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ORIGINAL ARTICLE

A sensitive, simple and rapid HPLC–MS/MS method for simultaneous quantification of buprenorpine and its N-dealkylated metabolite norbuprenorphine in human plasma

Yi-Ya Wang[a], Xiao-Hang Shen[b], Hao Li[b], Feng-Ju Chen[b], Yan Fu[b], Li Ding[a],*

[a]Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b]Bioanalytical Service, WuXi AppTec Co., Ltd., 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, China

Abstract   A sensitive, simple and rapid high performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method was developed and fully validated for the simultaneous quantification of buprenorphine (BUP) and its N-dealkylated metabolite norbuprenorphine (NBUP) in 200 μL human plasma. Human plasma samples were prepared using liquid–liquid extraction, and then separated on a Shiseido MG C18 (5 μm, 2.0 mm × 50 mm) via 4.1 min gradient elution. Following electrospray ionization, the analytes were quantified on a triple–quadrupole mass spectrometer in multiple-reaction-monitoring (MRM) positive ion mode. Linearity was achieved from 25.0 to 10000 pg/mL for buprenorphine, from 20.0 to 8000 pg/mL for norbuprenorphine with $r^2 > 0.99$. The method was demonstrated with acceptable accuracy, precision and specificity for the detection of buprenorphine and norbuprenorphine. Recovery was 81.8–88.8% for buprenorphine and 77.0–84.6% for norbuprenorphine, and the matrix effect was 95.6–97.4% for buprenorphine and 94.0–96.9% for norbuprenorphine; all were not concentration dependent. With validated matrix and autosampler stability data, this method was successfully applied in a bioequivalence study to support abbreviated new drug application.

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*Corresponding author. Tel./fax: +86 25 8327 1289.
E-mail addresses: dinglidl@hotmail.com,
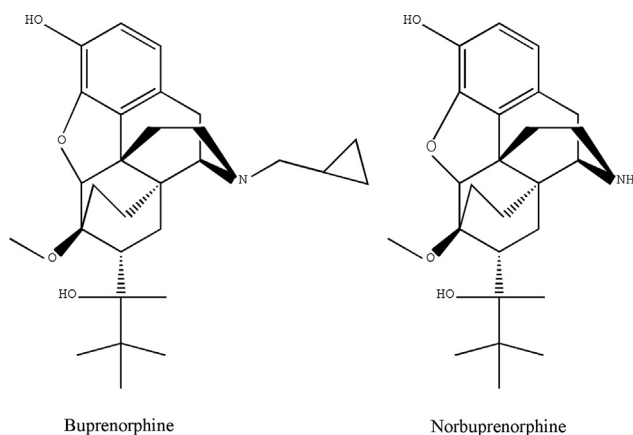dinglihg@sina.com (L. Ding).

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1.   Introduction

Buprenorphine (BUP) (Fig. 1), (2S)-2-[(-)-(5R, 6R, 7R, 14S)-9α-cyclopropylmethyl-4, 5-epoxy-6, 14-ethano-3-hydroxy-6-methoxy-morphinan-7-yl]-3, 3-dimethylbutan-2-ol, is a semi-synthetic opioid derived from thebaine. First marketed in the 1980s as an

Fig. 1 The chemical structures of buprenorphine (BUP) and norbuprenorphine (NBUP).

analgesic, BUP was indicated for the treatment of moderate to severe chronic pain [1]. In 2002, BUP was approved by the Food and Drug Administration (FDA) of the United States for detoxification and long-term replacement therapy in opioid dependency, and the drug is now used predominantly for this purpose [2].

BUP undergoes N-dealkylation to norbuprenorphine (NBUP, Fig. 1) mainly by cytochrome P450 3A4 in liver. Both BUP and NBUP are further metabolized by phase II glucuronidation to buprenorphine-glucuronide (BUP-Gluc) and norbuprenorphine-glucuronide (NBUP-Gluc) [3]. NBUP, BUP's main active metabolite, can cross the blood–brain-barrier similarly to BUP and likely contributes to pharmacologic profile of the parent drug [4].

Quantification of BUP and NBUP in human plasma is of great importance in pharmacokinetic evaluation and clinical drug monitoring [5]. Minimum effective concentration of the transdermal patch formulation of buprenorphine (Transtec®) was only 100 pg/mL in plasma and C_{max} was only 305 ± 117 pg/mL for 35 µg/h dose, which indicating the necessary for a highly sensitive method [6]. To date, liquid chromatography tandem mass spectrometry (LC-MS), with its advantages of high selectivity, sensitivity and accuracy, becomes a major technique for quantification of BUP and its metabolites in a wide variety of matrices, including urine [7–15], plasma [16–24], whole blood [9–11,25], hair [9–11,26–28], sweat [29], meconium [30], and breast milk [31]. Of the current available LC-MS methods applied in plasma, the lack of the requisite sensitivity and selectivity for accurate assessment of the drug concentrations is the major limitation. When triple–quadrupole MS applied, although BUP and NBUP can be well ionized in Q1, at low collision energy (CE) setting the little fragmentation was achieved, and at higher CE the multiple low intensity products were obtained. Therefore, only the surviving ion transition can be monitored [17,21], which often leads to a high baseline and limited sensitivity. There were also other reported methods using ion trap spectrometer, which were able to monitor more than one ion transitions of BUP and NBUP [19,20], but the lower limit of quantification (LLOQ, 0.1 ng/mL for BUP and 0.5 ng/mL for NBUP) was not desirable [20]. Limited sensitivity in turn required larger sample volume, which is also not practical in sample analysis. In order to achieve good separation from the high baseline caused by endogenous materials, though more advantageous instruments like UPLC were applied, longer run time of LC separation is still required [16–23]. A simpler, economic

sample treatment procedure with excellent reproducibility and high recovery is also in need to meet the requirement of high-throughput analysis. [16–18,21,24].

In this study, a sensitive, simple and rapid HPLC–MS/MS method was developed and fully validated for simultaneously quantification of BUP and NBUP in human plasma. The method demonstrated improved performance with simpler procedure, high extraction efficiency, rapid LC separation, high selectivity and sensitivity. Key factors to the success of this assay are also addressed.

2. Materials and methods

2.1. Chemicals and materials

BUP, NBUP and deuterated internal standards BUP-d4, NBUP-d3 ampoules at 100 µg/mL in methanol were obtained from Cerilliant (Austin, TX, USA). HPLC grade acetonitrile (ACN), methanol (MeOH) and isopropanol (IPA) were obtained from Merck (Darmstadt, Germany) and ethyl acetate (EtOAc) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Reagent grade ammonium trifluoroacetate (NH4TFA) and formic acid (FA) were from Sigma-Aldrich (St. Louis, MO, USA). Acetone and ammonia hydroxide (NH_4OH) were obtained from Sinopharm (Shanghai, China). Water was deionized and purified in-house on an Elga purification system (Marlow, Bucks, UK). Human plasma (K_2EDTA) was obtained from Bioreclamation (Hicksville, NY, USA), which will be used as blank matrix for the preparation of calibration standards (Cs), quality control samples (QCs) and blank samples.

2.2. Preparation of standard and quality control samples

Eight calibration standards, six levels of QCs and internal standard (IS) working solution were prepared by diluting the stock solutions with 50% MeOH.

Calibration standards were prepared by 20-fold spiking the working solutions with blank plasma at the concentrations of 25.0, 50.0, 250, 500, 1500, 6000, 9000, 10,000 pg/mL for BUP and 20.0, 40.0, 200, 400, 1200, 4800, 7200, 8000 pg/mL for NBUP. Lower limit of quantification QC (LLOQ QC), low QC (LQC), geometric QC (GMQC), medium QC (MQC), high QC (HQC) and dilution QC (DQC) were prepared with the same spiking procedure at concentrations of 25.0, 75.0, 750, 4000, 8000, 50,000 pg/mL for BUP and 20.0, 60.0, 600, 3200, 6400, 40,000 pg/mL for NBUP.

2.3. Sample preparation

An aliquot of plasma (200 µL) was placed in a 96-well plate followed by 30 µL IS working solution (10.0 ng/mL for both BUP and NBUP). After adding 30.0 µL of 5 M NH_4OH, samples were vortexed well for 2 min and followed by LLE with 800 µL EtOAc. After vortexing and centrifugation (4000 rpm, 4 °C for 10 min), 550 µL of organic layer was transferred to a clean 96-well receiving plate and evaporated to dryness under a gentle stream of nitrogen at 50 °C. Dried extracts were reconstituted with 100 µL mobile phase, containing 60% mobile phase A (3 mM NH4TFA in water with 0.004% FA) and 40% mobile phase B (3 mM NH4TFA in 75% MeOH with 0.004% FA). A 20.0 µL aliquot of

well-mixed extracts was injected into the LC-MS/MS. All experiments must be done under yellow light condition.

2.4. Instrument conditions

Samples were injected into the LC-MS/MS system using an HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). LC-MS/MS analysis was performed with an Agilent Model 1200 separation system (Santa Clara, CA, USA) interfaced to an API 5000 (Applied Biosystems, Foster City, CA, USA) with an electrospray ion (ESI) source. The temperature of autosampler was maintained at 8 °C, and the column compartment at 50 °C.

Chromatographic separation was performed on an MG C18 (2.0 mm × 50 mm, 5 μm, Shiseido, Tokyo, Japan) column and C18 security guard cartridges (4.0 mm × 3.0 mm, Phenomenex, Torrance, CA, USA). A 4.1 min HPLC gradient consisting of mobile phases A and B at a 400 μL/min flow rate was applied. A Valco Valve Diverter (Houston, TX, USA) was used to divert the initial 1.2 min elution. The gradient elution performed is shown in Table 1.

Mass spectrometric data were acquired in positive ion mode with the following ESI-MS parameters: IonSpray voltage: 2000 V; source temperature: 550 °C; curtain gas, gas 1 and gas 2 (nitrogen): 30, 30 and 40 units, respectively; All gases were nitrogen. Both Q1 and Q3 were set at unit resolution. Data were recorded in MRM mode. The precursor ions, product ions, and LC-MS/MS parameters are displayed in Table 2.

Data acquisition and peak integration were assigned to a computer work station running Analyst™ Software1.4.2 (Applied Biosystems, Foster City, CA, USA). Validated Watson LIMS 7.2.0.02 (Thermo Scientific, San Jose, CA, USA) was applied to regression and calculation.

2.5. Method validation

The analytical procedure was validated in terms of linearity, LLOQ, intra-batch and inter-batch precision and accuracy, specificity, selectivity, dilution integrity, extraction recovery, matrix effect and stability. All validation experiments were designed according to the principles outlined in the FDA industry guidance on bioanalytical method validation [32].

Calibration curves for each analyte were individually constructed by least-squares linear regression analysis of an eight-point calibration curve by plotting analyte-to-IS peak area ratio versus its nominal concentration, using $1/x^2$ as a weighting factor. The LLOQ was established based on the lowest QC results with acceptable precision ($\leq 20\%$), accuracy ($\pm 20\%$) and satisfactory signal to noise ratio of greater than 10.

Accuracy and precision were investigated by at four levels (LLOQ QC, LQC, GMQC, MQC and HQC) in a single batch and over three different batches. Coefficient of variation (CV%) and bias were calculated to evaluate the precision and accuracy of the method.

The interference of one analyte to the other analyte and IS, interference from IS to analyte and interference from endogenous material were determined in this validation.

Selectivity was demonstrated to establish the lack of interference from commonly used potentially co-administered

Table 1 Mobile phase composition.

Time (min)	A[a] (%)	B[b] (%)	Flow rate (μL/min)
0.01	55	45	400
0.20	55	45	400
2.00	0	100	400
3.00	0	100	400
3.10	55	45	400
4.10	55	45	400

[a]Mobile phase A: 3 mM NH4TFA in water with 0.004% FA.
[b]Mobile phase B: 3 mM NH4TFA in 75% MeOH with 0.004% FA.

Table 2 LC-MS/MS parameters for buprenorphine, norbuprenorphine, buprenorphine-d4 and norbuprenorphine-d3.

| Analytes and internal standards	Transitions (m/z)	Dwell time (ms)	DP[a] (V)	CE[b] (V)	EP[c] (V)
Buprenorphine	468.3/396.3	70	95	54	9
Buprenorphine-d4	472.3/415.3	70	108	49	8
Norbuprenorphine	414.2/187.1	70	80	52	10
Norbuprenorphine-d3	417.3/343.3	70	80	41	8

[a]Declustering potential.
[b]Collision energy.
[c]Entrance potential.

medications (including acetaminophen, acetylsalicylic acid, caffeine, chlorpheniramine maleate, ibuprofen, naproxen, estradiol, progesterone, naltrexone, 6-β-naltrexol, pseudoephedrine, naloxone and naloxone-3-β-D-glucuronide) at the concentrations equal or higher than the reported maximum plasma concentrations (C_{max}) of each medication.

The dilution integrity was validated by diluting quality control samples (DQC) by a dilution factor (1/10) to a concentration within the calibration range using blank human plasma in six replicates.

Extraction recovery and matrix effect were evaluated at three concentration levels (LQC, MQC and HQC) for each analyte and at the working concentration for internal standards. Post-spiked QCs used in recovery and matrix effect test, were prepared by spiking the extracted blank matrix with analyte and IS to ensure that concentrations are equivalent to those in the LQC, MQC and HQC extracted samples. Extraction recovery was calculated by comparing the signal of analyte or IS of regular QCs with those of post-spiked QCs. Matrix effect was evaluated by comparing the signal of post-spiked QCs with those of the neat solutions at LQC, MQC and HQC levels.

Analyte stability was evaluated at two levels (LQC and HQC) in matrix and in sample extracts placed in autosampler (8 °C). For room temperature stability, long-term stability (≤ -15 °C) and five freeze-thaw cycles stability test, the concentration of each analyte after storage was compared to its nominal concentration. For autosampler stability test, stability samples were quantified against the re-injected calibration curves.
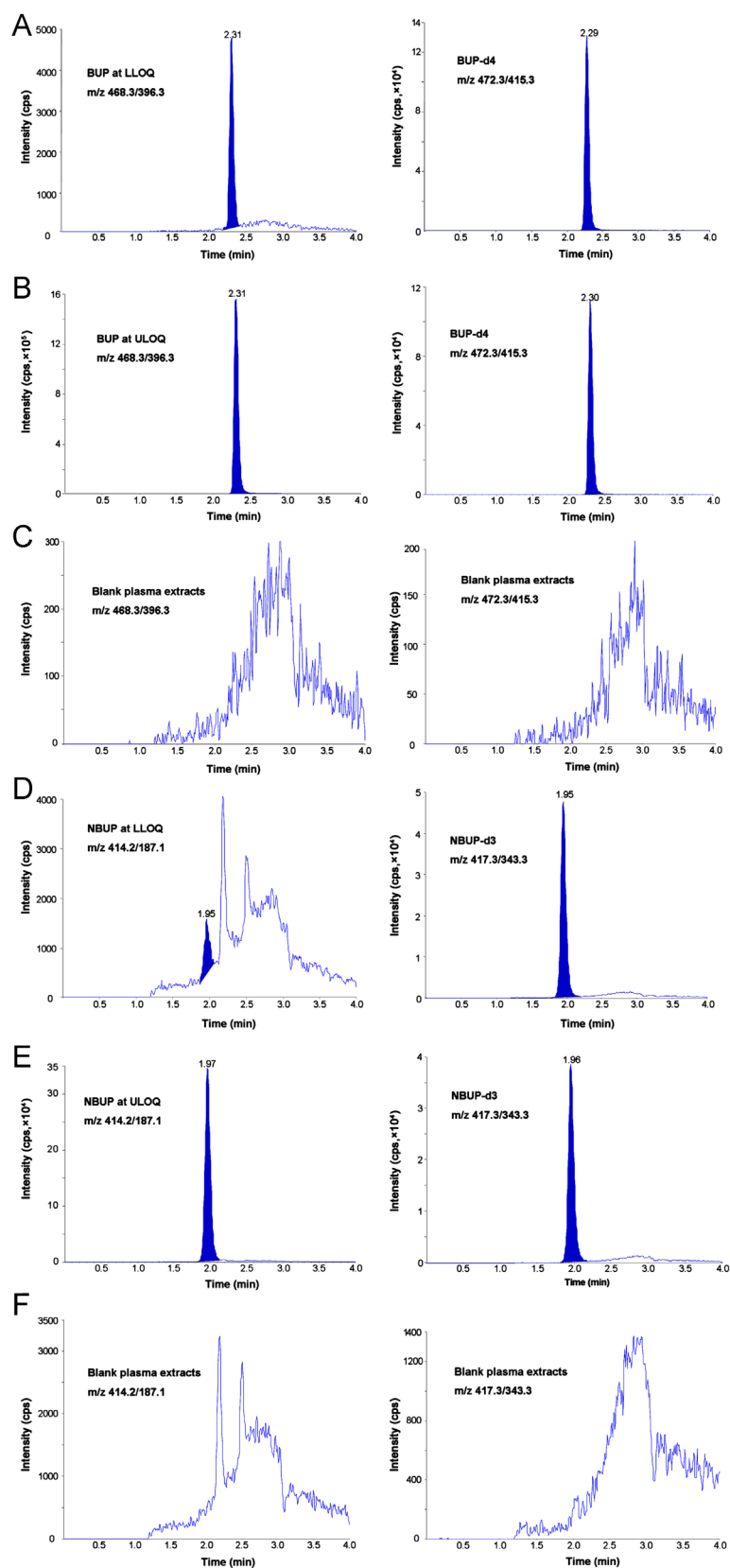
Fig. 2 Representative HPLC–MS/MS chromatograms of buprenorphine (BUP) and norbuprenorphine (NBUP) at LLOQ and ULOQ and blank plasma extracts. Pane (A) shows buprenorphine at LLOQ (25.0 pg/mL). Pane (B) shows buprenorphine at ULOQ (10,000 pg/mL). Pane (C) shows blank plasma extracts at the buprenorphine channel. Pane (D) shows norbuprenorphine at LLOQ (20.0 pg/mL). Pane (E) shows norbuprenorphine at ULOQ (8000 pg/mL). Pane (F) shows blank plasma extracts at the norbuprenorphine channel. The retention times for buprenorphine and norbuprenorphine were about 2.31 min and 1.96 min, respectively. ULOQ, upper limit of quantification; LLOQ, lower limit of quantification.

3. Results and discussion

3.1. Chromatography

Reported methods with more advantageous instruments like UPLC applied, have at least 7.8 min run time to simultaneously quantify BUP and NBUP, which are not ideally fit to analyzing pharmacokinetic samples in a high-throughput manner [23]. In this developed method, a good chromatographic separation of BUP and NBUP was achieved within 4.1 min by using a reverse-phase (C18) column in gradient mode.

Initially, different columns (including Polar-RP, C8 and C18) were tested to separate BUP and NBUP in method development. MG C18 (2.0 mm × 50 mm, 5 μm, Shiseido, Tokyo, Japan) column exhibited the most satisfactory performance. On this column, BUP and NBUP were well separated from the potential endogenous interferential substances, and showed suitable retention and excellent peak shape. Optimized mobile phase and gradient program reduced the analytical run time without compromising separation efficiency and peak shape. Addition of salt and acid helps to obtain symmetrical peak shape.

With the chromatographic conditions described above, the retention time ranges were 2.39 ± 0.08 min for BUP and 2.13 ± 0.07 min for NBUP. Typical chromatograms of the ULOQ, LLOQ and blank plasma extracts are shown in Fig. 2. The low background from the biological matrix and the sharp and symmetrical resolution of the peaks showed good selectivity for BUP and NBUP. Blank samples injected after ULOQ samples did not show quantifiable response, which demonstrated that the former injection had no impact on the quantification of the later one, the method has no carryover effect.

3.2. MS/MS optimization

One of the challenges in the method development was that collision energy in Q2 did not produce significant fragment ions from the precursor ions of the analytes, especially for NBUP. The protonated molecular ions $[M+H]^+$ were easily observed in the full scan mass spectra of BUP and NBUP when establishing MRM transition by infusing 200/160 ng/mL

of BUP/NBUP in 50% MeOH directly. However, at low collision energy NBUP showed little fragmentation and at high collision energy it broke down into multiple low intensity product ions. Therefore, only the surviving ion transition can be monitored [17,21], which often leads to a high baseline and limited sensitivity. The additives of mobile phase and value of CE were optimized in order to successfully monitor the ion transitions of BUP and NBUP. Suitable addition of NH_4TFA and FA contributed to the ionization of both analytes, and the optimal CE value gave the most stable product ions of them. NBUP exhibited abundant product ion peaks at 340.1 and 187.1 at the optimal value of CE. During the method development stage, the above two ion transitions were monitored. The product ion at 187.1 (m/z) was then selected to perform the quantitation of NBUP, because it showed higher instrument response and was well separated from the interference of endogenous materials as well. The results of MRM scan and the deduced structures of product ions are shown in Fig. 3.

In this HPLC–MS/MS method, the modified ion transitions were monitored to simultaneously determine BUP and NBUP in human plasma using triple–quadrupole MS.

3.3. Sample treatment

Limited sensitivity also caused difficulties in the sample treatment. SPE (solid-phase extraction) was applied in many methods to clean up the plasma sample, which made the sample treatment highly cost and time consuming [23]. LLE (liquid–liquid extraction) was a simpler choice for sample treatment; however, larger sample volume together with large volume of extraction solvent was required, which was not suitable for green chemistry and made the procedures not practical in high-throughput analysis [24].

During the method development, the extraction solvents and volume selection of LLE were optimized. It was concluded that EtOAc with similar polarity to the two analytes is more suitable for the LLE. Extraction efficiency of both analytes was satisfactory and did not show significant difference when the volume ratios of sample and extraction solvent changed from 1:3 to 1:6. Since small volume of extraction solvent (1:3) may lead to insufficient extraction, and large
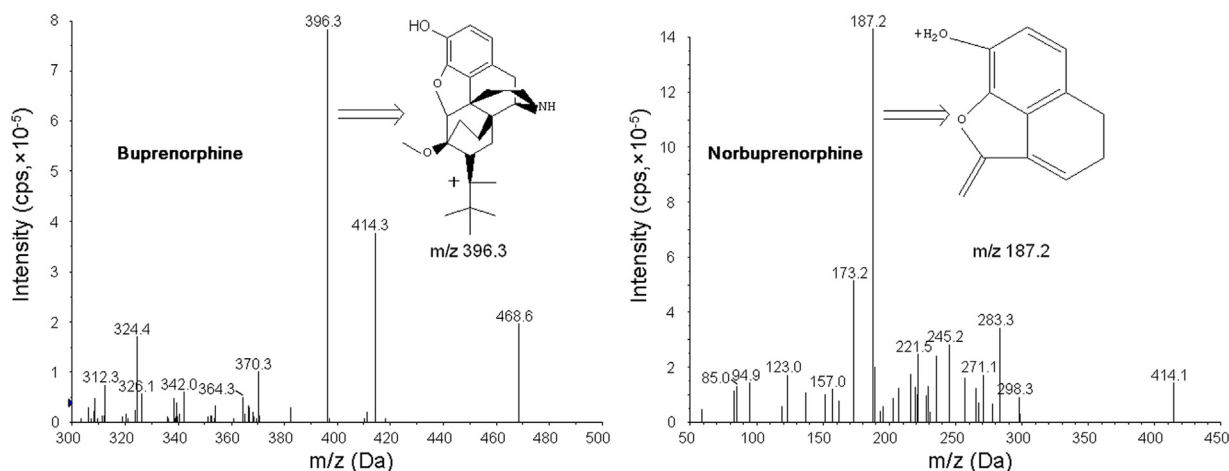

Fig. 3 MS/MS spectra of buprenorphine (BUP) and norbuprenorphine (NBUP) in 50% MeOH.

Table 3 Intra-batch and inter-batch accuracy and precision of BUP and NBUP in human plasma.

Analyte	Nominal concentration (pg/mL)	Precision (%)				Accuracy (%)			
		Intra-batch (n=6)			Inter-batch (n=18)	Intra-batch (n=6)			Inter-batch (n=18)
BUP	25.0 (LLOQ QC)	10.2	10.4	10.5	10.1	−0.8	−6.4	−6.0	−4.4
	75.0 (LQC)	3.5	5.0	9.8	6.8	4.0	7.7	0.5	4.1
	750 (GMQC)	1.5	2.3	10.0	6.1	7.5	8.0	1.7	5.7
	4000 (MQC)	2.7	1.2	8.0	4.7	5.3	7.8	5.5	6.3
	8000 (HQC)	5.0	2.5	9.0	5.8	6.0	9.4	7.8	7.8
NBUP	20.0 (LLOQ QC)	12.4	9.1	9.1	11.3	9.5	−0.5	13.5	7.5
	60.0 (LQC)	4.7	5.4	7.2	5.6	4.5	2.7	4.2	3.8
	600 (GMQC)	5.4	1.8	4.5	4.9	8.5	5.3	1.2	5.0
	3200 (MQC)	3.7	4.8	1.5	3.7	5.0	3.1	1.6	3.1
	6400 (HQC)	3.2	3.9	1.6	2.9	2.8	1.3	2.7	2.2

volume may cause insufficient vortexing, the volume ratio of sample and extraction was set to 1:4. Meanwhile, the sample pH was adjusted according to the pKa of the two analytes. Thirty microliter of 5 M NH_4OH is added for adjusting the pH value to keep most of the analytes in molecular state. Vigorous vortex was needed in order to get high recovery. Poor recovery of BUP was observed with insufficient vortex. After a series of method optimization on extraction solvent, sample pH and vortex mixing, a LLE treatment with simpler procedure and higher recovery was developed.

3.4. Method performance

Linearity was achieved in plasma over the concentration ranges of 25.0–10,000 pg/mL for BUP and 20.0–8000 pg/mL for NBUP. Calibration curve parameters (including slope and intercept of the calibration function $y=ax+b$) showed high reproducibility with $r^2 > 0.99$. The assay LLOQ was determined to be 25.0 pg/mL for BUP and 20.0 pg/mL for NBUP.

For BUP, intra-batch precision ranged from 1.2 to 10.5% and inter-batch precision ranged from 4.7 to 10.1%. For NBUP, intra-batch precision ranged from 1.5 to 12.4% and inter-batch precision ranged from 2.9 to 11.3%. For all target concentrations, accuracy ranged from −6.4 to 9.4% for BUP and −0.5 to 13.5% for NBUP. The accuracy and precision results of the method, presented in Table 3, were acceptable.

The analytical method was demonstrated specific for BUP and NBUP. Under described conditions, no interference with any extractable endogenous compound in plasma was observed. The interference from IS to the corresponding analyte was within 0.5% of the LLOQ concentration calibration standards response. There was no significant interference from one analyte to the other one. Method selectivity was also demonstrated: BUP and NBUP were quantified with 3.0% and −2.5% of its nominal concentration in LQC samples with addition of 13 potentially co-administered medications.

BUP and NBUP were quantified within −8.4 and 8.8% and −2.0 and 3.0% of the nominal concentration in DQC samples respectively, confirming dilution integrity.

Higher and consistent recoveries using this extraction method were obtained, with 81.8–88.8% for BUP and 77.0–84.6% for NBUP at LQC, MQC and HQC levels, respectively.

Table 4 Absolute recoveries and matrix effect of buprenorphine and norbuprenorphine in human plasma.

Analyte	Nominal concentration (pg/mL)	Recovery (n=6)		Matrix effect (n=9)	
		Mean value (%)	CV (%)	Mean value (%)	CV (%)
BUP	75.0	88.8	4.4	97.4	3.6
	4000	81.8	1.0	96.2	2.7
	8000	83.2	2.5	95.6	1.6
NBUP	60.0	84.6	5.8	95.8	3.0
	3200	77.0	4.1	94.0	3.9
	6400	83.9	2.2	96.9	1.9

Matrix effect was 95.6–97.4% for BUP and 94.0–96.9% for NBUP, indicating that the plasma extract did not cause significant ionization suppression or enhancement for both analytes in different lots of plasma. Results shown in Table 4 indicate that recovery and matrix effect were not concentration dependent.

Analytes were stable after five freeze-thaw cycles in plasma samples, when stored at room temperature (25 °C) for 24.5 h and stored in freezer ($\leq$−15 °C) for 67 days. Both BUP and NBUP were demonstrated good short-term stability in the autosampler (8 °C), with precision within 5.7% after 72 h. Detail stability data are shown in Table 5.

4. Conclusion

The LLE procedure gives a high recovery and is simple to apply. Efficient chromatography separation can be achieved in a shorter run time. Modified monitoring ion transitions improve the sensitivity and specificity of this method. Furthermore, given the sensitivity and the relatively small sample volume (200 μL human plasma), the present assay is also feasible and has been applied in bioequivalence study to support abbreviated new drug application submitted to FDA.

Table 5 Matrix stability of buprenorphine and norbuprenorphine under different conditions.

Stability (n=6)	BUP				NBUP			
	Nominal conc. (pg/mL)	Mean conc. (pg/mL)	Bias (%)	CV (%)	Nominal conc. (pg/mL)	Mean conc. (pg/mL)	Bias (%)	CV (%)
RT stability[a]	75.0	78.2	4.3	3.5	60.0	64.0	6.7	4.4
	8000	8700	8.8	3.0	6400	6630	3.6	2.8
Freeze-thaw stability[b]	75.0	79.1	5.5	4.8	60.0	57.9	−3.5	8.2
	8000	8390	4.9	3.0	6400	6640	3.8	2.2
Long term stability[c]	75.0	75.2	0.3	4.1	60.0	63.2	5.3	6.1
	8000	8160	2.0	1.7	6400	6890	7.7	3.7
Autosampler stability[d]	75.0	80.0	6.7	3.9	60.0	61.0	1.7	5.7
	8000	8500	6.3	3.2	6400	6570	2.7	2.1

[a]Room temperature (RT) for 24 h in polypropylene tube.
[b]Five freeze-thaw cycles (5 F/T).
[c]Long term stability ($\leq -15\,^{\circ}C$) for 67 days in polypropylene tube.
[d]Autosampler stability (8 °C) for 72 h in polypropylene plates.

Overall, the method presented here allows the rapid, selective, and sensitive quantification of BUP and its active metabolite NBUP, and it is ideally suited towards high-throughput samples analysis.

References

[1] D.E. Moody, J.D. Laycock, A.C. Spanbauer, et al., Determination of buprenorphine in human plasma by gas chromatography-positive ion chemical ionization mass spectrometry and liquid chromatography-tandem mass spectrometry, J. Anal. Toxicol. 21 (6) (1997) 406–414.

[2] Drug Addiction Treatment Act of 2000, USA, 2000, 111.

[3] E.J. Cone, C.W. Gorodetzky, D. Yousefnejad, et al., The metabolism and excretion of buprenorphine in humans, Drug Metab. Distrib. 12 (5) (1984) 577–581.

[4] M.L. Jensen, D. Foster, R. Upton, et al., Comparison of cerebral pharmacokinetics of buprenorphine and norbuprenorphine in an in vivo sheep model, Xenobiotica 37 (4) (2007) 441–457.

[5] H.G. Kress, Clinical update on the pharmacology, efficacy and safety of transdermal buprenorphine, Eur. J. Pain 13 (3) (2008) 219–230.

[6] R. Terlinden, T. Stadler, Pharmacokinetic study on single application of buprenorphine transdermal system (TDS), in: Proceedings of the Third EFIC Congress "Pain in Europe III, 2000: Advances in Pain Research and Therapy", Nice, France, 2000.

[7] S. Hegstad, H.Z. Khiabani, E.L. Øiestad, et al., Rapid quantification of buprenorphine-glucuronide and norbuprenorphine-glucuronide in human urine by LC-MS-MS, J. Anal. Toxicol. 31 (4) (2007) 214–219.

[8] R. Kronstrand, T.G. Seldén, M. Josefsson, Analysis of buprenorphine, norbuprenorphine, and their glucuronides in urine by liquid chromatography-mass spectrometry, J. Anal. Toxicol. 27 (7) (2003) 464–470.

[9] W. Huang, D.E. Moody, E.F. McCance-Katz, The in vivo glucuronidation of buprenorphine and norbuprenorphine determined by liquid chromatography-electrospray ionization-tandem mass spectrometry, Ther. Drug Monit. 28 (2) (2006) 245–251.

[10] A. Tracqui, P. Kintz, P. Mangin, HPLC/MS determination of buprenorphine and norbuprenorphine in biological fluids and hair samples, J. Forensic Sci. 42 (1) (1997) 111–114.

[11] D. Favretto, G. Frison, S. Vogliardi, et al., Potentials of ion trap collisional spectrometry for liquid chromatography/electrospray ionization tandem mass spectrometry determination of buprenorphine and nor-buprenorphine in urine, blood and hair samples, Rapid Commun. Mass Spectrom. 20 (8) (2006) 1257–1265.

[12] A.C. Liu, T.Y. Lin, L.W. Su, et al., Online solid-phase extraction liquid chromatography-electrospray-tandem mass spectrometry analysis of buprenorphine and three metabolites in human urine, Talanta 75 (1) (2008) 198–204.

[13] M.J. Hull, M.F. Bierer, D.A. Griggs, et al., Urinary buprenorphine concentrations in patients treated with suboxone as determined by liquid chromatography-mass spectrometry and CEDIA immunoassay, J. Anal. Toxicol. 32 (7) (2008) 516–521.

[14] S.L. Kacinko, M. Concheiro-Guisan, D.M. Shakleya, et al., Development and validation of a liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of buprenorphine, norbuprenorphine, and metabolites in human urine, Anal. Bioanal. Chem. 392 (5) (2008) 903–911.

[15] H. Hoja, P. Marquet, B. Verneuil, et al., Buprenorphine and norbuprenorphine determination in urine and serum by liquid chromatography-mass spectrometry with electrospray-type ionization, Analusis 24 (4) (1996) 104–107.

[16] H.F. Yue, M.R. Borensteinb, S.A. Jansena, et al., Liquid chromatography-mass spectrometric analysis of buprenorphine and its N-dealkylated metabolite norbuprenorphine in rat brain tissue and plasma, J. Pharmacol. Toxicol. Methods 52 (3) (2005) 314–322.

[17] A. Ceccato, R. Klinkenberg, P. Hubert, et al., Sensitive determination of buprenorphine and its N-dealkylated metabolite norbuprenorphine in human plasma by liquid chromatography coupled to tandem mass spectrometry, J. Pharm. Biomed. Anal. 32 (4–5) (2003) 619–631.

[18] S. Pirnay, F. Hervé, S. Bouchonnet, et al., Liquid chromatographic-electrospray ionization mass spectrometric quantitative analysis of buprenorphine, norbuprenorphine, nordiazepam and oxazepam in rat plasma, J. Pharm. Biomed. Anal. 41 (4) (2006) 1135–1145.

[19] M. Concheiro, D.M. Shakleya, M.A. Huestis, Simultaneous quantification of buprenorphine, norbuprenorphine, buprenorphine-glucuronide and norbuprenorphine-glucuronide in human umbilical cord by liquid chromatography tandem mass spectrometry, Forensic Sci. Int. 188 (1–3) (2009) 144–151.

[20] M. Concheiro, H. Jones, R.E. Johnson, et al., Confirmatory analysis of buprenorphine, norbuprenorphine, and glucuronide metabolites in plasma by LCMSMS. Application to umbilical

cord plasma from buprenorphine-maintained pregnant women, J. Chromatogr. B 878 (1) (2010) 13–20.

[21] A. Polettini, M.A. Huestis, Simultaneous determination of buprenorphine, norbuprenorphine, and buprenorphine-glucuronide in plasma by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Biomed. Sci. Appl. 754 (2) (2001) 447–459.

[22] M.E. Rodriguez-Rosas, M.R. Lofwall, E.C. Strain, et al., Simultaneous determination of buprenorphine, norbuprenorphine and the enantiomers of methadone and its metabolite (EDDP) in human plasma by liquid chromatography/mass spectrometry, J. Chromatogr. B 850 (1–2) (2007) 538–543.

[23] Q. Guo, Z. Du, Development of a rapid and simultaneous detection method for buprenorphine, norbuprenorphine and naloxone in human plasma using ultra-high performance liquid chromatography-tandem mass spectrometer with solid-phase extraction, Chin. J. Chem. 29 (9) (2011) 1922–1926.

[24] T.Y. Chiang, L.H. Pao, C.H. Hsiong, et al., Simultaneous determination of buprenorphine, norbuprenorphine and naloxone in human plasma by LC-MS-MS, Chromatographia 74 (7–8) (2011) 575–583.

[25] H. Hoja, P. Marquet, B. Verneuil, et al., Determination of buprenorphine and norbuprenorphine in whole blood by liquid chromatography-mass spectrometry, J. Anal. Toxicol. 21 (2) (1997) 160–165.

[26] R.S. Goodwin, D.G. Wilkins, O. Averin, et al., Buprenorphine and norbuprenorphine in hair of pregnant women and their infants after controlled buprenorphine administration, Clin. Chem. 53 (12) (2007) 2136–2143.

[27] D.G. Wilkins, D.E. Rollins, A.S. Valdez, et al., A retrospective study of buprenorphine and norbuprenorphine in human hair after multiple doses, J. Anal. Toxicol. 23 (6) (1999) 409–415.

[28] D. Thieme, H. Sachs, M. Thevis, Formation of the N-methylpyridinium derivative to improve the detection of buprenorphine by liquid chromatography-mass spectrometry, J. Mass Spectrom. 43 (7) (2008) 974–979.

[29] P. Kintz, A. Tracqui, P. Mangin, et al., Sweat testing in opioid users with a sweat patch, J. Anal. Toxicol. 20 (6) (1996) 393–397.

[30] S.L. Kacinko, D.M. Shakleya, M.A. Huestis, Validation and application of a method for the determination of buprenorphine, norbuprenorphine, and their glucuronide conjugates in human meconium, Anal. Chem. 80 (1) (2008) 246–252.

[31] D. Grimm, E. Pauly, J. Pöschl, et al., Buprenorphine and norbuprenorphine concentrations in human breast milk samples determined by liquid chromatography-tandem mass spectrometry, Ther. Drug Monit. 27 (4) (2005) 526–530.

[32] Guidance for Industry on Bioanalytical Method Validation, Food and Drug Administration of the United States, USA, 2001.