Simultaneous Quantitation of 78 Drugs and Metabolites in Urine with a Dilute-And-Shoot LC–MS-MS Assay

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A novel LC-MS-MS assay that simultaneously detects and quantitates 78 drugs and metabolites was developed and validated for chronic pain management. Urine specimen was diluted and mixed with internal standards (ISs) before injected into LC-MS-MS. Seventy-two analytes were detected with positive electrospray ionization mode and the remaining six analytes with negative mode. Two separate gradient elution chromatographic programs were established with the same mobile phases on the same bi-phenyl HPLC column. The assay was linear for all analytes with linear regression coefficient ranging 0.994-1.000. The intra-assay precision was between 1.7 and 8.8% and inter-assay precision between 1.9 and 12.2%, with bias <20% for all but six analytes. All analytes in urine specimens were stable for 7 days at 4°C, and no significant matrix effect or carryover was observed. A suboptimal recovery rate (60.0-156.8%) was observed for six analytes, potentially due to the lack of available deuterated ISs, requiring comparison to a chemically different IS. Method comparison using patient and proficiency testing samples demonstrated that this assay was sensitive and accurate. The assay improves on currently existing assays by including glucuronide conjugates, allowing direct detection of metabolites that might otherwise be missed by existing methods.

Introduction

Both prescribed drug misuse and illicit drug abuse risks exist in patients in chronic pain management programs (1). It was reported that 75% of patients in chronic pain management programs were found non-compliant with their prescription and 11% tested positive for illicit drug use (2). It is worth noting that abuse of prescription medications is increasing faster than illicit drugs. The incidence rate of prescription drug abuse has increased over 80% from 2000 to 2006, exceeding the incidence rate of abuse of the illicit drugs such as cocaine, heroin, marijuana and ecstasy combined (3). Drug compliance testing provides an objective measure of a patient's pattern of drug use. It may aid in identifying patients who are inappropriately taking prescribed medications or illicit drugs, which may interfere with treatment. Without drug testing, it could be challenging to identify drug abusers as obvious signs, behaviors and symptoms are not always present in those patients (4, 5).

Urine drug testing is a useful tool for pain management providers to assist in diagnostic and therapeutic decision-making. The main advantages of choosing urine as the specimen for drug testing include noninvasive sampling, drugs and metabolites present in high concentrations and relatively long detection windows (5). Adherence monitoring with urine drug testing has become a common practice in recent years. It has been shown that random urine drug testing increased compliant use of opioids with concomitant decreased illicit drug use in pain management practices (6, 7). Immunoassay drug tests are most commonly used in initial urine drug screens. They are based on interactions between antibodies and drugs of interest and are designed to classify substances as either present or absent in patient urine. However, because of the nature of immunoassays, they are prone to false positives caused by cross reactions and false negatives because of limited sensitivity (8). Therefore, results of immunoassay drug screens are presumptive and cannot be used solely to determine compliance status. Gas chromatographymass spectrometry (GC-MS) methods are considered the gold standard for confirmatory testing. However, GC-MS methods are time consuming and in many cases require a derivatization step that contributes to sample loss (9). Compared with GC-MS, the liquid chromatography-tandem mass spectrometry (LC-MS-MS) assays generally require much simpler sample preparation with reasonably high sensitivity and specificity, are subjected to fewer interferences and have the potential to quantitate multiple analytes in a single method (10).

Many pain medications, such as opiates, opioids and benzodiazepines, are metabolized and conjugated in the liver. Parent drugs and metabolites are excreted in urine in both free and glucuronide conjugated forms (11). The urine concentration of conjugates can vary substantially depending on individual metabolism rates and sample collection time (8, 11). To improve assay sensitivity and reduce the number of monitored transitions, glucuronide hydrolysis may be included in sample preparation prior to LC-MS-MS. Chemical hydrolysis, such as acid hydrolysis, is fast and efficient, but its efficiency is subject to variables like acid concentration, temperature and pressure (12). Enzymatic hydrolysis (β-glucuronidase) is more specific, but can lead to incomplete hydrolysis if the enzyme concentration or incubation time is not properly optimized (12, 13). Solid-phase extraction (SPE) is frequently used for urine sample cleanup in recent LC-MS-MS method reports of urine drug testing (8, 9, 14, 15). However, SPE sample extraction typically involves laborious procedures and has inconsistent recoveries for all analytes, especially when many analytes were measured simultaneously (8, 15). Alternatively, direct injection of diluted urine samples into LC-MS-MS, or dilute-and-shoot methods, have been successfully employed in a several recent pain management urine drug testing assay (2, 16, 17). Although these reported dilute-and-shoot based assays were subject to various amounts of ion suppression due to matrix effects, it provides a simple but robust solution to develop an LC-MS-MS pain medication panel.

In this study, we developed and validated a simple and costeffective LC–MS-MS assay which is able to simultaneously quantitate 78 drugs and metabolites that cover major categories of illicit drugs and drugs commonly prescribed in chronic pain patients. This dilute-and-shoot assay involves minimum sample preparation by including glucuronide conjugates with commercially available standards, eliminating the need for an extra hydrolysis step. To maximize ionization efficiency, two separate LC–MS-MS methods, one employing positive and the other negative ionization, were built to measure 72 and 6 analytes, respectively. Isotope-labeled internal standards (ISs) were incorporated to facilitate reliable quantitation of the samples. As a major urinary metabolite of propoxyphene, norpropoxyphene has been reported to be unstable and become cyclized degradation products (8, 18, 19). To ensure accurate quantitation, both norpropoxyphene and its degradation products were quantified and summed before results reported.

Materials and methods

Chemicals and reagents

All certified reference standards and isotope-labeled IS solutions were purchased from Cerilliant (Round Rock, TX, USA). Methanol (Optima[®] LC/MS grade) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Trazodone (T-030, 1 mg/mL) and its metabolite *meta*-chlorophenylpiperazine (*m*-CPP, C-089, 1 mg/mL) methanol stocks were purchased from Cerilliant. Formic acid (LC/MS grade, 98%) was purchased from Sigma-Aldrich (St Louis, MO, USA). LiquichekTM Urine Toxicology Negative Control (blank human urine) was purchased from Bio-Rad (Hercules, CA, USA). Deionized water was generated with a Milli-Q water purification system from Millipore (Billerica, MA, USA).

Instrumentation and conditions

LC-MS-MS analysis was performed on an AB Sciex 5500 O-trap mass spectrometer (Framingham, MA, USA) coupled with a Shimadzu Nexera X2 ultra-high pressure liquid chromatography (UHPLC) system (Kyoto, Japan). The temperature of the thermostatted column and the autosampler were set at 40 and 5°C, respectively. Of the 78 drugs and metabolites measured, 72 were detected in a positive ionization method and 6 in a negative ionization method (Table I). In both the methods, the chromatography separation was performed with a RaptorTM Bi-phenyl column, 3.0×50 mm, 2.7μ m (Restek, Bellefonte, PA, USA) and gradient elution comprising 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). A RaptorTM EXP[®] Guard Column Cartridge (2.7 μ m, 3.0 \times 5 mm) was installed preceding the bi-phenyl analytical column for the sake of sample cleanup. The gradient program for positive ionization method started from 5% mobile phase B at 0-0.2 min, increasing to 25% at 3 min. The mobile phase B content was further increased to 100% at 7.5 min, and held until 9.0 min, after which it was dropped to 5% at 9.5 min and held until 11.0 min. The total run time was 11.1 min with a flow rate of 0.6 mL/min. The gradient program for positive ionization method started from 10% mobile phase B at 0-0.2 min, increasing to 40% at 0.7 min. The mobile phase B content was further increased to 99% at 3.0 min, and held until 3.5 min, after which it was dropped to 10% at 3.6 min and held until 4.5 min. The total run time was 4.5 min with a flow rate of 0.7 mL/min.

Analytes were detected by mass spectrometry using scheduled multiple reaction monitoring (MRM) in either positive or negative electrosprav ionization (ESI) modes. All analytes were monitored within a ± 0.5 min retention time window. The dwell time was automatically calculated by the software Analyst® under the dynamic MRM mode with a total cycle time of 0.3 and 0.4 ms, respectively, for positive and negative ionization modes. For the positive ionization method, the source parameters were: curtain gas, 35 L/min; collisional activated dissociation (CAD), medium; heated nebulizer temperature, 550°C; nebulizing gas (GS1), 50 L/min and heater gas (GS2), 60 L/min. For the negative ionization method, the source parameters were: curtain gas, 35 L/min: CAD, medium; heated nebulizer temperature, 600°C; GS1, 50 L/ min and GS2, 50 L/min. Two characteristic MRM transitions were monitored for each analyte, with the exception of amphetamine, buprenorphine-3β-D-glucuronide, norbuprenorphine and norbuprenorphine-3β-D-glucuronide for which only one transition was available. The MRM ratios, which are defined as the peak area ratios between primary and secondary ion transitions, were only acceptable within $\pm 30\%$ or better for all analytes. All data were collected using the AB Sciex Analyst® software and quantified with the MultiQuant[®] 2.1 software.

Preparation of calibrators and quality control materials

All calibrators and quality controls (QCs) were prepared separately for positive and negative ionization methods, comprising 72 and 6 compounds, respectively. First, the $40 \times$ positive or negative working solution was prepared by mixing each individual compound in 50% methanol in water. The concentration of each compound in the above working solutions was 40 times of their individual cutoff value. Different predetermined cutoffs were used for different analytes, based on their distinct clinical significance. The $40 \times$ working solution was then diluted with 50% methanol in water, producing five calibrator solutions: calibrator 5 ($8\times$), calibrator 4 (4× cutoff), calibrator 3 (1× cutoff), calibrator 2 (0.4× cutoff) and calibrator 1 ($0.2 \times$ cutoff). High-level (QC-H, $3 \times$ cutoff) and low-level (QC-L, $0.5 \times$ cutoff) QCs were diluted from separately prepared $40 \times$ working solutions with 50% methanol in water. The calibrator and QC bulk solutions were then aliquoted into microcentrifuge tubes (2.0 mL) and stored at -20° C.

Similarly, IS mix was also prepared separately for positive and negative ionization methods, by adding individual deuterated IS in 100% methanol. IS mix was aliquoted and stored at -20° C.

Sample preparation

One hundred microliter urine specimens, calibrators or controls were centrifuged for 3 min at 10,000 rpm (Eppendorf centrifuge model 5430). After centrifugation, 10 μ L of urine specimen supernatant (or calibrator, QC), and 10 μ L of IS mix, was diluted in 480 μ L of sample diluent (95% mobile phase A + 5% mobile phase B, for patient samples) or 470 μ L of sample diluent plus 10 μ L of Bio-Rad blank urine (for calibrators and QCs) before injection. The purpose of adding 10 μ L of Bio-Rad blank urine for calibrators and QCs is to ensure the matrix resemblance to patient samples in the final preparation.

Assay validation

The method was validated for linearity, limit of detection (LOD), lowest limit of quantitation (LLOQ), precision, accuracy,

 Table I

 MRM Transitions, Retention Time and Compound Tuning Parameters

2.4	Compound	Precursor ion (m/z)	Primary ion (m/z)	Secondary ion (m/z)	Retention time (min)	DP	CE	lonization mode
Bit Mine Amanda Magnetin 180 15.1 22.2 15.1 100 4.0 Particle Amanda Magnetin 180 15.1 22.2 15.1 100 4.0 Particle Amanda Magnetin 180 15.1 22.2 15.1 100 4.0 Particle Beraphetine 20.1 183.1 15.1 2.1 100 15.0 Particle Beraphetine 20.1 183.1 15.1 2.1 100 100 Particle Classageonic 20.1 27.0 105.1 2.2 100 100 Particle Classageonic 20.2 100.1 100.1 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 <td>2-Hydroxyethylflurazepam</td> <td>333.2</td> <td>211.2</td> <td>109.0</td> <td>7.1</td> <td>110</td> <td>55</td> <td>Positive</td>	2-Hydroxyethylflurazepam	333.2	211.2	109.0	7.1	110	55	Positive
Żwinschwarzene 286.2 121.1 222.2 5.5 100 4.4 Perkler Aperatom 106.2 701.2 202.7 7.0 100 50 Perkler Aperatom 106.2 701.2 202.7 7.0 100 50 Perkler Breenophre 202.1 100 50 Perkler 100 50 Perkler Breenophre 403.1 105.1 100 50 Perkler 100 100 Perkler Calorization 443.1 464.1 100 100 Perkler 100 100 Perkler 100 Perkler 100 100 100 Perkler 100 100 Perkler 100 100 100 100 100 100 100 100 100 100 100 100		328.1	165.0	191.0		100		Positive
Zohnschweigen 280.2 121.1 222.2 5.5 100 4.4 Perkles Aparatin 300.2 21.3 22.2 6.1 100 6.3 Perkles Aparatin 300.2 21.0 22.2 7.5 100 6.3 Perkles Aparatin 300.2 21.0 22.2 7.5 100 5.5 Perkles Bernsphran 803.3 90.3 5.5 7.0 5.5 Perkles Aparatin 7.0 7.41 7.0 7.41 7.0 7.41 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.44 7.0 7.4 7.0 7	6B-Naltrexol	344.1	308.3	326.0	4.6	100	40	Positive
Aburschnin 392 2812 282 7.5 100 5.0 Parlies Arushterinin 12.0 118.0 116.0 12.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0	7-Aminoclonazepam	286.2	121.1	222.2		100	41	Positive
Arenbarnine 15.0 15.0 2.3 2.0 13 Parisine Parisine Decompring Bornvolgrone 40.1 38.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 <td>7-Aminoflunitrazepam</td> <td>284.2</td> <td>135.1</td> <td>227.2</td> <td>6.1</td> <td>100</td> <td>40</td> <td>Positive</td>	7-Aminoflunitrazepam	284.2	135.1	227.2	6.1	100	40	Positive
Arenbarnine 15.0 15.0 2.3 2.0 13 Parisine Parisine Decompring Bornvolgrone 40.1 38.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 <td>Alprazolam</td> <td>309.2</td> <td>281.2</td> <td>205.2</td> <td>7.5</td> <td>160</td> <td>50</td> <td>Positive</td>	Alprazolam	309.2	281.2	205.2	7.5	160	50	Positive
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Megrodopante219.215.8.297.25.51012.5PonitveMethanphetamine150.091.2119.03.6201.6PonitveMethanphetamine28.129.1124.01.82.51007.8PonitveMorphine-5-glucuronide46.2.228.6201.22.01804.4PonitveMorphine-5-glucuronide46.2.228.6201.22.82.04.7PonitveMorphine-5-glucuronide42.228.6201.22.82.04.7PonitveNalexone32.127.225.0-5.61004.0PonitveNorbupenorphine41.315.2.0-5.61004.0PonitveNorbupenorphine27.1.2140.1165.17.21803.0PonitveNorbupenorphine27.1.2140.1155.17.21803.0PonitveNorbupenorphine32.34.2.156.05.2804.2PonitveNorbupenorphine32.34.2.156.05.2804.2PonitveNorphorphine32.34.125.25.04.5PonitveNorphorphine32.34.2.156.05.2804.2PonitveNorphorphine32.34.125.2804.2PonitveNorphorphine32.34.2.156.05.2804.5PonitveNorphorphine32.3 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>								
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Morphine-3-glucuronide 482.2 286.2 201.2 2.0 80 9.4 Positive Natoxone 328.1 212.2 253.2 3.9 80 5.1 Positive Natoxone 328.1 212.2 253.0 4.4 100 40 Positive Natoxone 328.1 212.2 255.0 4.4 100 40.0 Positive Notoxperenphine-3p-g-glucuronide 590.4 414.4 - 4.9 30 52 Positive Nordizepan 27.1 21.4 10.1 165.1 72.2 160 39 Positive Nordizepan 27.1 284.1 42.0 4.1 120 30 Positive Nordizepan 32.3 44.1 252.3 62.3 80 62.6 Positive Noreoxocone 32.6.3 44.1 252.3 62.3 80 62.6 Positive Noreoxocone 32.6.3 44.1 252.3 80.3 80	Midazolam	326.1	291.1	249.1		100	48	Positive
Morphnes-E-glucuronide 48.2.2 28.6 20 4.7 Positive Naloxone 38.1 212.2 253.2 3.9 80 51 Positive Naloxone 342.1 267.2 255.0 4.4 100 40 Positive Norbugrenorphine 44.3 152.0 - 56 100 129 Positive Nordizargan 213.1 104.0 155.0 4.9 30 52 Positive Nordizargan 233.1 104.0 55.0 4.9 80 32 Positive Nordizargan 233.1 104.0 55.0 4.9 80 42 Positive Nordizargan 233.1 242.1 36.0 52 80 42 Positive Nordizargan 233.1 242.1 133.0 63 80 25 Positive Nordizargan 232.1 227.1 284.1 39 100 74 Positive Nordizargan	Morphine	286.2	152.0	128.0	2.5	100	78	Positive
Nakowa 328.1 212.2 253.2 3.9 80 51 Positive Natreane 34.1 267.2 55.0 4.4 100 40 Positive Norbuperprophine 414.3 152.0 - 5.6 100 129 Positive Norbuperprophine-3β-a-glucuronide 590.4 414.4 - 4.9 30.0 52 Positive Norfinerany 271.2 140.1 165.1 7.2 180.39 Positive Norrigocodne 286.1 199.1 241.1 4.1 1 120.30 Positive Norreportine degradent 308.3 100.0 143.0 6.3 80.2 Positive Norreportine degradent 308.3 100.0 143.0 7.0 150.3 Positive Oxazepam 250.2 58.1 42.0 4.1 4.3 160.5 9.0 Positive Oxazepam-glucuronide 463.1 287.2 241.1 143.3 160.5 9.0 Pos	Morphine-3-glucuronide	462.2	286.2	201.2	2.0	80	44	Positive
Natiresone 342.1 267.2 55.0 4.4 100 40 Positive Norbuprenorphine-3β-n-glucuronide 590.4 414.4 - 4.9 30 52 Positive Norbuprenorphine-3β-n-glucuronide 590.4 414.4 - 4.9 30 52 Positive Norbuprenorphine-3β-n-glucuronide 233.1 84.0 55.0 4.9 80 32 Positive Norbuprenorphine 286.1 199.1 241.1 4.1 120 30 Positive Normeperidne 236.3 44.1 252 60 45 Positive Norporpoxynene 302.1 227.1 284.1 3.9 120 41 Positive Operatryl Tamadol 250.2 58.1 42.0 4.1 50 14 Positive Oxazepam 287.2 241.1 104.0 7.0 150 30 Positive Oxazepam_glucuronide 463.1 287.2 27.1 1.8 80	Morphine-6-glucuronide	462.2	286.2	201.2	2.8	20	47	Positive
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Nortgreprophine-3βglucuronide 590.4 414.4 - 4.9 30 52 Positive Nordiareparm 213.1 140.1 165.1 7.2 160 39 Positive Nordiareparm 213.1 84.0 55.0 4.9 80 32 Positive Northytocodone 236.1 198.1 241.1 4.1 120 30 Positive Norreportinitie 234.3 42.1 56.0 5.2 80 42 Positive Norreportinitie 236.3 44.1 252.3 6.2 50 45 Positive Norpoposyphene 308.3 100.0 143.0 6.3 80 26 Positive Oberatylf Tramadu 250.2 58.1 42.0 4.1 104.0 70 150 30 Positive Oxazeparn glucuronide 478.3 272.2 211.1 241.1 4.3 160 58 Positive Oxymorphone 302.1 227.0 1	Naltrexone				4.4	100	40	Positive
Norbigrenorphine-3β-oglucuronide 590.4 414.4 - 4.9 30 52 Positive Nordiacepan 213.1 140.0 155.1 7.2 160 39 Positive Nordiacepan 233.1 84.0 55.0 4.9 80 32 Positive Northytocodone 236.1 199.1 241.1 4.1 120 30 Positive Northytocodone 302.1 227.1 284.1 3.9 20 41 Positive Northytoprophythen 36.3 44.1 252.3 6.2 50 45 Positive NDP (norbuprenorphine degradent) 308.3 100.0 143.0 6.3 80 26 Positive Oxazepan 287.2 241.1 104.0 7.0 150 30 Positive Oxazepan-glucuronide 483.1 287.2 212.1 241.1 4.3 160 58 Positive Oxymorphone 302.1 270.2 198.1 2.7	Norbuprenorphine	414.3	152.0	_	5.6	100	129	Positive
Nordizepami 271.2 140.1 165.1 7.2 160 39 Positive Norfnetanyl 231 84.0 55.0 4.9 80 32 Positive Norfnetanyl 233.1 84.0 55.0 4.9 80 32 Positive Norreperfidine 286.1 199.1 241.1 4.1 120 30 Positive Norreportsponde 302.1 222.1 244.1 3.8 120 44 Positive Norreportsponder 306.3 100.0 143.0 6.3 80 25 Positive Obacepan-jucuronide 463.1 287.2 241.1 104.0 7.0 150 30 Positive Oxacepan-jucuronide 473.1 241.0 6.6 100 24 Positive Oxymorphone-3-glucuronide 478.3 284.2 227.1 1.8 80 40 Positive Oxymorphone-3-glucuronide 478.3 284.2 27.7 100 38	Norbuprenorphine-3B-D-glucuronide	590.4	414.4	_	4.9	30	52	Positive
Norfentanyl 233.1 84.0 55.0 4.9 80 32 Positive Norhydrocodone 286.1 199.1 241.1 4.1 120 30 Positive Norreperidine 234.3 42.1 56.0 5.2 80 42 Positive Norreportyphene 302.1 227.1 284.1 3.9 120 41 Positive NPD (norbuprenorphene digradent) 308.3 100.0 143.0 6.3 80 26 Positive O-Bernettyl Tranadol 250.2 58.1 42.0 4.1 50 14 Positive Oxazepam 287.2 241.1 104.0 7.0 150 30 Positive Oxycodore 316.2 212.1 241.1 4.3 160 24 Positive Oxymorphone-S-glucuronide 478.3 284.2 277.1 1.8 80 40 Positive Propoxythene 300.3 266.3 57.7 6.2 80 13		271.2	140.1	165.1	7.2	160		Positive
Normsperidine 234.3 42.1 56.0 5.2 80 42. Positive Noraxycodone 302.1 227.1 284.1 3.9 120 41 Positive Norpropsychene 328.3 44.1 252.3 6.2 50 45 Positive NPD (norbuprenorphine degradent) 308.3 100.0 143.0 6.3 80 26 Positive Obzerephy Tramadol 250.2 58.1 42.0 4.1 50 14 Positive Oxazepam-glucunonide 463.1 287.2 241.1 104.0 7.0 150 30 Positive Oxycodone 316.2 212.1 241.1 4.3 160 58 Positive Oxycodone 302.1 227.0 198.1 2.7 18 80 40 Positive Oxymorphone-3-glucunonide 478.3 284.2 272.1 1.8 80 40 Positive Propsynthene 340.3 266.3 57.7	Norfentanyl	233.1	84.0	55.0		80		Positive
Normsperidine 234.3 42.1 56.0 5.2 80 42. Positive Noraxycodone 302.1 227.1 284.1 3.9 120 41 Positive Norpropsychene 328.3 44.1 252.3 6.2 50 45 Positive NPD (norbuprenorphine degradent) 308.3 100.0 143.0 6.3 80 26 Positive Obzerephy Tramadol 250.2 58.1 42.0 4.1 50 14 Positive Oxazepam-glucunonide 463.1 287.2 241.1 104.0 7.0 150 30 Positive Oxycodone 316.2 212.1 241.1 4.3 160 58 Positive Oxycodone 302.1 227.0 198.1 2.7 18 80 40 Positive Oxymorphone-3-glucunonide 478.3 284.2 272.1 1.8 80 40 Positive Propsynthene 340.3 266.3 57.7	Norhydrocodone	286.1	199.1	241.1	4.1	120	30	Positive
Νσαχνοdone 302.1 227.1 284.1 3.9 120 41 Positive Narpopoxyhene 326.3 44.1 252.3 6.2 50 45 Positive D/D (norbupenorphine degradent) 308.3 100.0 143.0 6.3 80 26 Positive Oxazepam 250.2 58.1 42.0 4.1 50 14 Positive Oxazepam-glucuronide 463.1 287.2 241.0 6.6 100 24 Positive Oxycoorphone 316.2 212.1 241.1 4.3 160 58 Positive Oxymorphone 302.1 227.0 198.1 2.7 100 38 Positive PCP (Phencyclidine) 243.3 91.0 159.0 6.1 40 24 Positive Pregabalin 160.1 97.1 83.1 24 70 20 Positive Temazepam 304.2 258.2 117.2 7.4 150 55		234.3	42.1	56.0	5.2	80		Positive
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	Nortriptyline						29	Positive
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1 nonoodionaan 201.1 42.1 100.0 2.4 - 50 - 54 Negalive	Phenobarbital	231.1	42.1	188.0	2.4	- 90	- 54	Negative

(continued)

Table I Continued							
Compound	Precursor ion (m/z)	Primary ion (m/z)	Secondary ion (m/z)	Retention time (min)	DP	CE	Ionization mode
Pentobarbital	225.0	42.0	182.1	2.6	- 95	-56	Negative
Secobarbital	237.1	42.0	194.1	2.7	- 95	-54	Negative
THC-COOH	343.1	245.2	107.1	3.6	-115	- 40	Negative
THC-COOH glucuronide	519.2	343.1	299.2	3.5	- 55	-34	Negative

DP, declustering potential; CE, collision energy.

interference and matrix effect, sample stability, carryover and correlation studies.

Linearity

Standard curve linearity was measured using the ratio of the analyte peak area to the IS area versus nominal concentration of standards by weighted linear regression $(1/X^2)$. The acceptance criterion for a calibration curve was a correlation coefficient *R* of 0.990 or better.

LOD, LLOQ and dilution validation

LODs were evaluated by analyzing 15 Bio-Rad blank urine specimens fortified with IS over 3 days. An LOD was defined as the average concentration of the negative specimens plus three times the standard deviations (SDs) of the negative specimens. LLOQs were evaluated by analyzing serially diluted standards in 50% methanol urine specimen that were repeated 15 times over 5 days. The LLOQs were defined as the concentration at which the percent coefficient variation (% CV) was $\leq 20\%$. The accuracy acceptance criterion in LLOQ determination was $\pm 20\%$. The dilution validation was evaluated by 10-fold dilution of $40 \times$ working solution with Bio-Rad blank urine, which was repeated nine times over 3 days. The acceptance criterion for dilution validation was that the inter-assay percent CV was $\leq 20\%$ and the % bias was within $\pm 20\%$.

Sample stability

The stability of the analytes in urine was assessed by storing urine samples (from three different negative patients) that have been fortified at their individual cutoff concentrations at 4° C for up to 7 days.

Carryover

Carryover was assessed by running four calibrator 1 samples (L_1-L_4) immediately after injecting three calibrator 5 (H_1-H_3) samples to verify the minimal sample carryover. Carryover was calculated as 100 * $((L_1 - (L_3 + L_4)/2)/((H_2 + H_3)/2 - (L_3 + L_4)/2))$ and must be <1% to be acceptable.

Interference and matrix effect

To evaluate interference, urine samples from 10 drug-negative patients (determined by in-house qualitative drug abuse screening on a Roche Cobas[®] 8000 platform) were analyzed separately to ensure that no visible interferences were present at the retention time of all analytes. The matrix effect was assessed by simultaneous post-column infusion (or 'tee-infusion') of standard compounds into the MS-MS detector during the chromatographic analysis of 10 separate patient negative urine samples (20). All

Table II

IS MRM Transitions, Retention Time and Compound Tuning Parameters

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6-Acetylmorphine 331.1 165.1 4.2 100 51 Positive 7-Aminoflunitrazepam-D4 290.1 121.1 5.5 100 41 Positive Arminoflunitrazepam-D5 314.2 286.1 7.5 160 39 Positive Arphetamine-D5 141.1 124.0 2.9 20 13 Positive Benzoylegonine-D8 298.2 171.1 5.4 100 27 Positive Buprenorphine-D4 472.3 59.1 6.0 70 97 Positive Diazepam-D5 290.2 198.2 7.7 160 44 Positive EoDP-D3 281.2 233.7 6.3 100 45 Positive Gabepentin-D10 182.3 55.0 3.1 80 33 Positive MD4-D5 195.2 110.0 3.6 60 30 Positive MD4-D5 199.2 154.8 4.2 80 25 Positive <	Compound				DP	CE	
6-Acetylmorphine 331.1 165.1 4.2 100 51 Positive (6-MAM)-D3 290.1 121.1 5.5 100 41 Positive 7-Aminoflunitrazepam-D7 291.2 138.1 6.1 100 40 Positive Apprazolam-D5 314.2 286.1 7.5 160 39 Positive Barzoylegopine-D8 298.2 171.1 5.4 100 27 Positive Buprenorphine-D4 472.3 59.1 6.0 70 97 Positive Diazepam-D5 290.2 198.2 7.7 160 44 Positive EDP-D3 281.2 233.7 6.3 100 42 Positive Gabepertin-D10 182.3 55.0 3.1 80 33 Positive MD4-D5 199.2 164.8 4.2 80 25 Positive MDA-D5 199.2 164.8 4.2 80 25 Positive MDA-D5 <td>2-Hydroxyethylflurazepam-D4</td> <td>337.2</td> <td>113.0</td> <td>7.1</td> <td>110</td> <td>37</td> <td>Positive</td>	2-Hydroxyethylflurazepam-D4	337.2	113.0	7.1	110	37	Positive
(6-MAM)-D3 7-AminoClonazepam-D4 290.1 121.1 5.5 100 41 Positive Alprazolam-D5 314.2 286.1 7.5 160 39 Positive Alprazolam-D5 141.1 124.0 2.9 20 13 Positive Benzoylecgonine-D8 298.2 171.1 5.4 100 27 Positive Buprenorphine-D4 472.3 59.1 6.0 70 97 Positive Codeine-D3 303.3 115.1 4.1 100 100 Positive Diazepam-D5 290.2 198.2 7.7 160 44 Positive Gabepentin-D10 182.3 55.0 3.1 80 30 Positive Hydrocodone-D6 306.2 202.1 4.5 100 40 Positive MDA-D5 185.2 110.0 3.6 60 30 Positive MDA-D5 199.2 164.8 4.2 80 26 Positive MDA-D5 199.2 164.8 4.2 80 26		331.1	165.1	4.2	100	51	Positive
7-Aminoflunitrazepam-D7 291.2 138.1 6.1 100 40 Positive Alprazolam-D5 314.2 286.1 7.5 160 39 Positive Amphetamine-D5 141.1 124.0 2.9 20 13 Positive Buprenorphine-D4 472.3 59.1 6.0 70 97 Positive Codeine-D3 303.3 115.1 4.1 100 100 Positive Diazepam-D5 290.2 198.2 7.7 160 44 Positive Gabepentin-D10 182.3 55.0 3.1 80 30 Positive Gabepentin-D10 182.3 55.0 3.1 80 30 Positive MDA-D5 185.2 110.0 3.6 60 30 Positive MDA-D5 199.2 164.8 4.2 80 25 Positive MDFa-D5 131.4 268.0 6.5 80 26 Positive MDA-D5 199.2 155.2 121.2 3.6 20 15 Positive <td>(6-MAM)-D3</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	(6-MAM)-D3						
Alprazolam-D5314.2286.17.516039PositiveAmphetamine-D5141.1124.02.92013PositiveBenzoylecgonine-D8298.2171.15.410027PositiveCodeine-D3303.3115.14.1100100PositiveDiazepam-D5290.2198.27.716044PositiveEDDP-D3281.2233.76.310042PositiveGabepentin-D10182.355.03.18033PositiveHydrocodone-D6306.2202.14.510040PositiveHydrocodone-D6292.3185.03.012040PositiveMDA-D5185.2110.03.66030PositiveMDA-D5198.2110.03.66030PositiveMDA-D5198.2123.1105.14.64026PositiveMDA-D5198.2121.23.62015PositiveMethadone-D3313.4268.06.58026PositiveMorphine-6-glucuronide-D3289.2152.210074PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5292.3246.27	7-Aminoclonazepam-D4	290.1	121.1	5.5	100	41	Positive
Amphetamine-D5 141.1 124.0 2.9 20 13 Positive Benzoylecgonine-D8 298.2 171.1 5.4 100 27 Positive Buprenorphine-D4 472.3 59.1 6.0 70 97 Positive Codeine-D3 303.3 115.1 4.1 100 100 Positive EDDP-D3 281.2 233.7 6.3 100 42 Positive Gabepentin-D10 182.3 55.0 3.1 80 33 Positive Hydrocodone-D6 306.2 202.1 4.5 100 40 Positive MDEA-D5 185.2 110.0 3.6 60 30 Positive MDEA-D5 199.2 164.8 4.2 80 25 Positive Morphine-D3 313.4 268.0 6.5 80 26 Positive Morphine-D3 289.2 152.2 2.8 20 47 Positive Morphine-D3	7-Aminoflunitrazepam-D7	291.2	138.1	6.1	100	40	Positive
Benzoylecgonine-D8298.2171.15.410027PositiveBuprenorphine-D4472.359.16.07097PositiveCodeine-D3303.3115.14.1100100PositiveDiazepam-D5290.2198.27.716044PositiveEDDP-D3281.2233.76.310042PositiveFentanyl-D5342.3188.36.010045PositiveGabepentin-D10182.355.03.18033PositiveHydrocodone-D6306.2202.14.510040PositiveMDA-D5185.2110.03.66030PositiveMDA-D5199.2164.84.28025PositiveMDMA-D5199.2164.84.28026PositiveMeperidine-D4252.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMorphine-6-glucuronide-D3465.2289.22.82047PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5276.364.24.15014PositiveNordiazepam-D5292.3246.2 <td< td=""><td>Alprazolam-D5</td><td>314.2</td><td>286.1</td><td>7.5</td><td>160</td><td>39</td><td>Positive</td></td<>	Alprazolam-D5	314.2	286.1	7.5	160	39	Positive
Buprenorphine-D4472.359.16.07097PositiveCodeine-D3303.3115.14.1100100PositiveDiazepam-D5290.2198.27.716044PositiveEDDP-D3281.2233.76.310042PositiveGabepentin-D10182.355.03.18033PositiveHydrocodne-D6306.2202.14.510040PositiveHydrocodne-D6292.3185.03.012040PositiveMDA-D5185.2110.03.66030PositiveMDA-D5185.2110.03.66030PositiveMDA-D5199.2164.84.28025PositiveMeperidine-D425.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMorphine-D3289.2152.22.82047PositiveMorphine-D3282.2152.28042PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5282.1213.284.04.98032PositiveNordiazepam-D5236.364.24.15014PositiveNordiazepam-D5237.1140.07.216039PositiveNordiazepam-D5236.364.24.150	Amphetamine-D5	141.1	124.0	2.9	20	13	Positive
Codeine-D3 303.3 115.1 4.1 100 100 Positive Diazpam-D5 290.2 198.2 7.7 160 44 Positive EDDP-D3 281.2 233.7 6.3 100 42 Positive Fentanyl-D5 342.3 188.3 6.0 100 45 Positive Gabepentin-D10 182.3 55.0 3.1 80 33 Positive Hydrocodone-D6 306.2 202.1 4.5 100 40 Positive MDA-D5 185.2 110.0 3.6 60 30 Positive MDA-D5 199.2 164.8 4.2 80 25 Positive Methadone-D3 313.4 268.0 6.5 80 26 Positive Morphine-D3 289.2 152.2 2.8 20 47 Positive Nordiazepam-D5 276.1 140.0 7.2 160 39 Positive Norentanyl-D5	Benzoylecgonine-D8	298.2	171.1	5.4	100	27	Positive
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Buprenorphine-D4	472.3	59.1	6.0	70	97	Positive
EDDP-D3281.2233.76.310042PositiveFentanyl-D5342.3188.36.010045PositiveGabepentin-D10182.355.03.18033PositiveHydrocodone-D6306.2202.14.510040PositiveMDA-D5185.2110.03.66030PositiveMDA-D5185.2110.03.66030PositiveMDA-D5199.2164.84.28025PositiveMeperidine-D4252.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMorphine-6-glucuronide-D3465.2289.22.82015PositiveMorphine-D3289.2152.22.510078PositiveNordiazepam-D5276.1140.07.216039PositiveNorderacepam-D5292.3246.27.015030PositiveNorderacepam-D5292.3246.27.015030PositiveNorgeone-D6322.1218.24.316058PositiveNymorphone-D3305.2230.32.710038PositiveNorgeone-D6322.1218.24.316058PositiveNorgeone-D6322.1218.24.316058PositiveNorgeone-D6322.1218.24.3 <t< td=""><td>Codeine-D3</td><td>303.3</td><td>115.1</td><td>4.1</td><td>100</td><td>100</td><td>Positive</td></t<>	Codeine-D3	303.3	115.1	4.1	100	100	Positive
Fentaryl-D5342.3188.36.010045PositiveGabepentin-D10182.355.03.18033PositiveHydrocodone-D6306.2202.14.510040PositiveMDA-D5185.2110.03.66030PositiveMDA-D5185.2110.03.66030PositiveMDA-D5185.2110.03.66030PositiveMDA-D5199.2164.84.28025PositiveMeperidine-D425.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMorphine-G-glucuronide-D3465.229.22.82047PositiveMorphine-D5276.1140.07.216039PositiveNordiazepam-D5276.1140.07.216039PositiveNormeperidine-D4238.242.05.28042PositiveNormeperidine-D4238.223.02.710038PositiveNormeperidine-D5305.2230.32.710038PositiveNymophone-D3305.2230.32.710038PositiveOxymorphone-D3305.2230.32.710038PositiveOxymophone-D3305.2230.32.710038PositiveZolpidem-D7315.1242.15.9 <td>Diazepam-D5</td> <td>290.2</td> <td>198.2</td> <td>7.7</td> <td>160</td> <td>44</td> <td>Positive</td>	Diazepam-D5	290.2	198.2	7.7	160	44	Positive
Gabepentin-D10 182.3 55.0 3.1 80 33 Positive Hydrocodone-D6 306.2 202.1 4.5 100 40 Positive MDA-D5 185.2 110.0 3.6 60 30 Positive MDA-D5 185.2 110.0 3.6 60 30 Positive MDMA-D5 199.2 164.8 4.2 80 25 Positive Meperidine-D4 252.1 93.0 5.3 100 72 Positive Methanghetamine-D5 155.2 121.2 3.6 20 15 Positive Morphine-G-glucuronide-D3 465.2 289.2 2.8 20 47 Positive Nordiazepam-D5 276.1 140.0 7.2 160 39 Positive Normeperidine-D4 238.2 42.0 5.2 80 42 Positive Normeperidine-D4 238.2 42.0 5.2 80 42 Positive Nore	EDDP-D3	281.2	233.7	6.3	100	42	Positive
Hydrocodone-D6 306.2 202.1 4.5 100 40 PositiveHydromorphone-D6 292.3 185.0 3.0 120 40 PositiveMDA-D5 185.2 110.0 3.6 60 30 PositiveMDEA-D5 213.1 105.1 4.6 40 26 PositiveMDMA-D5 199.2 164.8 4.2 80 25 PositiveMeperidine-D4 252.1 93.0 5.3 100 72 PositiveMethadone-D3 313.4 288.0 6.5 80 26 PositiveMorphine-6-glucuronide-D3 465.2 289.2 2.8 20 47 PositiveMorphine-03 289.2 152.2 2.5 100 78 PositiveNorfiazepam-D5 276.1 140.0 7.2 160 39 PositiveNorfiazepam-D5 2276.1 140.0 7.2 160 39 PositiveNorfentanyl-D5 238.2 84.0 4.9 80 32 PositiveNorgenthyl Tramadol-D6 256.3 64.2 4.1 50 14 PositiveOxycodone-D6 322.1 218.2 4.3 160 58 PositiveOxymorphone-D3 305.2 230.3 2.7 100 38 PositiveOxymorphone-D3 305.2 230.3 2.7 100 35 PositiveCalpidem-D7 315.1 242.1 5.9 100 45 Po	Fentanyl-D5	342.3	188.3	6.0	100	45	Positive
Hydromorphone-D6292.3185.03.012040PositiveMDA-D5185.2110.03.66030PositiveMDA-D513.1105.14.64025PositiveMDA-D5199.2164.84.28025PositiveMeperidine-D4252.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMethamphetamine-D5155.2121.23.62015PositiveMorphine-6-glucuronide-D3289.2152.22.510078PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5238.284.04.98032PositiveNordentanyl-D5238.242.05.28042PositiveNordentanyl-D5292.3246.27.015030PositiveOxycodone-D6322.1218.24.316058PositiveOxycodone-D6322.1218.24.316058PositiveVycodone-D6322.1218.24.316058PositiveColpidem-D7315.1242.15.910045PositiveZopidome-D4393.1245.05.48525PositiveZopidome-D7315.1242.15.910045PositiveZopidome-D3284.261.06.3<	Gabepentin-D10	182.3	55.0	3.1	80	33	Positive
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Hydrocodone-D6	306.2	202.1	4.5	100	40	Positive
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Hydromorphone-D6	292.3	185.0	3.0	120	40	Positive
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	MDA-D5	185.2	110.0	3.6	60	30	Positive
Meperidine-D4252.193.05.310072PositiveMethadone-D3313.4268.06.58026PositiveMethamphetamine-D5155.2121.23.62015PositiveMorphine-Glucuronide-D3465.2289.22.82047PositiveNordiazepam-D3289.2152.22.510078PositiveNordiazepam-D5276.1140.07.216039PositiveNorfentanyl-D5238.284.04.98032PositiveO-Demethyl Tramadol-D6256.364.24.15014PositiveOxazepam-D5292.3246.27.015030PositiveOxycodone-D6322.1218.24.316058PositiveOxycodone-D6322.1218.24.316058PositivePCP (Phencyclidine)-D5249.496.06.14024PositivePropoxyphene-D11351.3277.36.38013PositiveZolpidem-D7315.1242.15.910045PositiveZolpidem-D7315.1242.15.910045PositiveZolpidem-D7315.1242.05.48525PositiveZolpidem-D4393.1245.05.485PositiveArHydroxytriazolam-D5230.042.02.5 -95 -18NegativePhenobarbital-D5 <td>MDEA-D5</td> <td>213.1</td> <td>105.1</td> <td>4.6</td> <td>40</td> <td>26</td> <td>Positive</td>	MDEA-D5	213.1	105.1	4.6	40	26	Positive
Methadone-D3313.4268.06.58026PositiveMethamphetamine-D5155.2121.23.62015PositiveMorphine-6-glucuronide-D3465.2289.22.82047PositiveMorphine-D3289.2152.22.510078PositiveNordiazepam-D5276.1140.07.216039PositiveNordiazepam-D5238.284.04.98032PositiveNormeperidine-D4238.242.05.28042PositiveOvazepam-D5292.3246.27.015030PositiveOxycodone-D6322.1218.24.316058PositiveOxycodone-D6322.1218.24.316058PositiveVycodone-D6322.1218.24.316058PositiveOxymorphone-D3305.2230.32.710038PositivePropoxyphene-D11351.3277.36.38013PositiveZopidom-D7315.1242.15.910045PositiveZopidome-D4393.1245.05.48525PositiveZopidome-D3284.261.06.39063PositiveZopidome-D4393.1245.05.48525PositiveArHydroxytriazolam-D5280.0185.22.5-95-18NegativePhenobarbital-D52	MDMA-D5	199.2	164.8	4.2	80	25	Positive
Methamphetamine-D5155.2121.23.62015PositiveMorphine-Gglucuronide-D3465.2289.22.82047PositiveMorphine-D3289.2152.22.510078PositiveNordiazepam-D5276.1140.07.216039PositiveNormenp-ID5238.284.04.98032PositiveNormeperidine-D4238.242.05.28042PositiveOxazepam-D5292.3246.27.015030PositiveOxazepam-D5292.3246.27.015030PositiveOxycodone-D6322.1218.24.316058PositiveOxymorphone-D3305.2230.32.710038PositivePCP (Phencyclidine)-D5249.496.06.14024PositivePropoxyphene-D11351.3277.36.38013PositiveZolpidem-D7315.1242.15.910045PositiveZolpidem-D7315.1245.05.48525PositiveCarHydroxytarzolam-D5330.3302.37.116037PositiveInipramine-D3284.261.06.39063PositiveButalbital-D5236.042.02.4 -70 -54 NegativePhenobarbital-D5230.042.02.4 -70 -54 NegativePhen	Meperidine-D4	252.1	93.0	5.3	100	72	Positive
Morphine-O-glucuronide-D3 465.2 289.2 2.8 20 47 Positive Morphine-D3 289.2 152.2 2.5 100 78 Positive Nordiazepam-D5 276.1 140.0 7.2 160 39 Positive Normeperdine-D4 238.2 84.0 4.9 80 32 Positive Obstrangl-D5 292.3 246.2 7.0 150 30 Positive Oxazepam-D5 292.3 246.2 7.0 150 30 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxymorphone-D3 305.2 230.3 2.7 100 38 Positive Coxymorphone-D13 351.3 277.3 6.3 80 13 Positive Copidem-D7 315.1 242.1 5.9 100 45 Positive Zopidom-D7 315.1 242.1 5.9 100 45 Positive Zopiclon	Methadone-D3	313.4	268.0	6.5	80	26	Positive
$\begin{array}{l c c c c c c c c c c c c c c c c c c c$	Methamphetamine-D5	155.2	121.2	3.6	20	15	Positive
Nordiazepam-D5 276.1 140.0 7.2 160 39 Positive Norfentanyl-D5 238.2 84.0 4.9 80 32 Positive Normeperidine-D4 238.2 42.0 5.2 80 42 Positive O-Demethyl Tramadol-D6 256.3 64.2 4.1 50 14 Positive Oxazepam-D5 292.3 246.2 7.0 150 30 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive Zopiclone-D4 393.1 245.0 5.4 85 Positive a <t< td=""><td>Morphine-6-glucuronide-D3</td><td>465.2</td><td>289.2</td><td>2.8</td><td>20</td><td>47</td><td>Positive</td></t<>	Morphine-6-glucuronide-D3	465.2	289.2	2.8	20	47	Positive
Norfentanyl-D5 238.2 84.0 4.9 80 32 Positive Normeperidine-D4 238.2 42.0 5.2 80 42 Positive O-Demethyl Tramadol-D6 256.3 64.2 4.1 50 14 Positive Oxazepam-D5 292.3 246.2 7.0 150 30 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxymorphone-D3 305.2 230.3 2.7 100 38 Positive Propxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zopichone-D4 393.1 245.0 5.4 85 25 Positive Zopichone-D4 393.1 245.0 5.4 85 Positive CarHydroxytrizzolam-D4 363.1 335.1 7.1 100 37 Positive Imipra	Morphine-D3	289.2	152.2	2.5	100	78	Positive
Normeperidine-D4 238.2 42.0 5.2 80 42 Positive O-Demethyl Tramadol-D6 256.3 64.2 4.1 50 14 Positive Oxazepam-D5 292.3 246.2 7.0 150 30 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxymorphone-D3 305.2 230.3 2.7 100 38 Positive PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Zopidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive Zopiclone-D3 284.2 61.0 6.3 90 63 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive	Nordiazepam-D5	276.1	140.0	7.2	160	39	Positive
0-Demethyl Tramadol-D6 256.3 64.2 4.1 50 14 Positive 0xazepam-D5 292.3 246.2 7.0 150 30 Positive 0xycodone-D6 322.1 218.2 4.3 160 58 Positive 0xymorphone-D3 305.2 230.3 2.7 100 38 Positive PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Zopidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive α-Hydroxytrizzolam-D4 363.1 335.1 7.1 100 37 Positive mipramine-D3 284.2 61.0 6.3 90 63 Positive α-Hydroxytrizzolam-D5 236.0 42.0 2.4 -70 -54 Negative <td>Norfentanyl-D5</td> <td>238.2</td> <td>84.0</td> <td>4.9</td> <td>80</td> <td>32</td> <td>Positive</td>	Norfentanyl-D5	238.2	84.0	4.9	80	32	Positive
Oxazepam-D5 292.3 246.2 7.0 150 30 Positive Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxymorphone-D3 305.2 230.3 2.7 100 38 Positive PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Chydroxylaprazolam-D5 30.3 302.3 7.1 160 35 Positive α-Hydroxylrizzolam-D4 363.1 335.1 7.1 100 37 Positive mipramine-D3 284.2 61.0 6.3 90 63 Positive Phenobarbital-D5 230.0 42.0 2.4 -70 -54 Negative <td>Normeperidine-D4</td> <td>238.2</td> <td>42.0</td> <td>5.2</td> <td>80</td> <td>42</td> <td>Positive</td>	Normeperidine-D4	238.2	42.0	5.2	80	42	Positive
Oxycodone-D6 322.1 218.2 4.3 160 58 Positive Oxymorphone-D3 305.2 230.3 2.7 100 38 Positive PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive ArHydroxytrizzolam-D4 363.1 335.1 7.1 160 35 Positive mipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 236.0 42.0 2.4 -70 -54 Negative Phenobarbital-D5 230.0 42.1 2.6 -110 -56 Negative	O-Demethyl Tramadol-D6	256.3	64.2	4.1	50	14	Positive
Ox/morphone-D3 305.2 230.3 2.7 100 38 Positive PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive ArHydroxylaprazolam-D5 330.3 302.3 7.1 160 37 Positive minpramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 236.0 42.0 2.4 -70 -54 Negative Phenobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative	Oxazepam-D5	292.3	246.2	7.0	150	30	
PCP (Phencyclidine)-D5 249.4 96.0 6.1 40 24 Positive Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive α-Hydroxylaprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxyriazolam-D4 363.1 335.1 7.1 100 37 Positive Mipramine-D3 284.2 61.0 6.3 90 63 Positive Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Phenobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18			218.2	4.3	160	58	Positive
Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopidem-D7 315.1 242.0 5.4 85 25 Positive α-Hydroxyalprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxytriazolam-D4 363.1 335.1 7.1 100 37 Positive mipramine-D3 284.2 61.0 6.3 90 63 Positive Mutabital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 230.0 42.0 2.4 -70 -54 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative <td>Oxymorphone-D3</td> <td>305.2</td> <td>230.3</td> <td>2.7</td> <td>100</td> <td>38</td> <td>Positive</td>	Oxymorphone-D3	305.2	230.3	2.7	100	38	Positive
Propoxyphene-D11 351.3 277.3 6.3 80 13 Positive Tramadol-13C, D3 268.0 58.0 5.1 50 35 Positive Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopidem-D7 315.1 242.0 5.4 85 25 Positive α-Hydroxyalprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxytriazolam-D4 363.1 335.1 7.1 100 37 Positive mipramine-D3 284.2 61.0 6.3 90 63 Positive Mutabital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 230.0 42.0 2.4 -70 -54 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative <td>PCP (Phencyclidine)-D5</td> <td>249.4</td> <td>96.0</td> <td>6.1</td> <td>40</td> <td>24</td> <td>Positive</td>	PCP (Phencyclidine)-D5	249.4	96.0	6.1	40	24	Positive
Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive α-Hydroxyalprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxyalprazolam-D4 363.1 335.1 7.1 100 37 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 230.0 42.0 2.4 -70 -54 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative					80	13	
Zolpidem-D7 315.1 242.1 5.9 100 45 Positive Zopiclone-D4 393.1 245.0 5.4 85 25 Positive α-Hydroxyalprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxyalprazolam-D4 363.1 335.1 7.1 100 37 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Secobarbital D5-S 242.0 199.0 2.7 -115 -18 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative	Tramadol-13C, D3	268.0	58.0	5.1	50	35	Positive
Zopiclone-D4 393.1 245.0 5.4 85 25 Positive α-Hydroxylaprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxylaprazolam-D4 363.1 335.1 7.1 100 37 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Pentobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Pentobarbital-D5 230.0 42.1 2.6 -110 -56 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative		315.1	242.1	5.9	100	45	Positive
α-Hydroxyalprazolam-D5 330.3 302.3 7.1 160 35 Positive α-Hydroxytriazolam-D4 363.1 335.1 7.1 100 37 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbial-D5 228.0 185.2 2.5 -95 -18 Negative Pentobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Secobarbital D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-CO0H-D3 346.1 248.1 3.6 -115 -40 Negative				5.4		25	
α-Hydroxytriazolam-D4 363.1 335.1 7.1 100 37 Positive Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Secobarbital D5-IS 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative	α-Hydroxyalprazolam-D5	330.3	302.3	7.1	160	35	Positive
Imipramine-D3 284.2 61.0 6.3 90 63 Positive Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Secobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative		363.1	335.1	7.1	100	37	Positive
Butalbital-D5 228.0 185.2 2.5 -95 -18 Negative Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Pentobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-C00H-D3 346.1 248.1 3.6 -115 -40 Negative							
Phenobarbital-D5 236.0 42.0 2.4 -70 -54 Negative Pentobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative							
Pentobarbital-D5 230.0 42.1 2.6 -110 -56 Negative Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative	Phenobarbital-D5					- 54	•
Secobarbital D5-IS 242.0 199.0 2.7 -115 -18 Negative THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative							
THC-COOH-D3 346.1 248.1 3.6 -115 -40 Negative							
	THC-COOH glucuronide-D3						Negative

DP, declustering potential; CE, collision energy.

analytes (0.1 μ g/mL) were mixed in 50% methanol with 0.1% formic acid and infused at a flow rate of 10 μ L/min in the course of the chromatographic analysis of the negative urine sample. The chromatographic signals of each selected MS-MS transition are examined to check for any signal perturbation (or ion suppression) of the MS-MS signal at the analytes' retention times.

Accuracy

To assess accuracy, analytes standard mix (each at their cutoff concentrations) was fortified in three sets of drug-negative

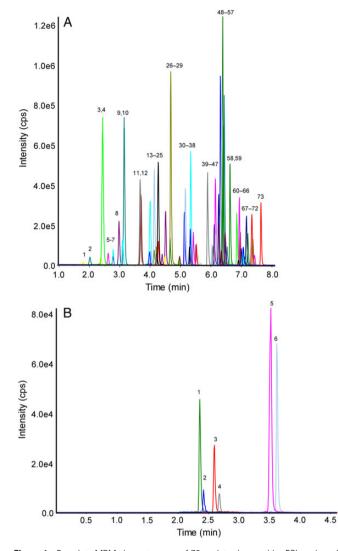


Figure 1. Complete MRM chromatograms of 73 analytes in a positive ESI mode and 6 in a negative ESI mode. (A) Analytes detected in a positive mode: 1. Oxymorphone-3-glucuronide, 2. Morphine-3-glucuronide, 3. Hydromorphone-3-glucuronide, 4. Pregabalin, 5. Morphine, 6. Oxymorphone, 7. Morphine-6-8. Amphetamine, 9. Hydromorphone, 10. alucuronide Gabapentin 11. Methamphetamine, 12. MDA, 13. Noroxycodone, 14. Naloxone, 15. Norhydrocodone, 16. O-Demethyl Tramadol, 17. Codeine, 18. MDMA, 19. 6-Acetylmorphine (6-MAM), 20. Codeine-6-glucuronide, 21. Oxycodone, 22. Naltrexone, 23. Hydrocodone, 24. 6B-Naltrexol, 25. MDEA, 26. Norbuprenorphine-3B-D-glucuronide, 27. Norfentanyl, Tramadol, 29. Zolpidem-phenyl carboxylic acid, 30. Normeperidine, 28 Meperidine, 32. Benzoylecgonine, 33. Zopiclone, 34. 7-Aminoclonazepam, 31. 35. Buprenorphine-3β-D-glucuronide, 36. Meprobamate, 37. Norbuprenorphine, 38 Zolpidem, 39. Fentanyl, 40. Buprenorphine, 41. 7-Aminoflunitrazepam, 42. Flurazepam, 43. PCP (Phencyclidine), 44. Chlordiazepoxide, 45. Norpropoxyphene, 46. Propoxyphene, 47. Desipramine, 48. Sufentanil, 49. Midazolam, 50. Imipramine, 51. EDDP, 52. NPD (norbuprenorphine degradent), 53. Nortriptyline, 54. Amitriptyline, 55. Carisoprodol, 56. Lorazepam-glucuronide, 57. Methadone, 58. Oxazepamglucuronide, 59. α -Hydroxymidazolam, 60. Lorazepam, 61. Oxazenam 62 Clonazenam Temazepam-glucuronide, 64. α-Hydroxytriazolam, 2-Hydroxyethylflurazepam, 66. α-Hydroxyalprazolam, 67. Nordiazepam, 65. 68. Zaleplon, 69. Flunitrazepam, 70. Triazolam, 71. Temazepam, 72. Alprazolam, 73. Diazepam (B) Analytes detected in a negative mode: 1. Phenobarbital, 2. Butalbital, 3. Pentobarbital, 4. Secobarbital, 5. THC-COOH glucuronide, 6. THC-COOH.

urine specimens from six different patients. Accuracy was determined by the ratio of recovered analyte concentration to nominal concentration.

Precision

Intra-assay imprecision was estimated by analyzing 10 control specimens at two levels (QC-H and QC-L) on the same day. Inter-assay imprecision was estimated by analyzing 20 control specimens at two levels (QC-H and QC-L) over 20 days with a positive ionization method or 5 days with a negative ionization method.

Correlation studies

To ensure accuracy of the results, correlation studies were performed in two studies. One study involved splitting 20 patient urine samples and had them tested by our method and by the national reference laboratory ARUP (Salt Lake City, UT, USA). The ARUP assay included a screening test by high-resolution time-of flight or immunoassay and confirmation/quantitation by either GC–MS or LC–MS-MS for positive samples. A second study was performed testing two urine proficiency testing (PT) samples from the 2014 Drug Monitoring for Pain Management (DMPM)—a program offered by the College of American Pathologists (CAP). These test results were compared against the peer group averages using LC–MS-MS-based methods.

Trazodone and m-CPP interference study

To investigate a potential false-positive amphetamine result from the comparison method, the methanol stocks of trazodone or *m*-CPP (1 mg/mL) were separately diluted to five different concentrations using blank urine: 1, 5, 10, 20 and 50 μ g/mL, followed by Amphetamine II immunoassay on Roche Cobas[®] 8000 module c502 (positive cutoff 1 μ g/mL).

Results and discussion

Assay optimization

THC-COOH, its glucuronide, and barbiturates were reported to have better ionization efficiency when analyzed with a negative ESI mode (8, 9, 21-23). The same ionization approach was employed in this study. Recent improvements in sensitivity and selectivity with negative ESI have contributed to its widespread use in pharmacokinetic, drug metabolism and pesticide residue studies (24-26). Weak organic acids, such as acetic or formic acid, are often added to mobile phases in positive ESI methods. It is commonly accepted that the acidic environment promotes protonation of analytes in a positive ionization mode. And it is a reasonable assumption that adding base to mobile phase solutions would help deprotonation in a negative-ion mode. However, previous studies with a negative ESI mode showed that adding volatile bases resulted in limited sensitivity and poor solution stability (24). On the contrary, addition of 0.1–0.2% acetic or formic acid has been shown to increase a signal-to-noise ratio (27). Although acetic acid was demonstrated to have better ionization efficiency than formic acid in negative ESI (24), 0.1% formic acid was chosen for mobile phases in our negative ESI method to eliminate the need of switching mobile phases between positive and negative ionization methods. The negative method sensitivity was acceptable with 0.1% formic acid added in mobile phases (see detailed discussion below about LOD and LLOQ).

MRM transitions were optimized by a direct infusion of each analyte at 10-100 ng/mL prepared in 50% methanol in water fortified with 0.1% formic acid. The ion transition

Table III

IS Paring Table For All Analytes

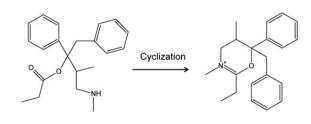
Compound	IS applied	Concentration (ng/mL)
2-Hydroxyethylflurazepam	2-Hydroxyethylflurazepam-D4	50
6-Acetylmorphine (6-MAM)	6-Acetylmorphine (6-MAM)-D3	50
6β-Naltrexol	Oxycodone-D6	50
7-Aminoclonazepam	7-Aminoclonazepam-D4	30
7-Aminoflunitrazepam	7-Aminoflunitrazepam-D7	10
Alprazolam	Alprazolam-D5	30
Amphetamine	Amphetamine-D5	50 50
Benzoylecgonine Buprenorphine	Benzoylecgonine-D8 Buprenorphine-D4	10
Buprenorphine-3B-D-glucuronide	Buprenorphine-D4	10
Carisoprodol	Gabepentin-D10	200
Chlordiazepoxide	Nordiazepam-D5	50
Clonazepam	Oxazepam-D5	50
Codeine	Codeine-D3	50
Codeine-6-glucuronide	Morphine-6G-D3	50
Diazepam	Diazepam-D5	50
EDDP	EDDP-D3	30
Fentanyl Flunitrazepam	Fentanyl-D5 Alprazolam-D5	5 30
Flurazepam	α-Hydroxyalprazolam-D5	30
Gabapentin	Gabepentin-D10	200
Hydrocodone	Hydrocodone-D6	50
Hydromorphone	Hydromorphone-D6	50
Hydromorphone-3-glucuronide	Morphine-6G-D3	50
Lorazepam	α -Hydroxyalprazolam-D5	30
Lorazepam-glucuronide	α -Hydroxyalprazolam-D5	30
MDA	MDA-D5	100
MDEA	MDEA-D5	100
MDMA Meperidine	MDMA-D5 Meperidine-D4	100 30
Meprobamate	Gabepentin-D10	200
Methadone	Methadone-D3	30
Methamphetamine	Methamphetamine-D5	50
Midazolam	Nordiazepam-D5	50
Morphine	Morphine-D3	50
Morphine-3-glucuronide	Morphine-6G-D3	50
Morphine-6-glucuronide	Morphine-6G-D3	50
Naloxone	Oxycodone-D6	50
Naltrexone	Oxycodone-D6	50
Norbuprenorphine Norbuprenorphine-3 _B -D-glucuronide	Buprenorphine-D4 Buprenorphine-D4	10 10
Nordiazepam	Nordiazepam-D5	50
Norfentanyl	Norfentanyl-D5	5
Norhydrocodone	Hydrocodone-D6	50
Normeperidine	Normeperidine-D4	30
Noroxycodone	Oxycodone-D6	50
Norpropoxyphene	Propoxyphene-D11	50
NPD (norbuprenorphine degradent)	Propoxyphene-D11	50
O-Demethyl Tramadol	O-desmethyltramadol-D6	50
Oxazepam Ovazepam duguranida	Oxazepam-D5	50 50
Oxazepam-glucuronide Oxycodone	Oxazepam-D5 Oxycodone-D6	50
Oxymorphone	Oxymorphone-D3	50
Oxymorphone-3-glucuronide	Morphine-6G-D3	50
PCP (Phencyclidine)	PCP (Phencyclidine)-D5	30
Pregabalin	Gabepentin-D10	200
Propoxyphene	Propoxyphene-D11	50
Sufentanil	Fentanyl-D5	5
Temazepam	Nordiazepam-D5	50
Temazepam-glucuronide	Nordiazepam-D5	50
Tramadol Triazolam	Tramadol-13C, D3	50
α -Hydroxyalprazolam	Oxazepam-D5 α-Hydroxyalprazolam-D5	50 30
α-Hydroxymidazolam	Alprazolam-D5	30
α-Hydroxytriazolam	α-Hydroxytriazolam-D4	50
Zaleplon	Zolpidem-D7	30
Zopiclone	Zopiclone-D4	10
Zolpidem	Zolpidem-D7	30
Zolpidem-Phenyl Carboxylic acid	Zolpidem-D7	30
Amitriptyline	Imipramine-D3	100
Desipramine	Imipramine-D3	100
Imipramine Nortriptyling	Imipramine-D3 Imipramine-D3	100
Nortriptyline Butalbital	Imipramine-D3 Butalbital-D5	100 100
Phenobarbital	Phenobarbital-D5	100
		(continued)

Assay validation

The linearity range was designed based on individual cutoff concentrations, with a dynamic range spanning $0.2 \times$ to $8 \times$ cutoff of each analyte (Table IV). As summarized in Table IV, all standard curves were linear with a linear regression coefficient *R* of

Table III	Continued
Compound	b

Compound	IS applied	Concentration (ng/mL)
Pentobarbital	Pentobarbital-D5	100
Secobarbital	Secobarbital D5	100
THC-COOH	THC-COOH-D3	50
THC-COOH glucuronide	THC-COOH glucuronide-D3	50



Norpropoxyphene m/z=326.3

NPD m/z=308.3

Figure 2. Conversion of norproposyphene to its cyclic form NPD.

determination was based on combination of high ion intensity, avoiding isobaric transitions, and low noise background in the presence of urine matrix. For most of the analytes, two MRM transitions were selected to monitor: the primary transition (quantifier) for concentration determination and the secondary transition (qualifier) for confirmatory analysis. The exceptions are amphetamine, buprenorphine-3 β -D-glucuronide, norbuprenorphine and norbuprenorphine-3 β -D-glucuronide, for which only one pair of ion transition was monitored. This was due to either the signal of non-primary ion transitions being too low or high noise background associated with mobile phases and/ or urine matrix. MRM transitions and associated compound tuning parameters (such as declustering potential and collision energy) are included in Table I. IS transitions and compound tuning parameters are presented in Table II.

All 73 analytes detected in a positive mode and 6 analytes detected in a negative mode were well separated with a bi-phenyl reversed phase HPLC column. A bi-phenyl HPLC column with high hydrophobic retention and aromatic selectivity has been relatively widely used in pain management drug panels (2, 23, 28). The smoothed (two-point) chromatograms of standard mixtures in positive and negative modes are shown in Figure 1A and B, respectively. THC-COOH and its glucuronide, when made in aqueous solution, are known to stick to sample container surfaces made with different materials (29). This sample loss presents a hurdle in standard material preparations, such as calibrators and QCs, leading o inaccuracy and reproducibility issues. A simple and effective solution to this conundrum was to prepare THC-COOH and its glucuronide in methanol or methanol-based solution (8, 21, 23). In the current study, to avoid any other potential compound adsorption to container surfaces, both positive and negative standards were prepared in 50% methanol.

 Table IV

 Linearity, Cutoffs, LOD, LLOQ, Stability, Accuracy and Carryover in Assay Validation

Compound ^a	R ^b	Cutoff (ng/mL)	Linear range (ng/mL)	LOD (ng/mL)	LLOQ (ng/mL)	Stability ^c at 4°C	Accuracy ^d	Carry over (%)
2-Hydroxyethylflurazepam	0.998	20	4-160	0.2	2.5	102.9	100.4	ND
6-Acetylmorphine (6-MAM)	0.999	20	4-160	1.5	2.5	98.0	101.7	0.4
6β-Naltrexol	0.998	10	2-80	NQ	0.3	112.4	129.1	ND
7-Aminoclonazepam	0.993	10	2-80	0.2	1.3	76.8	95.7	ND
7-Aminoflunitrazepam	0.996	5	1-40	0.2	0.6	78.4	100.0	0.1
Alprazolam	1.000	10	2-80	0.4	1.3	105.8	94.4	0.1
Amphetamine	0.997	50	10-400	3.4	6.3	103.8	104.5	0.1
Benzoylecgonine	0.999	50	10-400	NQ	3.1	108.5	106.2	ND
Buprenorphine	0.996	10	2-80	0.3	2.5	111.8	92.9	ND
Buprenorphine-3β-D-glucuronide	0.999	10	2-80	0.1	0.6	103.9	104.0	ND
Carisoprodol Chlordiazepoxide	0.996 0.998	100 20	20-800 4-160	3.1 0.8	12.5 2.5	103.4 108.9	94.5 102.7	ND 0.1
Clonazepam	0.998	10	4-100 2-80	0.4	0.6	92.4	65.8	0.1
Codeine	0.997	20	4-160	0.9	2.5	99.9	109.2	ND
Codeine-6-glucuronide	0.996	20	4-160	0.2	2.5	99.7	98.6	ND
Diazepam	1.000	20	4-160	0.2	0.3	100.7	94.4	ND
EDDP	0.998	10	2-80	NQ	0.6	99.9	105.5	0.1
Fentanyl	0.997	2	0.4-16	NQ	0.5	94.3	97.4	ND
Flunitrazepam	0.999	5	1-40	0.2	0.6	88.3	78.9	ND
Flurazepam	0.998	10	2-80	NQ	0.6	99.4	80.9	ND
Gabapentin	1.000	500	100-4,000	NQ	62.5	102.7	97.4	0.1
Hydrocodone	0.999	20	4-160	0.8	1.3	107.2	101.8	ND
Hydromorphone	0.998	20	4-160	0.1	1.3	91.4	97.9	ND
Hydromorphone-3-glucuronide	0.997	20	4-160	1.1	2.5	94.7	99.1	ND
Lorazepam	0.999	20	4-160	0.2	1.3	95.0	83.8 05.0	ND
Lorazepam-glucuronide	0.998	40	8-320	1.8 2 E	5.0	104.9	85.6	ND
MDA MDEA	0.999 0.999	100 100	20-800 20-800	2.5 NQ	3.1 6.3	105.2 100.9	100.5 101.8	ND ND
MDMA	0.999	100	20-800	2.4	12.5	103.4	101.8	ND
Meperidine	0.997	10	2-80	0.4	1.3	107.1	99.6	ND
Meprobamate	0.999	500	100-4,000	NQ	125.0	106.9	78.0	0.2
Methadone	0.999	10	2-80	0.1	0.3	101.2	93.4	ND
Methamphetamine	1.000	50	10-400	0.8	3.1	100.6	99.1	ND
Midazolam	0.997	20	4-160	NQ	1.3	111.5	111.6	ND
Morphine	0.998	20	4-160	NΩ	2.5	107.6	101.3	ND
Morphine-3-glucuronide	0.995	20	4-160	0.1	2.5	103.6	102.2	ND
Morphine-6-glucuronide	0.997	20	4-160	0.2	2.5	96.6	91.5	0.1
Naloxone	0.997	100	20-800	3.7	25.0	111.0	105.9	0.0
Naltrexone	0.995	10	2-80	0.6	2.5	114.0	110.1	0.1
Norbuprenorphine	0.996	10	2-80	0.8	1.3	84.2	83.4	ND
Norbuprenorphine-3β-D-glucuronide	0.997	10	2-80	0.3	2.5	100.4	93.1	ND
Nordiazepam	0.999	20 5	4-160 1-40	0.3 0.1	0.6	99.3 105.0	91.4 100.7	ND ND
Norfentanyl Norhydrocodone	0.998 0.998	20	4-160	0.3	1.3 0.6	109.1	60.0	0.1
Normeperidine	0.998	10	2-80	0.2	0.6	97.0	97.1	0.1
Noroxycodone	0.997	20	4-160	0.1	1.3	116.2	80.1	ND
Norpropoxyphene and NPD	0.999	20	4-160	1.1	2.5	107.6	97.5	ND
<i>O</i> -Demethyl Tramadol	0.999	50	10-400	NQ	3.1	102.8	97.9	0.1
Oxazepam	0.999	20	4-160	0.4	2.5	95.3	90.4	ND
Oxazepam-glucuronide	0.998	20	4-160	3.6	5.0	106.9	100.9	ND
Oxycodone	0.997	20	4-160	0.5	1.3	103.5	85.3	ND
Oxymorphone	0.998	20	4-160	0.6	2.5	96.9	90.5	ND
Oxymorphone-3-glucuronide	0.997	20	4-160	0.9	2.5	98.0	105.1	ND
PCP (Phencyclidine)	0.997	10	2-80	0.3	0.6	100.7	91.5	ND
Pregabalin	0.999	500	100-4,000	2.4	7.8	100.5	93.4	ND
Propoxyphene	0.999	20	4-160	NQ	0.6	101.3	95.4 100.0	0.1
Sufentanil Temazepam	0.998 0.999	5 20	1-40 4-160	ΝΩ ΝΩ	0.6 0.6	99.6 107.6	100.0 103.0	ND ND
Temazepam-glucuronide	0.999	20	4-160	2.2	0.8 5.0	107.6	88.8	ND
Tramadol	0.999	20 50	4-160	0.2	5.0 1.6	106.4	88.8 84.2	ND
Triazolam	1.000	20	4-160	0.2	1.3	107.4	106.2	0.1
α-Hydroxyalprazolam	0.998	10	2-80	0.5	2.5	97.9	99.5	ND
α-Hydroxymidazolam	0.999	20	4-160	1.0	2.5	96.8	106.2	0.1
α-Hydroxytriazolam	0.995	20	4-160	NQ	2.5	103.0	112.2	0.2
Zaleplon	0.999	10	2-80	0.2	0.3	112.1	104.6	ND
Zopiclone	0.997	10	2-80	0.1	1.3	100.4	97.4	0.1
Zolpidem	1.000	10	2-80	0.1	0.6	104.0	99.0	ND
Zolpidem-Phenyl Carboxylic acid	0.997	10	2-80	0.1	0.3	123.2	156.8	0.1
Amitriptyline	0.999	50	10-400	NQ	3.1	98.5	85.5	0.1
Desipramine	0.999	50	10-400	0.1	6.3	100.3	89.2	0.1
Imipramine	0.999	50	10-400	NQ	3.1	107.3	91.7	0.1
Nortriptyline	0.998	50	10-400	0.6	3.1 12 F	98.2	88.0	0.1
Butalbital Phenobarbital	0.998 0.995	100 100	20-800 20-800	8.4 3.1	12.5 12.5	96.9 92.0	96.4 106.6	ND ND
Pentobarbital	0.995	100	20-800	3.1 10.4	25.0	92.0 84.6	106.6	ND
	0.000	100	20 000	TU.T	20.0	0.70	101.0	ND

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(continued)

Table IV Continued								
Compound ^a	R ^b	Cutoff (ng/mL)	Linear range (ng/mL)	LOD (ng/mL)	LLOQ (ng/mL)	Stability $^{\rm c}$ at $4^{\circ}{\rm C}$	Accuracy ^d	Carry over (%)
Secobarbital	0.997	100	20-800	4.6	12.5	103.2	107.0	0.2
THC-COOH	1.000	50	10-400	NQ	3.1	103.8	96.5	0.1
THC-COOH glucuronide	0.999	50	10-400	1.0	1.6	101.5	94.0	ND

NQ, not quantifiable; ND, not detectable.

^aAll calibrators and QCs are stored at -20° C for 1 year.

^bLinear regression coefficient was determined from the standard curve of each analyte.

^cStability is defined as the percentage of observed day-7 concentration normalized to that of day-0 specimen.

^dAccuracy is defined as observed concentration in fortified negative urine normalized to the nominal concentration.

 \geq 0.990 (with a range of 0.994–1.000). For all analytes, LODs were lower than LLOQ. With some analytes, LODs were not quantifiable, producing a false value of below zero (designated as 'NQ' in Table IV). LLOQs of all analytes were at least 25% of the cutoff concentration and were comparable or lower than those in previous reports of simultaneous multiple drug quantitation LC–MS-MS methods (2, 8, 15, 16). Detailed information of LODs and LLOQs are included in Table IV.

Urine drug testing cutoffs for pain management must be determined with care. If they are too high, false negative may result; if too low, the risk of reporting a positive drug test due to inadvertent exposure (such as pharmaceutical impurities) is increased (30, 31). Unlike immunoassay-based urine drug testing with relatively high cutoffs due to limited sensitivity, LC–MS-MS are capable of detecting drugs at low concentrations (see Table IV for LOD and LLOQ). Some reference laboratories offering LC– MS-MS drug panels use LLOQ as their cutoffs, which are the thresholds at which reliable quantitation can be made. To avoid potential false positives, comparable cutoffs were used in our assay although the LLOQs are at least 25% or lower of the cutoff values for each analyte.

The analytes were found to be stable in urine for at least 7 days at 4° C (n = 3), with a range of recovery 77–123% (acceptance range $100 \pm 25\%$). Although no significant carryover was observed between calibrator 5 and calibrator 1, it is recommended to be aware of potential carryover during routing sample analysis, especially for acute intoxicated patients whose urine drug concentration could be much higher than those tested in carryover studies.

Both of the positive mode and negative mode methods were found to be specific and no interference peaks were observed in 10 drug-negative urine controls. The same drug-negative urine specimens were used in the matrix effect investigation with a post-column infusion method. No substantial matrix effect or ion suppression was observed for any of the analytes with any of the negative urine specimens (data not shown). As presented in Table IV, accuracy of most analytes in spike-and-recovery experiment fell in the acceptance range 80-120%. The exceptions are 6β-naltrexol (129.1%), clonazepam (65.8%), flunitrazepam (78.9%), meprobamate (78.0%), norhydrocodone (60.0%) and zolpidem-phenyl carboxylic acid (156.8%). It is noteworthy that none of the six analytes had their own deuterium-labeled IS used in quantitation (Table III). Instead, shared ISs of different analytes from the same drug class that had close retention time were applied. Therefore, the observed matrix effect might be contributed from that fact the IS used for those analyes were not eluted at the same retention time. Including the analyte-specific IS might be warranted to alleviate their matrix

for its own non-labeled ones. However, ISs are not always commercially available and can also be costly. It is a common practice to have different analytes with close retention time share IS in multiple pain drug panel method development (2, 8, 15, 16). With the observed matrix effect for those six analytes, extra care should be taken in patient result interpretation. The combined results of the parent drugs or metabolites of the six analytes, namely naltrexone, 7-aminoclonazepam, 7-aminoflunitrazepam, carisoprodol, hydrocodone and zolpidem, which were also included in our panel and less subjective to matrix effect, could provide more reliable information in final interpretation. Norpropoxyphene is a major metabolite of propoxyphene

effects. Ideally, isotope-labeled IS compound should be used

found in urine. However, norpropoxyphene has been reported to be unstable not only in urine matrix but also in methanol stock (8, 18, 19). There is substantial conversion of norpropoxyphene to a cyclic degradation product (norbuprenorphine degradent, NPD), as shown in Figure 2. As this structural conversion is constantly on-going, it is impossible to predict the exact amount of norpropoxyphene and NPD in the manufacture's original stock at a particular time point, which presents difficulties when preparing QCs and calibrators. To circumvent this issue, the total norproposyphene was calculated by summing up an intact and cyclized product (NPD). Because of the molecular weight difference of intact (326 Da) and NPD (308 Da), the peak area of NPD was first converted to that of norpropoxyphene by multiplying the molar normalizing factor (326/ 308). Then, the peak area of norpropoxyphene and NPD was summed and divided by their common IS peak area. This combined peak area ratio was then used for constructing standard curves and quantification of routine urine samples. This manual calculation was successful in generating linear calibration curves, accurate and precise validation results (Tables IV and V).

The intra-assay CV for QC-L ranged 1.7-8.8% and for QC-H ranged 1.8-7.5%; the inter-assay CV for QC-L ranged 2.1-12.2% and for QC-H ranged 1.9-10.2%. The accuracy which was measured by % bias was within $\pm 20\%$ acceptance criteria at both QC-L and QC-H levels from intra- or inter-assay experiments (Table V). To establish the assay dilution linearity for samples with high concentrations of analytes, a 10-fold dilution validation using $40 \times$ working solution diluted with Bio-Rad blank urine was performed. The inter-assay CV for the dilution study ranged 1.6-8.7% and the assay % bias was within $\pm 20\%$ except for THC-COOH and THC-COOH glucuronide (data not shown). As expected, significant negative % bias (around -35%) was observed with THC metabolites after 10-fold dilution with Bio-Rad urine, due to the adsorption effect discussed

 Table V

 Precision and Accuracy at Two QC Levels

Compound	Intra-assay precis	ion and accuracy		Inter-assay precision and accuracy				
	QC-L ^a CV (%) ^b	QC-L bias (%) ^c	QC-H ^d CV (%)	QC-H bias (%)	QC-L CV (%)	QC-L bias (%)	QC-H CV (%)	QC-H bias (%
2-Hydroxyethylflurazepam	5.5	-1.0	3.5	2.3	6.2	-4.1	4.3	-1.4
6-Acetylmorphine (6-MAM)	5.9	-9.9	5.1	-2.8	8.2	-3.8	5.2	-1.3
6β-Naltrexol	5.1	9.5	3.5	6.6	4.3	19.5	5.0	15.1
7-Aminoclonazepam	2.4	0.6	4.3	1.2	4.1	1.2	3.0	1.3
7-Aminoflunitrazepam	5.5	-1.0	5.1	-3.3	6.3	-1.8	3.0	0.8
Alprazolam	3.6	-1.7	4.1	-0.6	4.3	1.9	4.2	-0.3
Amphetamine	3.2	-3.5	3.3	-3.8	4.4	-0.9	3.3	-1.1
Benzoylecgonine	2.4	-2.7	3.5	1.2	4.9	0.2	4.2	-2.0
Buprenorphine	7.9 3.2	10.1 16.8	7.5 4.8	4.9 12.1	8.5 5.5	6.4 6.4	5.6	2.3 10.0
Buprenorphine-3 _{β-D} -glucuronide Carisoprodol	3.Z 4.2	-2.2	4.8 3.3	- 4.3	5.5 6.7	0.4 1.4	6.4 4.9	-0.7
Chlordiazepoxide	4.2 5.3	-12.2	3.3 4.4	- 12.4	5.4	-10.2	4.9 5.4	- 8.3
Clonazepam	3.6	-9.0	3.7	-10.5	4.3	-0.9	4.6	- 2.5
Codeine	3.7	-3.2	6.5	3.1	5.8	- 1.5	5.5	-1.0
Codeine-6-glucuronide	4.9	1.5	3.5	6.5	9.5	-5.8	9.7	- 1.5
Diazepam	2.3	0.6	2.4	3.6	2.3	1.3	2.2	3.4
EDDP	4.3	2.3	4.5	5.3	5.0	3.1	4.8	4.7
Fentanyl	5.9	-4.2	3.8	-2.8	4.2	-5.5	4.0	-4.3
Flunitrazepam	5.0	8.2	3.3	10.4	4.2	13.2	1.9	10.7
Flurazepam	3.4	- 10.1	4.7	-12.7	6.8	-5.7	7.5	- 7.1
Gabapentin	3.5	-2.6	3.8	2.5	4.6	2.3	3.6	4.1
Hydrocodone	4.6	-5.7	4.3	0.4	4.2	-1.7	3.2	-0.4
Hydromorphone	5.7	-5.8	3.9	-1.9	9.7	-5.7	7.4	-2.4
Hydromorphone-3-glucuronide	4.1	3.9	3.5	6.0	8.5	-1.7	4.8	4.0
Lorazepam	4.4	-6.5	4.8	-6.3	5.3	-8.1	6.2	- 7.8
Lorazepam-glucuronide	5.0	-2.5	4.6	-3.4	4.8	0.5	6.3	- 1.6
MDA	2.9	0.4	3.3	2.2	2.9	0.3	3.8	1.5
MDEA	3.4	-0.5	2.9	3.6	2.5	2.1	2.6	2.3
MDMA	4.1	-3.8	4.5	-0.3	4.3	1.1	3.6	1.4
Meperidine	5.0	-5.0	4.2	-2.2	6.2	-1.1	3.7	-1.9
Meprobamate	3.1 3.9	-8.6 -3.4	1.8 2.8	-5.0 -1.4	6.9 4.5	-8.5 -1.9	5.7 3.6	-4.1 -1.0
Methadone Methamphetamine	3.9		3.8	- 1.4 - 2.9	4.5 2.1	- 1.9 0.2	3.0 2.7	- 1.0 1.2
Midazolam	5.6	-5.0	3.9	-5.5	6.3	2.8	5.3	-0.4
Morphine	4.0	-2.6	3.0	- 4.2	5.3	-2.4	4.5	-0.4
Morphine-3-glucuronide	4.4	4.9	3.5	10.0	7.2	-2.3	5.9	4.9
Morphine-6-glucuronide	4.9	3.8	5.0	3.3	5.8	-0.3	4.8	0.1
Naloxone	2.3	0.2	4.1	3.9	7.8	11.3	6.7	9.3
Naltrexone	3.6	-6.5	4.7	-3.3	12.3	1.7	7.0	6.2
Norbuprenorphine	8.3	2.2	5.7	4.6	7.6	8.4	4.5	5.1
Norbuprenorphine-3 _B -D-glucuronide	5.0	17.5	5.8	12.1	6.2	9.6	4.9	9.8
Nordiazepam	3.8	-5.3	2.8	-3.1	3.5	-4.1	3.8	-2.3
Norfentanyl	5.8	-2.4	4.7	-3.5	5.0	-0.2	4.6	0.2
Norhydrocodone	5.0	6.1	5.4	5.4	7.1	3.6	4.4	3.1
Normeperidine	5.7	-1.6	4.4	2.2	6.4	2.1	6.0	1.2
Noroxycodone	2.5	-4.9	7.0	-0.6	9.7	2.5	6.6	1.3
Norpropoxyphene and NPD	1.8	8.8	3.4	2.9	6.6	-0.8	4.1	-1.1
O-Demethyl Tramadol	3.3	-0.9	3.7	2.6	4.0	0.3	3.4	1.9
Oxazepam	3.7 6.1	-3.1	4.0	-0.6	3.6	0.2	3.3	-0.4 -0.6
Oxazepam-glucuronide Oxycodone	5.5	-12.4 0.9	5.0 5.3	-9.3 -0.1	5.0 8.1	-0.7 -0.5	5.3 5.5	- 0.8 - 1.3
Oxymorphone				2.9	4.3			3.2
Oxymorphone-3-glucuronide	4.9 4.4	6.1 1.4	5.1 3.5	15.0	8.9	2.9 4.8	4.4 7.5	5.0
PCP (Phencyclidine)	3.8	-7.1	4.3	-4.3	5.2	-1.5	4.2	-2.6
Pregabalin	2.7	-7.4	2.9	-4.6	5.7	1.9	3.9	1.7
Propoxyphene	3.0	-2.3	3.8	0.5	5.2	-3.4	3.5	-0.8
Sufentanil	5.6	-6.8	3.3	-5.4	5.2	-4.1	4.3	-0.4
Temazepam	5.6	5.1	4.3	7.9	6.2	2.5	4.1	3.4
Temazepam-glucuronide	7.7	-0.9	4.1	-1.7	9.1	-0.1	6.7	3.4
Tramadol	3.0	-2.2	4.3	-0.3	3.4	0.3	2.4	1.9
Triazolam	2.7	-3.1	4.5	-2.4	5.2	5.4	4.8	1.7
lpha-Hydroxyalprazolam	6.8	-5.9	2.8	-5.7	10.5	-0.5	5.6	0.3
lpha-Hydroxymidazolam	5.1	-12.1	2.7	-8.9	6.5	-1.1	3.2	-2.6
α -Hydroxytriazolam	5.2	10.6	4.7	9.6	6.3	14.0	5.1	9.0
Zaleplon	3.5	15.5	2.5	19.8	6.3	14.3	6.8	13.4
Zopiclone	3.0	-7.4	2.8	- 7.0	5.6	-8.1	3.2	- 9.3
Zolpidem	3.4	-1.3	2.8	1.8	3.2	-0.2	3.8	1.0
Zolpidem-Phenyl Carboxylic acid	2.6	18.8	3.0	18.9	2.2	18.3	4.2	14.7
Amitriptyline	3.3	0.2	3.9	1.7	4.6	1.3	4.0	3.3
Desipramine	3.1	0.8	3.6	-0.2	3.7	0.1	3.1	2.2
Imipramine	3.6	-1.3	3.1	2.7	4.2	1.4	2.5	3.0
Nortriptyline	3.1	-1.2	3.0	2.1	5.1	4.4	4.4	3.7
Butalbital	7.2	5.0	4.2	9.1	10.4	11.0	10.2	11.4

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(continued)

Compound	Intra-assay precis	Intra-assay precision and accuracy					Inter-assay precision and accuracy				
	QC-L ^a CV (%) ^b	QC-L bias (%) $^{\rm c}$	QC-H ^d CV (%)	QC-H bias (%)	QC-L CV (%)	QC-L bias (%)	QC-H CV (%)	QC-H bias (%)			
Phenobarbital	8.8	-0.9	5.4	4.6	7.7	0.9	6.5	3.3			
Pentobarbital	4.5	19.9	6.3	18.5	11.3	10.1	8.3	8.5			
Secobarbital	7.0	2.8	5.0	8.6	8.0	5.3	5.8	6.7			
THC-COOH	2.1	0.5	3.1	1.2	3.6	2.9	3.5	1.7			
THC-COOH glucuronide	2.9	7.4	2.4	8.7	3.0	8.6	3.4	8.7			

^aLow-level QC (QC-L) is at 50% of each analyte's cutoff concentration.

^bPrecision is defined as the percentage coefficient of variation (CV).

^cAccuracy is defined as the difference of observed concentration from the nominal concentration (percentage bias).

^dHigh-level QC (QC-H) is at three times of each analyte's cutoff concentration.

earlier. This dilution study result suggests that urine specimens can be diluted for all analytes except for the quantitation of THC metabolites.

Our assay was then evaluated by comparing patient sample testing results with those from a reference laboratory. The comparison results were summarized in Supplementary Table I. Twenty patient urine specimens that were screened positive for various drug classes by in-house immunoassays (Roche Cobas) were tested using our LC-MS-MS method and the reference laboratory screening and confirmation methods. Although the comparison results were largely consistent between all three methods, we did observe some inconsistencies, which are discussed below. VP3 was tested positive for amphetamine by immunoassay, but not detected by either the ARUP time-of-flight (TOF) MS or the in-house LC-MS-MS method. Instead, VP3 was tested positive for lorazepam-glucuronide only in our LC-MS-MS method, which is the only method of the three that can detect the conjugate of the sedative drug lorazepam. The VP3 specimen was from a patient from the emergency department who complained of severe insomnia lasting for a few days. Although the patient medical record did not indicate any medication taken before the hospital course, it is a reasonable guess that the patient could have taken a sedative or sleep inducing drug for his insomnia, for example, lorazepam or trazodone. Recently, trazodone metabolite m-CPP was found to have cross-reactivity with the Roche amphetamine II assay (32). Similarly, our in-house Roche amphetamine screening assay was found to be falsely positive with *m*-CPP of $10 \,\mu g/mL$ or higher (data not shown). Therefore, a possible explanation for the false-positive amphetamine result is that the patient might have been taking both trazodone and lorazepam for his insomnia, which resulted in positive amphetamine immunoassay and detection of lorazepam metabolite by LC-MS-MS. VP7 was tested positive for benzodiazepines and tricyclic antidepressant by the Roche immunoassay, but positive for tramadol by both ARUP TOF- MS and the in-house LC-MS-MS, showing the advantage of high specificity with mass spectrometry-based methods. In the analysis of specimen VP14, the immunoassay-positive benzodiazepine was detected as 7-aiminoclonazepam by in-house LC-MS-MS, but shown to be negative by the ARUP TOF method. Similar observation was made in VP10 analysis that was tested positive for barbiturates by Roche immunoassay, but negative by ARUP immunoassay. Consistent with Roche screening results, phenobarbital was detected positive by our LC-MS-MS method, showing the high sensitivity of urine drug screening by our LC-MS-MS.

Table VI

CAP DMPM PT Specimens Results

ID	Expected drugs	Acceptable range ^a (ng/mL)	Mean ^b of quantitation methods (ng/mL)	HMH LC– MS-MS (ng/mL)	Accuracy ^c (%)
DMPM-1 DMPM-2	6-MAM α-Hydroxyalprazolam Benzoylecgonine Carisoprodol Meprobamate Morphine Amphetamine Methamphetamine	169-434 952-1,752 477-777 964-2,632 3,011-5,971 19,417-88,965 184-298 1.627-3,200	304 1,352 626 1,798 4,491 54,191 241 2,413	380 1,507 653 1,811 4,673 51,891 240 2,820	127.9 104.4 106.9 100.7 104.1 99.7 98.8 118.4
	Lorazepam Oxycodone Oxymorphone	1,139–2,447 503–979 3,595–6,279	1,842 741 4,936	1,470 635 4,980	74.7 84.9 101.4

^aAcceptable range according to the CAP 2014 DMPM-A proficiency testing.

^bMean concentration reported by participating peer groups that employ GC-MS or LC-MS-MS. ^cAccuracy is defined as the percentage of observed concentration normalized to the mean concentrations.

As glucuronide hydrolysis is not included for the TOF-MS screening or in the opiate LC–MS-MS assay performed by ARUP, all glucuronide metabolites in screening and opiate glucuronides in confirmation/quantitation were not detected. This can lead to potentially false-negative screening results for some analytes that are mainly excreted in glucuronide forms, as seen for oxazepam, temazepam and codeine in VP1 analysis. Similarly, opiate quantitation by LC–MS-MS without hydrolysis were significantly underestimated as seen in the analyses of specimens VP2, 5, 8, 11, 12, 14 and 15 in Supplementary Table I. Besides higher sensitivity for glucuronide conjugates and avoiding various low de-glucuronidation efficiencies by glucuronidase, another advantage of directly detecting glucuronide conjugates is that it helps identify the adulterated urine specimens, as fortified drugs will not become glucuronide conjugated *in vitro*.

Two CAP DMPM PT samples were also tested with our method and quantitatively compared with the peer groups that employed GC–MS or LC–MS-MS methods for quantitation. As summarized in Table VI, all expected drugs were found to be positive in both specimens. More importantly, the determined concentrations by our method for all analytes ranged 74.7–127.9% compared with those of method mean from peer groups, which was within the PT program acceptance criteria $\pm 30\%$ or mean ± 2 SD.

In comparison with other published LC–MS-MS pain management panels that comprise multiple drug classes (2, 8, 15, 16, 21, 33), our assay has the most complete drug list for compliance testing and monitoring illicit use (excluding novel psychoactive substances) (15). With comparable performances, such as linearity, sensitivity, specificity, accuracy, precision etc., the assay presented involves little sample preparation by employing dilute-and-shoot and including glucuronide conjugates. Those commonly included laborious steps, such as SPE and glucuronide hydrolysis that both can bring in more variations, are avoided in our assay. With these advantages, lower cost and better turnaround time might be achieved in routine pain management testing.

Conclusion

An LC–MS-MS method has been established and validated that allows simultaneous detection of 78 pain management drugs and metabolites in urine samples. This method requires minimal sample preparation using a dilute-and-shoot strategy. Although the suboptimal recovery rate was observed for six analytes due to the lack of available ISs, this method could considerably reduce overall analysis cost and time, which are important factors for its application to routine urine drug compliance testing in pain management.

Supplementary Data

Supplementary data are available at *Journal of Analytical Toxicology* online.

References

- Cone, E.J., Caplan, Y.H., Black, D.L., Robert, T., Moser, F. (2008) Urine drug testing of chronic pain patients: licit and illicit drug patterns. *Journal of Analytical Toxicology*, 32, 530–543.
- Wang, J., Yang, Z., Lechago, J. (2013) Rapid and simultaneous determination of multiple classes of abused drugs and metabolites in human urine by a robust LC-MS/MS method—application to urine drug testing in pain clinics. *Biomedical Chromatography*, 27, 1463–1480.
- 3. Gourlay, D.L., Heit, H.A., Almahrezi, A. (2005) Universal precautions in pain medicine: a rational approach to the treatment of chronic pain. *Pain Medicine*, **6**, 107–112.
- Fleming, M.F., Balousek, S.L., Klessig, C.L., Mundt, M.P., Brown, D.D. (2007) Substance use disorders in a primary care sample receiving daily opioid therapy. *Journal of Pain*, 8, 573–582.
- Katz, N., Fanciullo, G.J. (2002) Role of urine toxicology testing in the management of chronic opioid therapy. *Clinical Journal of Pain*, 18, S76–S82.
- Manchikanti, L., Manchukonda, R., Pampati, V., Damron, K.S., Brandon, D.E., Cash, K.A. *et al.* (2006) Does random urine drug testing reduce illicit drug use in chronic pain patients receiving opioids? *Pain Physician*, 9, 123–129.
- Manchikanti, L., Manchukonda, R., Damron, K.S., Brandon, D., McManus, C.D., Cash, K.A. (2006) Does adherence monitoring reduce controlled substance abuse in chronic pain patients? *Pain Physician*, 9, 57–60.
- Yuan, C., Heideloff, C., Kozak, M., Wang, S. (2012) Simultaneous quantification of 19 drugs/metabolites in urine important for pain management by liquid chromatography-tandem mass spectrometry. *Clinical Chemistry and Laboratory Medicine*, **50**, 95–103.
- Feng, J., Wang, L., Dai, I., Harmon, T., Bernert, J.T. (2007) Simultaneous determination of multiple drugs of abuse and relevant metabolites in urine by LC-MS-MS. *Journal of Analytical Toxicology*, 31, 359–368.
- Pesce, A., West, C., West, R., Latyshev, S., Masters-Moore, D., Friel, P. et al. (2012) Analytical considerations when monitoring pain

medications by LC-MS/ MS. Journal of Analytical and Bioanalytical Techniques, **\$5**, 1–11.

- 11. Baselt, R.C. (2011) *Disposition of toxic drugs and chemicals in man*, 9th edition. Biomedical Publications: Seal Beach, CA.
- Wang, P., Stone, J.A., Chen, K.H., Gross, S.F., Haller, C.A., Wu, A.H. (2006) Incomplete recovery of prescription opioids in urine using enzymatic hydrolysis of glucuronide metabolites. *Journal of Analytical Toxicology*, **30**, 570–575.
- Duer, W.C., McFarland, S. (2007) Comments on 'Incomplete recovery of prescription opioids in urine using enzymatic hydrolysis of glucuronide metabolites'. *Journal of Analytical Toxicology*, **31**, 419–420.
- Marin, S.J., Coles, R., Merrell, M., McMillin, G.A. (2008) Quantitation of benzodiazepines in urine, serum, plasma, and meconium by LC-MS-MS. *Journal of Analytical Toxicology*, 32, 491–498.
- Tang, M.H., Ching, C.K., Lee, C.Y., Lam, Y.H., Mak, T.W. (2014) Simultaneous detection of 93 conventional and emerging drugs of abuse and their metabolites in urine by UHPLC-MS/MS. *Journal of Chromatography*, 969C, 272–284.
- Dickerson, J.A., Laha, T.J., Pagano, M.B., O'Donnell, B.R., Hoofnagle, A.N. (2012) Improved detection of opioid use in chronic pain patients through monitoring of opioid glucuronides in urine. *Journal* of Analytical Toxicology, 36, 541–547.
- Eichhorst, J.C., Etter, M.L., Rousseaux, N., Lehotay, D.C. (2009) Drugs of abuse testing by tandem mass spectrometry: a rapid, simple method to replace immunoassays. *Clinical Biochemistry*, 42, 1531–1542.
- Crews, B., Mikel, C., Latyshev, S., West, R., Pesce, A. (2009) Unstable propoxyphene metabolite excreted in human urine is detected by liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*, **33**, 379–383.
- Jian, H., Ybarra, S., Johnson, D., Dilek, I., Sreenivasan, U. (2009) An investigation of norproposyphene cyclization in methanol. Cerilliant. http://www.cerilliant.com/Shoponline/OpenDocument. aspx?DocumentId = 59 (accessed Nov 2, 2014).
- Annesley, T.M. (2003) Ion suppression in mass spectrometry. *Clinical Chemistry*, 49, 1041–1044.
- Jung, J., Kempf, J., Mahler, H., Weinmann, W. (2007) Detection of Delta9-tetrahydrocannabinolic acid A in human urine and blood serum by LC-MS/MS. *Journal of Mass Spectrometry*, 42, 354–360.
- 22. Spell, J.C., Srinivasan, K., Stewart, J.T., Bartlett, M.G. (1998) Supercritical fluid extraction and negative ion electrospray liquid chromatography tandem mass spectrometry analysis of phenobarbital, butalbital, pentobarbital and thiopental in human serum. *Rapid Communications in Mass Spectrometry*, **12**, 890–894.
- Scheidweiler, K.B., Desrosiers, N.A., Huestis, M.A. (2012) Simultaneous quantification of free and glucuronidated cannabinoids in human urine by liquid chromatography tandem mass spectrometry. *Clinica Chimica Acta*, 413, 1839–1847.
- Wu, Z., Gao, W., Phelps, M.A., Wu, D., Miller, D.D., Dalton, J.T. (2004) Favorable effects of weak acids on negative-ion electrospray ionization mass spectrometry. *Analytical Chemistry*, 76, 839–847.
- Thurman, E.M., Ferrer, I., Barcelo, D. (2001) Choosing between atmospheric pressure chemical ionization and electrospray ionization interfaces for the HPLC/MS analysis of pesticides. *Analytical Chemistry*, 73, 5441–5449.
- Yin, D., Xu, H., He, Y., Kirkovsky, L.I., Miller, D.D., Dalton, J.T. (2003) Pharmacology, pharmacokinetics, and metabolism of acetothiolutamide, a novel nonsteroidal agonist for the androgen receptor. *Journal of Pharmacology and Experimental Therapeutics*, **30**4, 1323–1333.
- Sanchez-Rabaneda, F., Jauregui, O., Casals, I., Andres-Lacueva, C., Izquierdo-Pulido, M., Lamuela-Raventos, R.M. (2003) Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry*, 38, 35–42.
- Poklis, J.L., Wolf, C.E., Goldstein, A., Wolfe, M.L., Poklis, A. (2012) Detection and quantification of tricyclic antidepressants and other psychoactive drugs in urine by HPLC/MS/MS for pain management compliance testing. *Journal of Clinical Laboratory Analysis*, 26, 286–294.
- Roth, K.D., Siegel, N.A., Johnson, R.W., Jr, Litauszki, L., Salvati, L., Jr, Harrington, C.A. *et al.* (1996) Investigation of the effects of solution

composition and container material type on the loss of 11-nor-delta 9-THC-9-carboxylic acid. *Journal of Analytical Toxicology*, **20**, 291–300.

- West, R., Crews, B., Mikel, C., Almazan, P., Latyshev, S., Pesce, A. *et al.* (2009) Anomalous observations of codeine in patients on morphine. *Therapeutic Drug Monitoring*, **31**, 776–778.
- West, R., West, C., Crews, B., Almazan, P., Latyshev, S., Rosenthal, M. et al. (2010) Anomalous observations of hydrocodone in patients on oxycodone. *Clinica Chimica Acta*, 412, 29–32.
- 32. Baron, J.M., Griggs, D.A., Nixon, A.L., Long, W.H., Flood, J.G. (2011) The trazodone metabolite meta-chlorophenylpiperazine can cause false-positive urine amphetamine immunoassay results. *Journal of Analytical Toxicology*, 35, 364–368.
- 33. Jang, M., Chang, H., Yang, W., Choi, H., Kim, E., Yu, B.H. et al. (2013) Development of an LC-MS/MS method for the simultaneous determination of 25 benzodiazepines and zolpidem in oral fluid and its application to authentic samples from regular drug users. *Journal of Pharmaceutical and Biomedical Analysis*, 74, 213–222.