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Simultaneous determination of opiates, methadone, buprenorphine and metabolites in human urine by superficially porous liquid chromatography tandem mass spectrometry

Huei-Ru Lin[a,b,c,*], Chin-Lun Chen[a], Chieh-Liang Huang[d], Shao-Tsu Chen[e,f], Ahai-Chuang Lua[a,b,c]

[a] Institute of Medical Biotechnology, Tzu Chi University, Hualien, Taiwan
[b] Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan
[c] Center for Drug Analysis, Tzu Chi University, Hualien, Taiwan
[d] Department of Psychiatry, China Medical University and Hospital, Taichung, Taiwan
[e] Department of Psychiatry, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[f] Department of Medicine, Tzu Chi University, Hualien, Taiwan

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ABSTRACT

For monitoring compliance of methadone or buprenorphine maintenance patient, a method for the simultaneous determination of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine, norbuprenorphine, opiates (morphine, codeine, 6-monoacetylmorphine) in urine by superficially porous liquid chromatography tandem mass spectrometry was developed and validated. After enzyme digestion and liquid–liquid extraction, reverse-phase separation was achieved in 5.2 min and quantification was performed by multiple reaction monitoring. Chromatographic separation was performed at 40 °C on a reversed phase Poroshell column with gradient elution. The mobile phase consisted of water and methanol, each containing 0.1% formic acid, at a flow rate of 0.32 mL/min. Intra-day and inter-day precision were less than 12.1% and accuracy was between −9.8% and 13.7%. Extraction efficiencies were more than 68%. Although ion suppression was detected, deuterated internal standards compensated for these effects. Carryover was minimal, less than 0.20%. All analytes were stable at room temperature for 16 h, 4 °C for 72 h, and after three freeze–thaw cycles. The assay also fulfilled compound identification criteria in accordance with the European Commission Decision 2002/657/EC. We analyzed 62 urine samples from patients received maintenance therapy and found that 54.8% of the patient samples tested were detected for morphine, codeine, or 6-monoacetylmorphine. This method provides a reliable and simultaneous quantification of opiates, maintenance drugs, and their metabolites in urine samples. It facilitates the routine monitoring in individuals prescribed the drug to ensure compliance and help therapeutic process.

1. Introduction

The use of heroin has increased worldwide, making dependence on this drug a major health and social problem. Among the many hazards faced by heroin addicts is high risk for HIV infection from sharing needles. According to statistics from the Centers of Disease Control in Taiwan, the percentage of HIV infected patients in Taiwan caused by injected drugs is 28.96% [1]. In Taiwan and most other countries, methadone and buprenorphine are recommended for detoxification from heroin and for opioid maintenance therapy because they offer several important advantages [2,3]. These treatments typically have longer half-lives and durations of effect, meaning that they are administered only once daily. They effectively minimize the withdrawal effects from ceasing heroin use, and they are designed for oral or sublingual administration instead of injection, avoiding additional risk of infectious disease.

In the body, heroin is rapidly hydrolyzed to 6-monoacetylmorphine (6-AM) and further metabolized to morphine, which is excreted through the urine principally in a conjugated form [4]. The glucuronide conjugate of morphine has been reported to be six-fold more abundant than free morphine in human urine [5]. Street heroin may also contain 1–5% acetylcodeine, which is metabolized to codeine [6]. Thus, morphine, codeine, and their glucuronide metabolites are the major

* Corresponding author at: Department of Laboratory Medicine and Biotechnology, Tzu Chi University, 701 Section 3, Jhongyang Road, Hualien 970, Taiwan. Tel.: +886 38 565301x2346; fax: +886 38 571917.
E-mail addresses: rita0107@mail.tcu.edu.tw, rita0107@gmail.com (H.-R. Lin).

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metabolites found in urine after heroin use, and the presence of 6-AM in urine in particular has the potential to serve as an indicator of recent heroin use [7].

Methadone is primarily administered for maintenance treatment of heroin addiction and has pharmacological properties similar to morphine [8]. Methadone undergoes N-demethylation by liver microsomal cytochrome P450 enzyme CYP3A4 to form its primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) [9]. The ratio of EDDP to methadone is a helpful parameter for distinguishing long-term and short-term administration [9]. Buprenorphine is a partial μ-opiate-receptor agonist and a κ-opiate receptor antagonist. It is used as a substitution drug for opioid addiction and for the treatment of moderate to severe pain. Buprenorphine is metabolized primarily in the liver, where it is N-dealkylated by CYP3A4 and CYP2C8 to form its major metabolite, norbuprenorphine [10]. These are subsequently conjugated to form buprenorphine-3-glucuronide (BUP-Glu) and norbuprenorphine-3-glucuronide (NBUP-Glu), respectively. It has been estimated that 80–90% of buprenorphine and norbuprenorphine excreted through the urine is glucuronide-conjugated [11].

Routine urine monitoring of patients receiving methadone or buprenorphine treatment is often performed through immunoassay or gas chromatography–mass spectrometry (GC–MS). Although immunoassays are rapid and amenable to automation, they are often expensive and tend to suffer from cross-reactivity with opiate drugs [12]. For GC–MS analysis, methadone converts to EDDP at the high temperatures of injector port [13] and buprenorphine and norbuprenorphine have been reported to poor reproducibility by undergoing chemical rearrangement during the derivatization procedure [14].

Liquid chromatography tandem mass spectrometry (LC–MS/MS) has become a powerful tool for the quantitative analysis of drugs that does not require derivatization [15–19]. Berg et al. used high pH mobile phase reversed phase LC–MS/MS to detect of opiates and cocaine in urine [15]. Concheiro et al. [16,17] reported the simultaneous determination of methadone, buprenorphine, opiates, cocaine, nicotine, and metabolites in oral fluid and sweat by LC–MS/MS. Using this alternative biological matrix for forensic toxicology, they developed a method that fulfilled EU identification guidelines. Gergov et al. [18] simultaneously quantified 25 opioid drugs in post-mortem blood and urine by LC–MS/MS, but were unable to evaluate major metabolites, EDDP, and norbuprenorphine. De Jager et al. [19] used an online extraction LC–MS/MS method for the quantitation of amphetamines, opiates, cocaine, cannabis, benzodiazepines, methadone, and metabolites. This assay offered simple sample preparation with online extraction but did not assess buprenorphine and norbuprenorphine in urine samples.

Recent developments in liquid chromatography have enabled rapid and efficient separation. Ultra-high-pressure liquid chromatography (UPLC) with sub-2 μm porous particles and sub-3 μm superficially porous particles (SPP) show great promise for small-molecular-weight compound analysis [20]. In this study, we used a SPP column with lower column back pressure applicable in a traditional HPLC instrument that would be more widely available in most labs.

Glucuronide metabolites are directly detectable by LC–MS/MS; however, glucuronide standards and relative deuterated analog internal standards are required for accurate quantification and compensation of matrix effects. No deuterated internal standards have been commercially available for BUP-Glu and NBUP-Glu until now. Kacinko et al. [21] reported that BUP-Glu and NBUP-Glu were not correctly quantified without the use of relative internal standards. Hydrolysis of glucuronide conjugates was usually assesses by acid or enzyme but the acid hydrolysis of the BUP-Glu and NBUP-Glu was ineffective [22,23]. In this study, we bypassed this problem as the glucuronide conjugates in urine sample. Samples were hydrolyzed by enzyme digestion and quantified in their unconjugated form.

We developed and validated a method for the simultaneous determination of opiates, methadone, buprenorphine, and their metabolites in urine using superficially porous LC–MS/MS. We anticipate that this method will be applicable to assessing compliance with addiction rehabilitation treatment.

2. Materials and methods

2.1. Chemicals and materials

Methadone, EDDP, buprenorphine, norbuprenorphine, morphine, codeine, 6-AM, and their deuterated internal standards were purchased from Cerilliant (Austin, TX, USA). Methanol and isobutanol were purchase from Mallinckrodt (Paris, KY). Acetic acid, sodium carbonate, and formic acid were purchased from Riedel-deHaën (Seelze, Germany). Sodium acetate, sodium bicarbonate, and dichloromethane were obtained from J. T. Baker (Phillipsburg, NJ). β-Glucuronidase (Helix pomatia, crude, Type H1 [G0751], 100,000 units/mL) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All organic solvents and chemicals were of reagent grade.

2.2. Standard solutions

The internal standard (ISTD) solution was prepared in methanol and contained 5 μg/mL each of methadone-d_9, EDDP-d_3, buprenorphine-d_4, norbuprenorphine-d_3, 500 ng/mL of 6-AM-d_3, and 15 μg/mL of morphine-d_3 and codeine-d_3. Stock solutions of each analyte were made in methanol. The calibration solution was prepared in blank urine at six concentrations in the ranges 0, 50, 100, 250, 500, 1000 ng/mL for methadone, EDDP, buprenorphine, and norbuprenorphine, 0, 5, 10, 25, 50, 100 ng/mL for 6-AM, and 0, 150, 300, 750, 1500, 3000 ng/mL for morphine and codeine. The low, medium, and high control solution is 40, 80, and 160 ng/mL for methadone, EDDP, buprenorphine, and norbuprenorphine, and 4, 8, 16 ng/mL for 6-AM, and 120, 240, 480 ng/mL for morphine and codeine.

2.3. Urine samples

Blank urine samples collected from laboratory personnel volunteers were used for method development and preparation of calibrators. 62 patient urine samples were collected from China Medical University and Hospital and Buddhist Tzu Chi General Hospital in May 2011. The study was approved by the Ethical Committee of Buddhist Tzu-Chi General Hospital. Samples were kept at −20 °C until analysis.

2.4. Sample preparation

Sample urine (2 mL) and ISTD solution (40 μL) were combined in a clean 12 mL screw-capped glass tube. The mixture was treated with 5000 units of β-glucuronidase (50,000 units/mL) in the presence of 1 mL of 1 M acetate buffer (pH 5.0) for 16 h at 60 °C with maximal hydrolysis efficacy as previously described [22–24]. After brief vortex mixing, 2 mL of 1.5 M bicarbonate buffer (pH 9.5) and 4 mL of 10% isobutanol in dichloromethane were added, and the solution was vortexed vigorously. The mixture was centrifuged at 2700 rpm for 4 min. The organic layer was carefully transferred to a clean screw-cap glass tube and evaporated to dryness under a stream of nitrogen at 60 °C. The dried extract was reconstituted in 200 μL of water and transferred to a vial for LC–MS/MS analysis.

While the concentration of analytes in patient urine sample was more than upper limit of linearity, the urine sample was diluted

Table 1
LC–MS/MS parameters and retention time (R.T.).

Analyte	R.T. (min)	Quantitation transition (CE, eV)	Qualifier transition (CE, eV)	Relative intensity (%)
Methadone	3.63	310 > 265 (15)	310 > 105 (29)	19.30
Methadone-d_9	3.63	319 > 268 (15)	319 > 105 (30)	
EDDP[a]	3.35	278 > 234 (30)	278 > 249 (24)	41.17
EDDP-d_3	3.35	281 > 234 (31)	281 > 249 (24)	
Buprenorphine	3.44	468 > 396 (37)	468 > 414 (33)	96.73
Buprenorphine-d_4	3.44	472 > 400 (39)	472 > 415 (32)	
Norbuprenophine	3.19	414 > 223 (39)	414 > 340 (29)	96.28
Norbuprenophine-d_3	3.19	417 > 343 (30)	417 > 399 (25)	
Morphine	0.78	286 > 152 (59)	286 > 201 (25)	99.21
Morphine-d_3	0.78	289 > 152 (56)	289 > 164 (37)	
Codeine	1.22	300 > 152 (63)	300 > 165 (39)	97.18
Codeine-d_3	1.22	303 > 152 (70)	303 > 215 (24)	
6-Acetylmorphine	1.54	328 > 165 (38)	328 > 211 (27)	78.61
6-Acetylmorphine-d_6	1.54	334 > 165 (38)	334 > 211 (25)	

[a] EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

at appropriate factor with water to satisfy the value between the linearity and re-analyzed.

2.5. Instrumentation

HPLC. A Thermo Accela LC system (Waltham, MA) consisting of an autosampler, a binary pump, and a column oven was used for chromatography. Chromatographic separation was performed with a superficially porous column, an Agilent Poroshell 120 EC-C_{18} (2.1 mm × 100 mm, 2.7 μm) reversed-phase column. A rapid resolution LC in-line filter (2.1 mm) coupled with 0.2 μm pore size of inlet frit was used. The mobile phase was composed of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in methanol (Solvent B).The flow rate was 0.32 mL/min. The gradient program was as follows: 0–2 min from 20% to 50% B; 2–3 min from 50% to 90% B; 3–4 min 90% B; 4–4.2 min 90% to 20% B; and 4.2–5.2 min column re-equilibration. The column temperature was held at 40 °C during analysis, and the injection volume was 10 μL.

MS–MS. A TSQ Quantum tandem triple-quadrupole MS equipped with an electrospray interface was used. Positive ionization was performed in multiple reaction monitoring (MRM) mode. The analysis was performed using the following instrument parameters: spray voltage was 5 kV; nitrogen was used as sheath and auxiliary gas at 40 and 0 units (arbitrary units); capillary temperature was 300 °C. The argon collision gas pressure was set to 1.0 mTorr. MS–MS conditions for individual analytes were optimized through post-column infusion of stock solution (1 μg/mL) using Quantum TuneMaster software (Thermo Fisher Scientific). The scan time was set to 15 ms. The MRM parameters are shown in Table 1.

2.6. Identification criteria

Identification criteria included a retention time within ±2.5% of the retention time of calibrator, the detection of two transitions, and relative ion intensities (% of base peak) within ±20% if the relative ion intensity was more than 50%, ±25% if it was 20–50%, ±30% if it was 10–20%, and ±50% if it was ≤10% [25]. Relative ion intensities were calculated on the basis of ion ratios (quantification transition divided by qualifier transition). These values were compared with the mean relative ion intensity of all calibrators.

2.7. Method validation

2.7.1. Linearity

The linear range for each analyte was evaluated using a series of standard solutions with internal standards. The calibration curves were constructed based on peak area ratios of the analytes to internal standard (analyte/ISTD) versus the corresponding concentration. Linearity was determined by linear regression with a $1/x$ weighting factor. Acceptable linearity was achieved when the coefficient of determination was ≥0.99 and the quantification of calibrators was within ±20% of target concentrations.

2.7.2. Limits of detection and quantification

Sensitivity was assessed by establishing the limit of detection (LOD) and limit of quantification (LOQ) for each analyte. In order to report a positive analyte result, the retention time, two transitions, and relative ion intensity must have satisfied the identification criteria described previously. The LOD was determined for all analytes as the lowest concentration exceeding a signal-to-noise (S/N) level of 3:1. The LOQ was defined as the lowest concentration that met S/N ratio of at least 10, and quantitated with bias less than 20%.

2.7.3. Carryover

Carryover was evaluated by injecting blank urine containing ISTD immediately after a sample spiked with 2500 ng/mL of all target analytes. The measured concentration of the blank sample was used to calculate the carryover rate. Carryover was considered negligible if the measured concentration was below the LOQ.

2.7.4. Extraction efficiency and matrix effects

The extraction efficiency was determined by injecting five replicates at low and high concentrations: 100/200 ng/mL for methadone, EDDP, buprenorphine and norbuprenorphine; 300/600 ng/mL for morphine and codeine; and 10/20 ng/mL for 6-AM. Blank urine was fortified with analyte solution and internal standard before and after liquid–liquid extraction. The percent extraction efficiency from urine was calculated as the mean peak area of samples spiked before extraction divided by that of samples spiked after extraction.

Matrix effects were evaluated according to the following procedure [26]: ten blank urine samples from ten different sources were extracted as described and were spiked with all the target analytes and ISTDs. Ten samples of water were spiked in the same manner to serve as references solutions. The reconstituted extracts and the reference solutions were analyzed, and the peak areas from the extracts were compared with the corresponding peak areas of the reference solutions. The matrix effect percentage was calculated as follows: (mean peak area of reconstituted solution/mean peak area of reference solution) ×100.

2.7.5. Precision and accuracy

The precision and accuracy were estimated at three concentration levels. The precision was expressed as the coefficient of variation of the measured value. The accuracy was presented as

Table 2
LOD, LOQ and calibration data.

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)	Slope ±SD	Intercept ±SD	R^2 ± SD
Methadone	0.50	3.33	3.33–1000	0.019 ± 0.004	−0.022 ± 0.024	0.9989 ± 0.0011
EDDP	0.50	1.25	1.25–1000	0.015 ± 0.002	0.005 ± 0.007	0.9998 ± 0.0001
Buprenorphine	0.83	5.00	5.00–1000	0.009 ± 0.001	0.003 ± 0.037	0.9962 ± 0.0028
Norbuprenorphine	0.83	5.00	5.00–1000	0.011 ± 0.002	-0.002 ± 0.019	0.9959 ± 0.0039
Morphine	1.50	3.75	3.75–3000	0.004 ± 0.001	0.001 ± 0.001	0.9994 ± 0.0003
Codeine	1.50	3.75	3.75–3000	0.002 ± 0.001	0.001 ± 0.001	0.9997 ± 0.0001
6-Acetylmorphime	0.05	0.50	0.50–100	0.196 ± 0.017	0.017 ± 0.016	0.9967 ± 0.004

percent error, calculated as follows: [(measured value − expected value)/expected value] ×100%. Intra-day precision and accuracy were assessed by preparing and analyzing five replicates on the same day. For inter-day precision and accuracy, one replicate was analyzed on five different days. A new calibration curve was generated each time using the controls.

2.7.6. Selectivity

Method selectivity was demonstrated by adding a high concentration (5000 ng/mL) of potentially interfering licit and illicit drugs to low-concentration control samples. The following drugs and metabolites were examined: pentazocine, benzoylecgonine, cocaine, nalorphine, amphetamine, methamphetamine, normeperidine, meperidine, 3,4-methylenedioxyamphetamine (MDA), (S)-(−)-cathinone, (S)-(−)-methcathinone, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA), delta-9-tetrahydrocannabinol (THC), methylenedioxyethylamphetamine (MDEA), ketamine, norketamine, dehydronorketamine, N-ethylamphetamine, ecogonine methyl ester, fenfluramine, normorphine, and levo-alpha-acetylmethadol (LAAM).

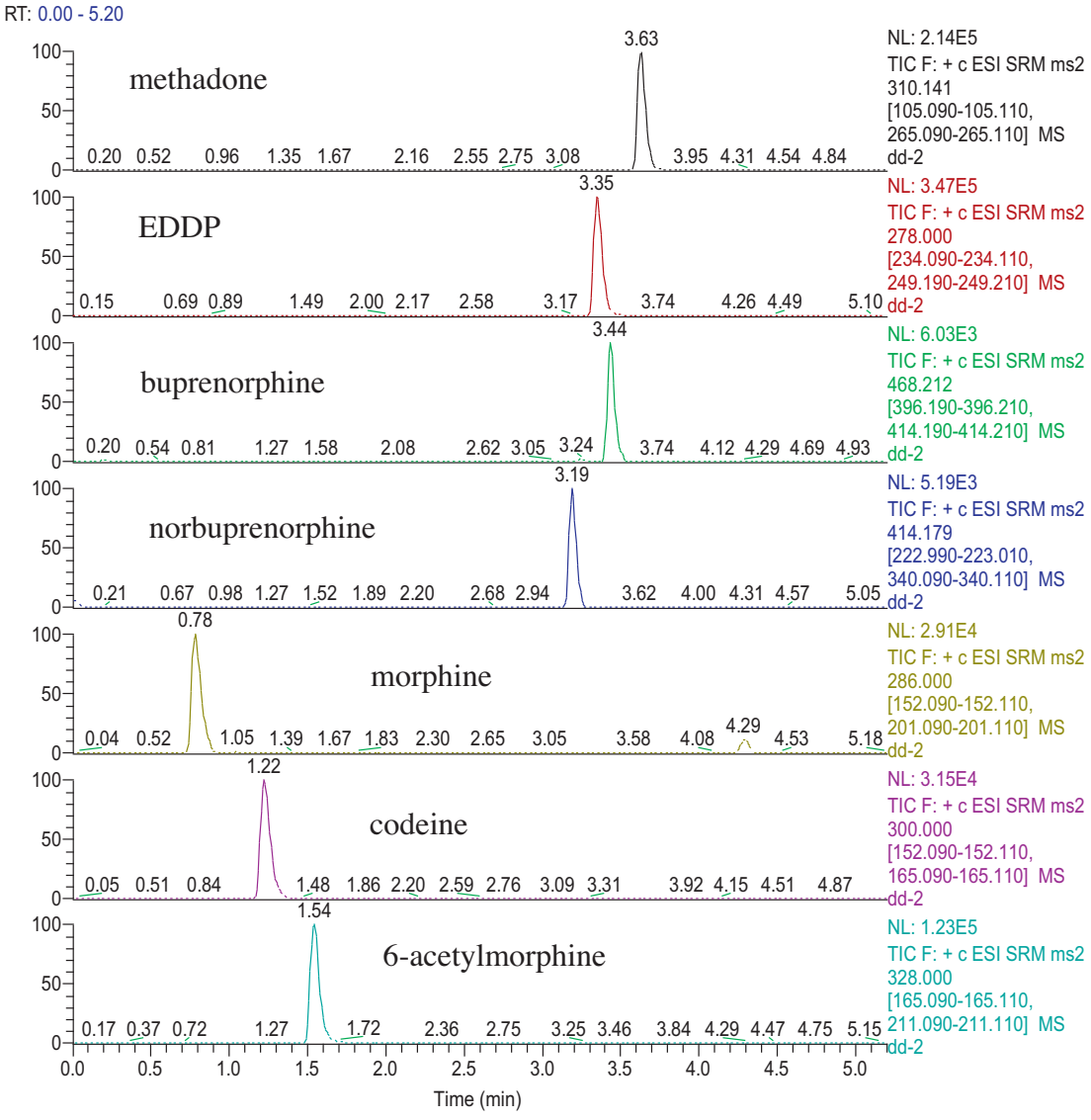

Fig. 1. MRM chromatogram of a negative sample spiked with 100 ng/mL of methadone, EDDP, buprenorphine, norbuprenorphine, and 10 ng/mL of 6-acetylmorphine, and 300 ng/mL of morphine and codeine.

Table 3
Extraction efficiency, and matrix effect.

Analyte	Extraction efficiency (%) low conc. (n = 5)	Extraction efficiency (%) high conc. (n = 5)	Matrix effect (%) (target response)	Matrix effect (%) (target/ISTD ratio)
Methadone	92.0	85.1	67 (19.2)	100 (3.1)
EDDP	83.8	85.3	71 (19.6)	102 (1.4)
Buprenorphine	88.7	87.8	101 (23.8)	107 (6.6)
Norbuprenorphine	84.7	86.4	81 (20.9)	101 (5.1)
Morphine	68.5	71.8	83 (11.3)	108 (9.0)
Codeine	92.0	83.3	92 (11.9)	103 (2.5)
6-Acetylmorphime	88.8	85.7	79 (19.1)	104 (4.9)

% RSD is given in parentheses.

2.7.7. Stability

Analyte stability was evaluated under a variety of conditions. Prepared sample stability was determined by triplicate measurements of low-concentration control samples stored at 4 °C for 48 and 72 h after preparation. Additionally, stability was demonstrated for spiked urine stored for 16 h at room temperature and for 72 h at 4 °C, as well as after three freeze–thaw cycles. Acceptable stability was achieved if the sample could be quantified to within ±15% of the expected value.

3. Results and discussion

We developed an LC–MS/MS method for the simultaneous identification and quantification of methadone, buprenorphine, opiates, and their metabolites, and we validated the method for linearity, sensitivity, carryover, extraction efficiency, matrix effects, precision, accuracy, process efficiency, and selectivity. Linear regression with $1/x$ weighting was used to construct the calibration curves, and the slopes, intercepts, and coefficients of determination (R^2) were determined and summarized in Table 2. For all analytes, R^2 was >0.99. The intercept of the calibration curves did not significantly differ from zero. The LOD and LOQ are shown in Table 2, and were determined to be 0.05–1.50 ng/mL and 0.50–5.00 ng/mL, respectively. Fig. 1 shows chromatograms of the quantification transitions for all analytes. The chromatograms of blank, LOQ, and middle control samples were shown in supplementary material Fig. S1–S3.

No analyte was detected in a blank sample injected immediately following the analysis of a 2500 ng/mL sample, except for EDDP. The carryover rate of EDDP was minimal at 0.20%.

The extraction efficiency is presented in Table 3. The extraction efficiency for all analytes ranged from 68.5% to 92.0%. The average values of the matrix effect shown in Table 3 indicate that the matrix effects of all analytes are different. The quantification ion intensities for all analytes varied among different blank samples were exhibited by CV values. With the addition of internal standards, the ion intensity ratio of quantification ions to that of the respective internal standards was calculated. The variation among the ratios was greatly reduced, leading us to conclude that matrix effects were eliminated by the use of isotope-labeled internal standard.

The method validation results for precision and accuracy are summarized in Table 4. Intra- and inter-day precision was less than 12.1% for all analytes. The accuracy was between −9.8% and 13.7%. The method selectivity was demonstrated by adding a high concentration (5000 ng/mL) of 22 potentially interfering drugs and metabolites to low-concentration control samples. All test samples were quantified within ±15% of the target, indicating no interference with the seven analytes of interest.

The stability of the analytes in urine and in analysis vials was reported as the percentage difference between the mean concentrations of fresh controls and of those kept under various conditions. The stability results are shown in Table 5. The analytes were stable under all storage conditions with the percentage difference from fresh controls ranging from −6.57% to 14.48%.

Table 4
Intra-day and inter-day precision and accuracy (n = 5).

analyte	Concentration (ng/mL)	Intra-day precision (CV, %)[a]	Intra-day accuracy (bias, %)[b]	Inter-day precision (CV, %) [a]	Inter-day accuracy (bias,%) [b]
Methadone	40	6.0	10.0	10.8	−1.3
	80	4.0	1.3	5.6	1.4
	160	2.6	0.4	7.8	0.3
EDDP	40	1.8	−6.8	4.5	−4.8
	80	4.9	−7.4	2.1	1.9
	160	3.4	−0.9	2.7	0.8
Buprenorphine	40	9.4	5.6	2.8	−0.7
	80	5.7	-7.2	7.3	−1.1
	160	5.3	-3.0	5.8	5.5
Norbuprenorphine	40	6.6	13.7	11.1	−1.8
	80	2.2	2.2	5.6	−5.0
	160	6.4	−5.1	5.1	6.6
Morphine	120	8.5	−9.8	2.7	−3.5
	240	5.3	−3.0	1.8	1.5
	480	1.4	−0.9	0.9	4.7
Codeine	120	3.2	−0.1	2.1	2.2
	240	3.4	−1.3	0.9	0.5
	480	1.1	2.1	3.8	2.7
6-AM	4	12.1	5.7	7.3	−4.3
	8	1.9	−1.9	10.2	1.3
	16	2.9	−2.1	8.9	2.2

[a] The coefficient of variance (% CV): SD/mean × 100%.
[b] Calculated as: [(calculate concentration − theoretical concentration)/theoretical concentration] ×100.

Table 5
The stability of analyte under different storage conditions; percentage difference from fresh controls ($n = 3$).

Analyte/storage condition	Conc. (ng/mL)	Prepared sample, 4 °C for 48 h	Prepared sample, 4 °C or 72 h	Three freeze–thaw cycles	RT for 16 h	4 °C for 72 h
Methadone	40	14.48	5.24	0.82	2.67	−1.05
EDDP	40	−1.15	4.71	5.30	9.33	0.90
Buprenorphine	40	0.51	8.43	−4.86	−4.77	−4.46
Norbuprenorphine	40	−6.57	4.97	−1.37	2.03	−2.59
Morphine	240	−2.14	2.39	5.63	8.70	3.85
Codeine	240	−0.31	1.46	2.63	5.97	0.73
6-AM	4	−4.00	1.63	2.72	0.60	−2.48

LC–MS/MS has become a powerful tool for quantitative analysis of drugs and has begun to find applications in the field of forensic toxicology and clinical toxicology. It is particularly suitable for the analysis of drugs and their metabolites, as there is a high percentage of the glucuronide metabolites of morphine, buprenorphine and norbuprenorphine in addition to the parent compounds found in urine samples.

The requisites of forensic analysis by LC–MS/MS with respect to EU guidelines include chromatographic separation, a minimum number of two MS/MS transitions to obtain the required identification points, and predefined thresholds for the variability of the relative intensities of the MS/MS transitions (MRM transitions) in samples and reference standards [25,27]. The validated data we obtained demonstrates that the criteria laid out by EU guidelines can be satisfied. Sixty-two urine samples collected from patients receiving methadone or buprenorphine maintenance treatment were analyzed. The concentration profile of all target analytes is shown in supplementary material Tables S1–S2. A chromatogram of methadone-treatment patient samples #29 was shown in supplementary material Fig. S4. Since 2006, buprenorphine has been used in Taiwan as a substitution drug for opioids and only three urine samples were collected from buprenorphine-maintenance patients. Morphine, codeine, or 6-monoacetylmorphine was detected in 54.8% (34/62) of all analyzed urine samples. Methadone or the metabolite, EDDP, was not detected, but morphine and codeine were found in two urine specimens (#6 and #57 in Table S2). 6-AM was excreted rapidly with half-life of 0.6 h after heroin administration and resulting in short detection time with 2–8 h. Detection of 6-AM in urine as a marker of recent heroin exposure [7]. 6-AM was detected in 17.7% (11/62) of urine samples, indicating the recent use of heroin in those patients. The ratio of EDDP to methadone in individuals compliant with therapy is typically higher than 0.6 [9]; in 53 methadone -positive samples, the ratios of EDDP and methadone were all higher than 0.6 except for #49 (Table S2). 6-AM was also detected in this sample, indicating that the patient had recent heroin use and was not in compliance with maintenance treatment. No methadone but low amount of EDDP was detected in patient sample #14, #20, and #38.

4. Conclusions

A simultaneous quantification method for the analysis of maintenance drugs and opiates in urine samples by LC–MS/MS was developed and validated. This validated method provides a rapid, sensitive, reliable, and simultaneous quantification of all target analytes in urine samples. It facilitates routine monitoring in individuals prescribed methadone or buprenorphine to ensure compliance and promote the therapeutic process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.02.020.

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