanylfentanyl-D5				
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $										
A00.3    340.2    18    100    A00.4    300.2    210.1    45    127      A00.3    368.3    14    100    300.2    221.1    40    127      Codeine    300.2    215.1    25    108    Hydrocodone    300.2    171.1    39    114      Codeine-D,    303.2    215.1    26    123    Hydrocodone    303.2    171.1    39    114      Codeine-D,    303.2    215.1    26    123    Hydrocodone    303.2    171.1    39    117	Carfentanil	395.2	335.2	18	100	HO-Alprazolam		216.1	39	117
Carfentanil-D <sub>5</sub> 400.3    368.3    14    110    HO-Alprazolam-D <sub>5</sub> 330.2    221.1    40    127      Codeine    300.2    215.1    25    108    Hydrocodone    300.2    171.1    39    114      Codeine-D <sub>5</sub> 303.2    215.1    26    123    Hydrocodone-D <sub>5</sub> 303.2    171.1    39    114      Codeine-D <sub>5</sub> 303.2    215.1    26    123    Hydrocodone-D <sub>5</sub> 303.2    171.1    39    117		395.2	363.2	13	100		325.1	205.1	45	117
400.3    368.3    14    110    300.2    221.1    40    127      Codeine    300.2    215.1    25    108    Hydrocodone    300.2    171.1    39    114      Codeine    300.2    165.1    41    108    Hydrocodone    300.2    241.1    27    114      Codeine-Da    303.2    215.1    26    123    Hydrocodone-Da    303.2    171.1    39    117	Carfentanil-D_	400.3	340.2	18	110	HO-Alprazolam-D	330.2	210.1	45	127
Codeine      300.2      165.1      41      108      Hydrocodone      300.2      241.1      27      114        Codeine-Da      303.2      215.1      26      123      Hydrocodone-Da      303.2      171.1      39      117	5	400.3	368.3	14	110	5	330.2	221.1	40	127
300.2  165.1  41  108  300.2  241.1  27  114    303.2  215.1  26  123  Hydrocodone-D,  303.2  171.1  39  117	Codeine	300.2	215.1	25	108	Hydrocodone	300.2	171.1	39	114
Codeine-D <sub>2</sub> Hydrocodone-D <sub>2</sub>	000000	300.2	165.1	41	108		300.2	241.1	27	114
303.2 165.1 41 123 303.2 241.1 27 117	Codeine D	303.2	215.1	26	123	Hydrocodope D	303.2	171.1	39	117
		303.2	165.1	41	123	Hydrocodone-D <sub>3</sub>	303.2	241.1	27	117

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

Compound	Precursor ( <i>m/z</i> )	Product ( <i>m/z</i> )	Collision energy (V)	RF lens (V)	Compound	Precursor (m/z)	Product (m/z)	Collision energy (V)	RF lens (V)
Hydromorphone	286.1	185.1	30	116	Methamphetamine-D <sub>11</sub>	161.2	97.1	19	80
riyaromorphone	286.1	157.1	41	116	Methamphetamme-D <sub>11</sub>	161.2	127.2	12	80
Hudromorphono D	289.2	185.1	30	134	Morphine	286.1	152.1	55	109
Hydromorphone-D <sub>3</sub>	289.2	157.1	42	134	Morphine	286.1	201.1	26	109
Isobutyrylfentanyl	351.2	188.2	24	101	Morphine-D	292.2	153.1	43	134
ISObutyrynentanyr	351.2	105.1	38	101	Morphine-D <sub>6</sub>	292.2	201.1	26	134
Katamina	238.1	125.0	27	80	Normanaridina	234.2	160.1	16	99
Ketamine	238.1	207.0	14	80	Normeperidine	234.2	56.1	23	99
3,4-Methylenedioxy-	180.1	105.1	22	83	Newser entrieling D	238.2	164.2	16	93
amphetamine (MDA)	180.1	135.1	19	83	Normeperidine-D <sub>4</sub>	238.2	58.1	23	93
	180.1	133.1	18	83		371.2	188.2	22	101
MDA-D <sub>5</sub>	185.1	110.1	23	102	Ocfentanil	371.2	105.1	38	101
3,4-Methylenedioxy-	185.1	138.1	19	102		244.3	86.2	13	68
methamphetamine (MDMA)	185.1	137.1	20	102	Phenylcyclidine (PCP)	244.3	91.1	31	68
	194.1	163.1	13	75		249.2	86.2	13	70
MDMA-D <sub>5</sub>	194.1	135.1	21	75	Phenylcyclidine-D <sub>5</sub>	249.2	96.1	32	70
	248.2	220.1	21	100		387.2	238.2	19	92
Meperidine	248.2	174.2	21	100	Sufentanil	387.2	111.0	36	92
	252.2	224.2	22	101		392.2	238.2	20	97
Meperidine-D <sub>4</sub>	252.2	178.2	21	101	Sufentantil-D <sub>5</sub>	392.2	111.0	36	97
	310.2	265.2	15	72		301.1	255.1	22	103
Methadone	310.2	105.0	27	72	Temazepam	301.1	177.0	38	103
	319.3	268.2	16	77		306.1	260.2	24	112
Methadone-D <sub>9</sub>	319.3	105.1	28	77	Temazepam-D <sub>5</sub>	306.1	177.0	38	112
	150.1	91.1	19	78		329.2	284.1	17	87
Methamphetamine	150.1	119.1	11	78	U-47700	329.2	173.0	32	87
	161.2	97.1	19	80		365.3	188.2	24	102
Methamphetamine-D <sub>11</sub>	161.2	127.2	12	80	Valerylfentanyl	365.3	105.1	39	102
	286.1	152.1	55	109		308.2	235.2	35	108
Morphine	286.1	201.1	26	109	Zolpidem	308.2	263.2	26	108
	292.2	153.1	43	134		315.2	242.2	36	104
$Morphine-D_6$	292.2	201.1	26	134	Zolpidem-D <sub>7</sub>	315.2	270.2	27	104

#### **Results and discussion**

Calibrations with good linearity (R<sup>2</sup> > 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 <sup>2</sup> values, lower limits of quantitation (LLC	OQ) and cutoff values
---	-----------------------

Compound	Internal standard	[Internal standard] (ng/mL)	Calibration range (ng/mL)	R <sup>2</sup>	LLOQ (ng/mL)	Cutoff (ng/mL)
4-ANPP	4-ANPP-D <sub>5</sub>	25	0.5 - 600	0.9956	1	2
Acetylfentanyl	Acetylfentanyl-13C6	25	0.5 - 600	0.9966	1	2
Acetylmorphine	Acetylmorphine-D <sub>6</sub>	100	7.5 – 900	0.9880	7.5	10
Acrylfentanyl	Acrylfentanyl-D <sub>5</sub>	25	0.5 - 600	0.9946	1	2
Alprazolam	Alprazolam-D <sub>5</sub>	100	7.5 – 1800	0.9968	3	10
Aminoclonazepam	Alprazolam-D <sub>5</sub>	100	7.5 – 1800	0.9803	30	10
Aminoflunitrazapem	Alprazolam-D <sub>5</sub>	100	7.5 – 1800	0.9809	75	5
Amphetamine	Amphetamine-D <sub>11</sub>	500	5 - 6000	0.9963	25	50
Benzoylecgonine	Benzoylecgonine-D <sub>8</sub>	500	5 - 6000	0.9964	10	50
Butyrylfentanyl	Acrylfentanyl-D <sub>5</sub>	25	0.5 - 600	0.9967	1	2
Carfentanil	Carfentanil-D₅	25	0.5 - 600	0.9970	1	2
Codeine	Codeine-D <sub>3</sub>	500	5 - 6000	0.9937	250	20
Cyclopropylfentanyl	Furanylfentanyl-D <sub>5</sub>	25	0.5 - 600	0.9913	1	2
Desalkylflurazepam	Desalkylflurazepam-D <sub>4</sub>	100	7.5 – 1800	0.9906	7.5	25
Diazepam	Alprazolam-D5	100	1.5 – 1800	0.9904	7.5	20
Ecgonine methyl ester	Ecgonine methyl ester-D <sub>3</sub>	500	5 - 6000	0.9953	25	50
EDDP	EDDP-D <sub>3</sub>	100	1.5 – 1800	0.9976	1.5	10
Ethylpentylone	Ethylpentylone-D <sub>5</sub>	100	1.5 – 1800	0.9974	3	30
Fentanyl	Fentanyl-D <sub>5</sub>	25	0.5 - 600	0.9956	1	2
FIBF	FIBF-D <sub>3</sub>	25	0.5 - 600	0.9936	0.5	2
Fluorofentanyl	Fluorofentanyl-D <sub>3</sub>	25	0.5 - 600	0.9983	0.5	2
Furanylfentanyl	Furanylfentanyl-D5	25	0.5 - 600	0.9966	1	2
HO-Alprazolam	HO-Alprazolam-D <sub>5</sub>	100	1.5 – 1800	0.9921	75	10
Hydrocodone	Hydrocodone-D <sub>3</sub>	500	5 - 6000	0.9975	10	20
Hydromorphone	Hydromorphone-D <sub>3</sub>	500	5 - 6000	0.9955	10	20
Isobutyrylfentanyl	Acrylfentanyl-D <sub>5</sub>	25	0.5 - 600	0.9967	1	2
Ketamine	Ethylpentylone-D <sub>5</sub>	100	1.5 – 1800	0.9967	1.5	30
MDA	MDA-D <sub>5</sub>	500	5 - 6000	0.9971	25	100
MDMA	MDMA-D <sub>5</sub>	500	5 - 6000	0.9971	5	100
Meperidine	Meperidine-D <sub>4</sub>	100	1.5 – 1800	0.9973	1.5	10
Methadone	Methadone-D <sub>9</sub>	100	1.5 – 1800	0.9970	1.5	10
Methamphetamine	Methamphetamine-D <sub>11</sub>	500	5 - 6000	0.9979	10	50
Morphine	Morphine-D <sub>6</sub>	500	25 – 3000	0.9800	250	20
Normeperidine	Normeperidine-D <sub>4</sub>	100	1.5 – 1800	0.9930	1.5	10
Ocfentanil	Fluorofentanyl-D <sub>3</sub>	25	0.5 - 600	0.9963	1	2
Phenylcyclidine	Phenylcyclidine-D <sub>5</sub>	100	1.5 – 1800	0.9947	1.5	10
Sufentanil	Sufentanil-D5	25	0.5 - 600	0.9952	1	2
Temazepam	Temazepam-D <sub>5</sub>	100	1.5 – 1800	0.9907	7.5	20
U-47700	Fluorofentanyl-D <sub>3</sub>	25	0.5 - 600	0.9932	2.5	2
Valerylfentanyl	Fluorofentanyl-D <sub>3</sub>	25	0.5 - 600	0.9847	10	2
Zolpidem	Zolpidem-D,	100	1.5 – 1800	0.9972	1.5	10

# thermo scientific

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

		QC		Medium QC				High QC				
Compound	[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.

#### References

- Liu, J.; Wang, H.; Manicke, N.E.; Lin, J-M.; Cooks, R.G.; Ouyang, Z. Development, characterization, and application of paper spray ionization. *Analytical Chemistry*. 2010, *82*(6), 2463–71.
- SWGTOX. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*. 2013, *37*, 452–74.

## Find out more at thermofisher.com/verispray

For Research Use Only. Not for use in diagnostic procedures. © 2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all locations. Please consult your local sales representative for details. TN73467-EN 0420S

