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

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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.

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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.
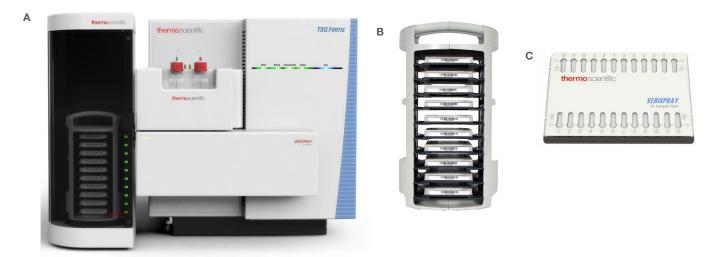


Figure 1. (A) VeriSpray PaperSpray system mounted to TSQ Fortis triple quadrupole MS, (B) magazine, and (C) VeriSpray cartridge

## 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:

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

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

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

## 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/x 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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