Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 538–543
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Simultaneous determination of buprenorphine, norbuprenorphine and the enantiomers of methadone and its metabolite (EDDP) in human plasma by liquid chromatography/mass spectrometry

Maria Esther Rodriguez-Rosas [a,*], Michelle R. Lofwall [b], Eric C. Strain [c], Danuta Siluk [a,d], Irving W. Wainer [a]

[a] *Bioanalytical and Drug Discovery Unit, Gerontology Research Center, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224-6825, USA*
[b] *University of Kentucky College of Medicine, Lexington, KY, USA*
[c] *Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[d] *Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Poland*

Received 7 July 2006; accepted 17 November 2006
Available online 1 December 2006

Abstract

A previously reported enantioselective LC–MS assay for the determination of (*R*)- and (*S*)-methadone [Met] and (*R*)- and (*S*)-2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine [EDDP] (the primary metabolite of Met) has been adapted for use in the simultaneous determination of the plasma concentrations of Met, EDDP, buprenorphine (Bu) and norbuprenorphine (norBu). All of the target compounds were separated within 15 min using an α_1-acid glycoprotein chiral stationary phase, a mobile phase composed of acetonitrile: ammonium acetate buffer [10 mM, pH 7.0] in a ratio of 18:82 (v/v), a flow rate of 0.9 ml/min at 25 °C. Deuterium labeled compounds were used as internal standards [d_4-Bu, d_3-norBu, (*R*,*S*)-d_3-Met and (*R*,*S*)-d_3-EDDP] and linear relationships between peak height ratios and drug concentrations were obtained for Bu and norBu in the range 0.2–12 ng/ml with correlation coefficients greater than 0.999. The relative standard deviations (%R.S.D.) for the intra- and inter-day precision of the method were <4.5% and for accuracy was <4.0%. The method was validated and used to analyze plasma samples obtained from opioid dependent methadone-maintained adults enrolled in a research study.

Keywords: Buprenorphine; Norbuprenorphine; (*S*)-methadone; (*R*)-methadone; (*S*)-EDDP; (*R*)-EDDP; Chiral chromatography; AGP chiral stationary phase; LC–MS

1. Introduction

Buprenorphine [Bu] (Fig. 1), 21-cyclopropyl-7-α-[(*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endoethano-6,7,8,14-tetrahydroororipavine, is a semi-synthetic oripavine derivative which is 25–50 times more potent than morphine [1]. In humans, Bu is *N*-dealkylated by cytochrome P450 3A4 (CYP 3A4) to form norbuprenorphine [norBu] (Fig. 1) [2].

Numerous methods have been published for the determination of Bu and norBu in biological matrices including immunoassays [3–6]; gas chromatography with electron capture detection (GC–ECD) [7,8]; liquid chromatography (LC) with electrochemical detection [9–11]; LC with fluorescence detection [12]; a variety of mass spectrometric (MS) methods which includes GC–MS [13–16], LC–MS [17–20] and LC–MS/MS [21–25].

Recently, Bu has been investigated as an alternative to methadone (Met) maintenance treatment for heroin addiction [26–28]. Bu is a partial agonist at the μ opioid receptor with antagonist effects at the δ and κ opioid receptors [29]. The observed agonist and antagonist effects of this agent can vary as a function of the administered Bu dose, as well as the level of physical dependence. In order to investigate the relationship between these two factors, a clinical study has been initiated to examine the effect of increasing doses of Bu on patients maintained on Met. Part of this study involves the determination of the pharmacokinetic profiles of Bu and as well as the Met and its primary metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenyl-

* Corresponding author. Tel.: +1 410 558 8255; fax: +1 410 558 8409.
E-mail address: rdrgzrss@yahoo.com (M.E. Rodriguez-Rosas).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.025

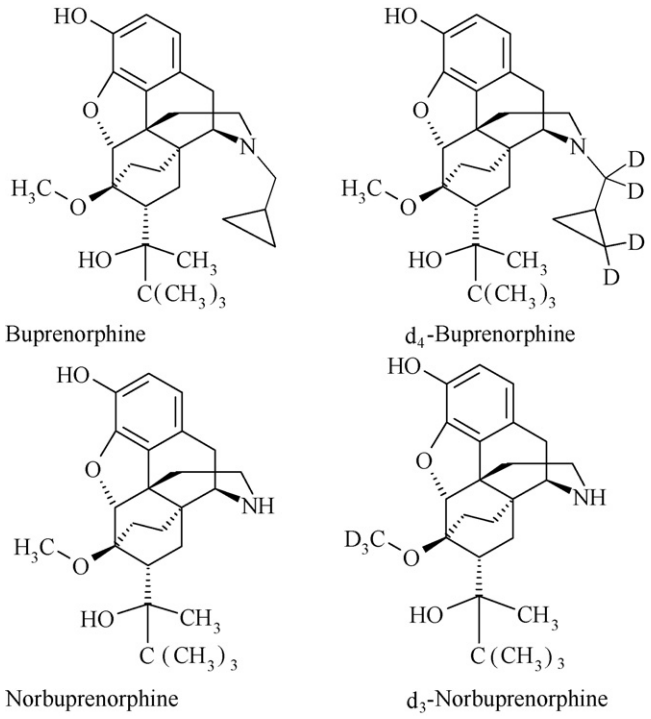

Fig. 1. The chemical structures of buprenorphine, d_4-buprenorphine, norbuprenorphine and d_3-norbuprenorphine.

pyrrolidine (EDDP). This paper describes the development and validation of an assay for the simultaneous determination of these compounds.

This paper describes the adaptation of previously reported enantioselective LC–MS assays for the determination of Met and EDDP enantiomers in saliva [30] and human plasma [31] for the simultaneous determination of Met, EDDP, Bu and norBu in human plasma. The resulting assay has greater sensitivity than previously reported methods, LLOQs for Bu and norBu were 0.2 ng/ml, and the run time was less than 15 min. The method is reproducible and accurate and has been applied to the analysis of plasma samples from participants in the clinical study.

2. Experimental

2.1. Chemicals, reagents and apparatus

Buprenorphine [Bu] (100 μg/ml methanol solution); norbuprenorphine [norBu] (100 μg/ml methanol solution); deuterium labeled-d_4-buprenorphine [d_4-Bu] (100 μg/ml methanol solution); deuterium labeled-d_3-norbuprenorphine [d_3-NorBu] (100 μg/ml methanol solution) were purchased from Cerilliant Corporation (Austin, TX, USA). Pooled drug-free human plasma obtained using sodium fluoride as anticoagulant was purchased from Valley Biomedical Inc. (Winchester, VA, USA). The sources of all of the other chemicals, reagents and apparatus were previously reported [31].

2.2. Chromatographic conditions

Analyses of Bu, norBu, d_4-Bu, d_3-norBu and enantioselective analyses of (*R*)- and (*S*)-Met, (*R*)- and (*S*)-EDDP, (*R*)- and (*S*)-d_3-Met and (*R*)- and (*S*)-d_3-EDDP were accomplished using a chiral stationary phase based upon immobilized α_1-acid glycoprotein (Chiral-AGP) from Advanced Separation Technologies (Whippany, NJ, USA). A Chiral-AGP guard column (10 mm × 2.0 mm i.d., 5 μm) and a chiral-AGP analytical column (100 mm × 4.0 mm i.d., 5 μm) were used in series. The mobile phase consisted of acetonitrile: ammonium acetate buffer [10 mM, pH 7.0 (adjusted with 0.5% aqueous ammonium hydroxide)], 18:82 (v/v). The flow rate was 0.9 ml/min, the injection volume was 20 μl, and the column temperature was maintained at 25 °C as indicated in our previously reported method [31].

2.3. Optimization of the mass selective detector (MSD) parameters

Mass spectra were recorded using a full scan in positive ion mode, with a scan range from *m/z* 100 to 600. Single ion monitoring (SIM) was used to quantify the target compounds. The chromatograms were monitored at *m/z* = 468.3 (Bu), *m/z* = 414.3 (norBu), *m/z* = 310.20 (Met), *m/z* = 278.20 (EDDP), *m/z* = 472.3 (d_4-Bu), *m/z* = 417.3 (d_3-norBu), *m/z* = 313.20 (d_3-Met) and *m/z* = 281.20 (d_3-EDDP). The procedure for the investigation of the optimal value for each MSD parameter was previously reported [31].

2.4. Preparation of stock solutions

Concentrated stock solutions of Bu [4.0 μg/ml as free base], norBu [4.0 μg/ml as free base], d_4-Bu [10 μg/ml as free base] and d_3-norBu [10 μg/ml as free base] were prepared in methanol, placed in capped polypropylene tubes, wrapped in aluminum foil and stored at −20 °C. Spiked standard solutions for the calibration curve and quality control samples (QCs) were made by serial dilutions with methanol starting with their respective concentrated stock solution. These spiked standards were placed in capped polypropylene tubes, wrapped in aluminum foil, and stored at 4 °C.

2.5. Preparation of calibration curve and quality control standards

The determinations of Bu and norBu were based on the internal standard method, using their respective deuterium labeled compounds as internal standards. Calibration and QC standards were prepared daily by spiking 50 μl of the corresponding spiked standard solutions to a 0.95 ml drug-free human plasma. Extraction was then performed as described in Section 2.6.2. In this way, 7-point calibration curves were prepared and ranged for Bu and norBu from 0.2 to 12 ng/ml (0.2, 1.0, 2.0, 4.0, 6.0, 10.0, 12.0 ng/ml) using constant concentrations of d_4-Bu [10.0 ng/ml] and d_3-norBu [10 ng/ml].

The linearity of each standard curve was determined using the "calibration settings" window in ChemStation (Rev A.10.01 [1635], 1990–2003, Hewlett-Packard) with the weighting function set at "Equal".

The QC standards used for Bu and norBu were low quality control, LQC = 2 ng/ml; medium quality control, MQC = 6 ng/ml; and high quality control, HQC = 12 ng/ml.

2.6. Sample preparation

2.6.1. Collection of plasma from patients

After obtaining informed consent plasma samples were collected from non-treatment seeking opioid dependent methadone-maintained adults enrolled in a research study #BPR00-03-10-01 entitled "The variability of agonist and antagonist effects as a function of level of physical dependence" at the Johns Hopkins Behavioral Pharmacology Research Unit (Baltimore, MD, USA).

Participants were stabilized on three different methadone dose levels (30, 60, and 90 mg by mouth once a day; randomly assigned order) for approximately 4-week periods at each level. While maintained at each methadone dose level, challenge sessions were conducted during which single intramuscular doses of placebo, naloxone, or Bu (0.5, 1, 2, and 4 mg in ascending order) were administered.

Plasma collection was scheduled as follows: during each four hour session eight blood samples (7 ml each) were collected using an intravenous catheter placed in the participants' non-dominant arm at 15 min prior to drug injection and 10, 20, 30, 40, 60, 120, and 240 min after drug administration. Samples were collected in vacutainer tubes containing sodium fluoride. The tubes were inverted 8–10 times, centrifuged at 3000 × *g* for 10 min, and the plasma layer transferred into cryotubes, which were stored at −20 °C until analyzed.

2.6.2. Extraction procedure

A 50 μl aliquot of a solution containing the deuterated internal standards d_4-Bu, d_3-norBu, d_3-Met and d_3-EDDP was added to 950 μl of plasma in a microcentrifuge tube. The resulting mixture was extracted as previously described [31].

2.7. Validation

2.7.1. Matrix effect, recovery and process efficiency

The matrix effect (ME) and recovery (RE) were studied by analyzing quality control standards at three levels (LQC, MQC, HQC) using five different drug-free plasma pools. Three sets of samples were prepared: Set A consisted of a set of neat quality control standards injected directly into the LC–MSD. Set B consisted of a set of drug-free plasma samples extracted by SPE, then spiked with quality control standards and finally injected into the LC–MSD. Set C consisted of a set of drug-free plasma samples spiked with quality control standards then extracted by SPE and finally injected into the LC–MSD system.

Matrix effect (ME) was obtained by comparing the concentration of the analytes found in Set B with respect to Set A. The ME was calculated using Eq. (1) [32]:

$$\mathrm{ME} = \frac{\text{Set B}}{\text{Set A}} \times 100 \tag{1}$$

Recovery (RE) was obtained by comparing the concentration of the analytes found in Set C with respect to Set B. The RE was calculated using Eq. (2) [32]:

$$\mathrm{RE} = \frac{\text{Set C}}{\text{Set B}} \times 100 \tag{2}$$

Process efficiency (PE) was calculated using Eq. (3) [32]:

$$\mathrm{PE} = \frac{\mathrm{RE} \times \mathrm{ME}}{100} \tag{3}$$

Results are expressed as the average value and the confidence interval was determined by the evaluation of the relative standard deviation (%R.S.D.) of these experimental values. The formula was: %R.S.D = (standard deviation/average) × 100.

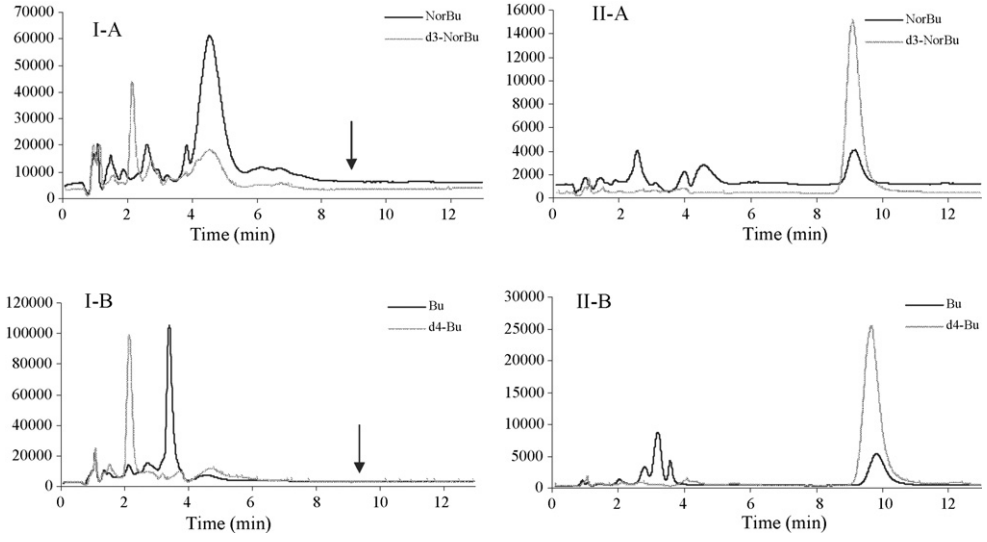

Fig. 2. Representative chromatograms of the blank human plasma. The chromatographic traces obtained using single ion monitoring at the following values: (I-A) at *m/z* = 414.30 (norBu) and at *m/z* = 417.30 (d_3-norBu); (I-B) at *m/z* = 468.30 (Bu) and at *m/z* = 472.30 (d_4-Bu). Representative chromatograms of the low quality control plasma sample (LQC) containing Bu [2 ng/ml]; d_4-Bu [10 ng/ml]; norBu [2 ng/ml]; d_3-norBu [10 ng/ml]. The chromatographic traces obtained using single ion monitoring at the following values: (II-A) at *m/z* = 414.30 (norBu) and at *m/z* = 417.30 (d_3-norBu); (II-B) at *m/z* = 468.30 (Bu) and at *m/z* = 472.30 (d_4-Bu).

Table 1
Results from the validation studies for buprenorphine and norbuprenorphine in human plasma

	Buprenorphine				Norbuprenorphine			
	LLOQ (0.2 ng/ml)	LQC (2 ng/ml)	MQC (6 ng/ml)	HQC (12 ng/ml)	LLOQ (0.2 ng/ml)	LQC (2 ng/ml)	MQC (6 ng/ml)	HQC (12 ng/ml)
Intra-day								
N	5	5	5	5	5	5	5	5
Average	0.2	2.0	5.7	12.3	0.2	2.0	6.0	11.8
S.D.	0.0	0.0	0.0	0.3	0.0	0.1	0.1	0.2
R.S.D. (%)	1.2	0.7	0.8	2.1	4.5	3.7	1.6	1.6
Inter-day								
N	7	15	15	15	7	15	15	15
Average	0.2	2.0	5.6	12.2	0.2	2.1	6.0	11.8
S.D.	0.0	0.0	0.1	0.3	0.0	0.1	0.2	0.5
R.S.D. (%)	1.5	1.9	1.0	2.3	3.9	3.4	3.0	4.0
Accuracy (%)	107.4	101.8	93.8	101.9	99.0	104.2	99.5	98.1

2.7.2. Intra- and inter-day validation studies

The intra- and inter-day validation studies for precision and accuracy were performed in quintuplicate with QC standards using five different plasma pools at concentrations specified in Section 2.5. The analyses were carried out over a period of 3 days for the inter-day validation. The curves were constructed by plotting the peak height ratio Bu/d_4-Bu or norBu/d_3-norBu against its concentration.

Accuracy was determined by comparing the observed concentrations of the QC standards (calculated from the calibration curve) to their nominal concentrations.

The specificity of the method for each analyte was examined by individually screening Bu, norBu, Met, EDDP, d_4-Bu, d_3-norBu, d_3-Met and, d_3-EDDP after spiking in pooled human plasma.

3. Results and discussion

3.1. Chromatographic conditions

The mobile phase composition for the validation and clinical studies was set at acetonitrile:ammonium acetate buffer [10 mM, pH 7.0], 18:82 (v/v) [31]. Under these conditions, the simultaneous analysis of the four drugs along with their internal standards was accomplished in 15 min. The observed retention time of norBu was 9.1 min (Fig. 2II-A) and for Bu was 9.8 min (Fig. 2II-B). In addition, the analysis of five different drug-free plasma pools at these *m*/*z* values detected no interfering peaks, representative chromatograms are presented in Fig. 2I-A and -B.

3.2. 3.2.Optimization of mass spectrometric detection

A full scan mass spectra of each compound injected individually was obtained. Based on their specific *m*/*z* value, the signals were monitored on eight separate channels and no overlaps were found. We also investigated the contribution and cross-talk of the unlabeled compounds into the internal standard channels and no contribution or cross-talk was observed.

The optimized parameters, based on the maximum signal for Bu and norBu, were: fragmentor, 60 V; drying gas flow rate, 11.0 l/min; nebulizer pressure, 25 psig; drying gas temperature, 350 °C and capillary voltage, 1000 V.

3.3. Linearity and detection limits

Calibration curves for Bu and norBu were generated by weighted ($1/x$) least squares linear regression. The linear relationships between peak height ratio and drug concentration in the range 0.2–12.0 ng/ml were described by the following equations, for Bu: $y = 1.1427x - 0.0376$, $r^2 = 0.9995$ and for norBu: $y = 1.0303x - 0.0239$, $r^2 = 0.9995$. The data were based on three replicates of a 7-point calibration curve.

The lower limit of quantification (LLOQ) is the concentration of the drug in the matrix that can be determined with a high percentage of accuracy (80–120%) [33]. LLOQs for Bu and norBu were 0.2 ng/ml. The limit of detection (LOD) at signal versus noise ratio (S/N) = 3, for Bu was 0.05 ng/ml and for norBu was 0.04 ng/ml. The sensitivity achieved as reported in this manuscript was better compared to the sensitivity reported by Murphy and coworkers using an LC/ESI–MS/MS method [23] where their LLOQs for Bu and norBu were 0.6 ng/ml and their LODs were 0.3 ng/ml for both compounds.

3.4. Matrix effect, recovery and process efficiency

The matrix effects were determined using Eq. (1) [32]. The results indicated that there were no significant effects at ME = 100.4 ± 2.8% (Bu) and ME = 98.7 ± 3.3% (norBu).

The plasma samples were extracted using the method previously described for the analysis of Met and EDDP [31]. The recoveries were determined using Eq. (2) [32], where the average recovery for Bu was 99.2 ± 2.3% and for norBu was 100.5 ± 4.9%.

The process efficiencies were calculated using Eq. (3) [32], where the average process efficiency for Bu was 99.6 ± 2.3% and for norBu was 99.0 ± 2.0%.

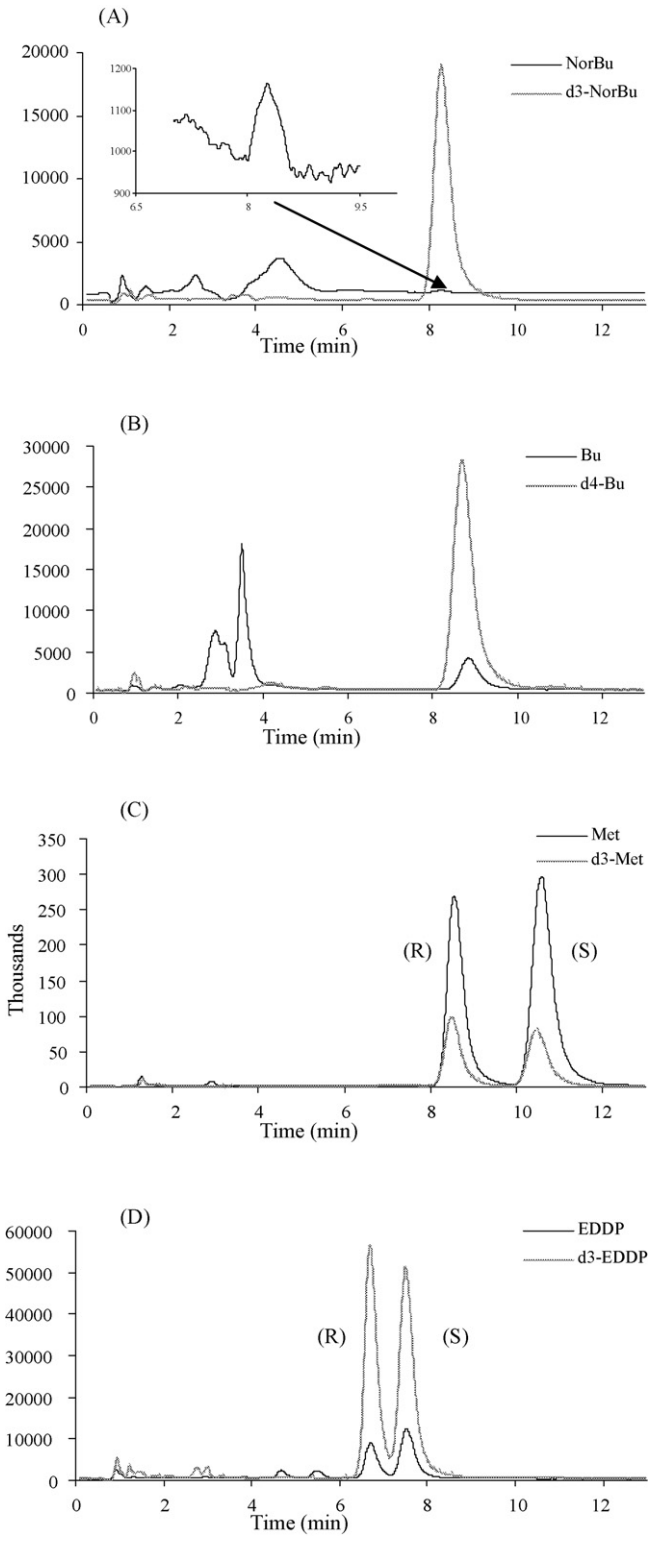

Fig. 3. Representative chromatograms of the analysis of a plasma sample obtained from a participant stabilized on methadone and 40 min after the intramuscular administration of a 4.0 mg dose of buprenorphine. The concentrations measured were: Bu = 1.5 ng/ml; norBu = 0.3 ng/ml; (*R*)-Met = 33.3 ng/ml; (*S*)-Met = 45.0 ng/ml; (*R*)-EDDP = 1.9 ng/ml; (*S*)-EDDP = 2.8 ng/ml. The chromatographic traces obtained using single ion monitoring at the following values: (A) at $m/z = 414.30$ (norBu) and at $m/z = 417.30$ (d_3-norBu); (B) at $m/z = 468.30$ (Bu) and at $m/z = 472.30$ (d_4-Bu); (C) at $m/z = 310.20$ ((*R*,*S*)-methadone) and at $m/z = 313.20$ ((*R*,*S*)-d_3-methadone) and (D) at $m/z = 278.20$ ((*R*,*S*)-EDDP) and at $m/z = 281.20$ ((*R*,*S*)-d_3-EDDP).

3.5. Accuracy and precision

Accuracy and precision of the method for Bu and norBu were evaluated from quintuplicate analysis of each QC standard level (LQC, MQC and HQC) and repeated for 3 days. The calculated average accuracy was $99.2 \pm 2.3\%$ for Bu and $100.6 \pm 4.0\%$ for norBu, Table 1. The intra- and inter-day precision of the method were determined as relative standard deviation (%R.S.D.). The results were $\leq 2.3\%$ for Bu and $\leq 4.5\%$ for norBu. The results of the validation studies in Table 1 demonstrate that the method has acceptable accuracy and precision.

3.6. Stability studies

The Bu and norBu standards were frozen at −20 °C for 2 weeks, defrosted and analyzed. There was no observable degradation of either analyte.

Stability of Bu and norBu were determined after three freeze and thaw cycles. The spiked plasma samples at three quality control levels (LQC, MQC and HQC) were stored at −20 °C for 24 h and thawed unassisted at room temperature ($n = 3$). When completely thawed, the samples were refrozen two more times, then analyzed. There was no observable degradation of either analyte. The LQCs, MQCs and HQCs for Bu and norBu were placed in a thermostated autosampler at 4 °C and assayed at 0, 8, 12, 16, 20 and 24 h. There was no observable degradation of either analyte during this period.

3.7. Application to clinical samples

The validated method was applied to the analysis of plasma samples obtained from patients who had been stabilized on Met

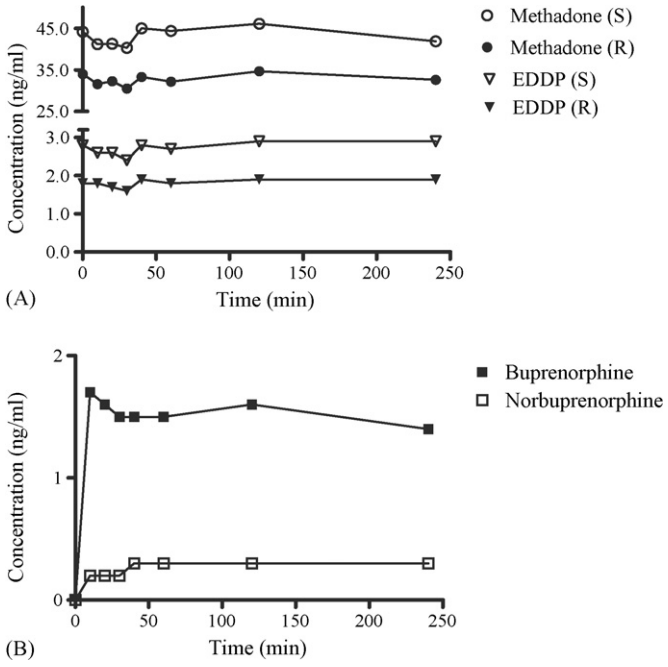

Fig. 4. Plasma drug concentration-time profile obtained after intramuscular administration of 4.0 mg Bu to a participant. Where (A) the results for Met (*S*), Met (*R*), EDDP (*S*), and EDDP (*R*); (B) the results for Bu and norBu.

and who then participated in a series of challenge sessions in which intramuscular placebo, naloxone, and ascending doses of Bu (0.5, 1, 2, and 4 mg) were administered.

Representative chromatograms of the analysis of a plasma sample from a participant stabilized on Met obtained 40 min after the intramuscular (i.m.) administration of a 4.0 mg dose of Bu are presented in Fig. 3. The concentrations measured were: Bu = 1.5 ng/ml; norBu = 0.3 ng/ml; (*R*)-Met = 33.3 ng/ml; (*S*)-Met = 45.0 ng/ml; (*R*)-EDDP = 1.9 ng/ml; (*S*)-EDDP = 2.8 ng/ml. The plasma drug concentration–time profile from 0 to 240 min after the i.m. administration of 4.0 mg Bu to this participant is presented in Fig. 4.

4. Conclusions

The bioanalytical assay reported in this manuscript is a simple, sensitive, accurate, rapid and reproducible LC/MS method for the simultaneous determination of Bu, norBu and the enantiomers of Met and EDDP in human plasma obtained from opioid dependent methadone-maintained adults. The method has been validated and is being applied to the analyses of samples from a methadone-maintenance program.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, National Institute on Aging and grants DA08045 and DA00332 from the National Institute on Drug Abuse. M.E.R.R. gratefully thanks Dr. J.T. Lee from Advanced Separation Technologies, Inc. and Mr. Philip Kim from Waters Corporation for their helpful suggestions and technical expertise.

References

[1] S.L. Walsh, K.L. Preston, G.E. Bigelow, M.L. Stitzer, J. Pharmacol. Exp. Ther. 274 (1995) 361.
[2] K. Kobayashi, T. Yamamoto, K. Chiba, M. Tani, N. Shimada, T. Ishizaki, Y. Kuroiwa, Drug Metab. Dispos. 26 (1998) 818.
[3] V. Cirimele, P. Kintz, S. Lohner, B. Ludes, J. Anal. Toxicol. 27 (March (2)) (2003) 103.
[4] N. De Giovanni, N. Fucci, S. Scarlata, G. Donzelli, Clin. Chem. Lab. Med. 43 (12) (2005) 1377.
[5] C.W. Hand, D. Baldwin, R.A. Moore, M.C. Allen, H.J. McQuay, Ann. Clin. Biochem. 23 (1986) 47.
[6] M. Bottcher, O. Beck, J. Anal. Toxicol. 29 (November–December (8)) (2005) 769.
[7] E.J. Cone, C.W. Gorodetzky, D. Yousefnejad, W.D. Darwin, J. Chromatogr. 337 (1985) 291.
[8] E.T. Everhart, P. Cheung, P. Shwonek, K. Zabel, E.C. Tisdale, P. Jacob, J. Mendelson, R.T. Jones, Clin. Chem. 43 (1997) 2292.
[9] L. Debrabandere, M. Van Boven, P. Daenens, J. Forensic Sci. 37 (1991) 82.
[10] E. Schleyer, R. Lohmann, C. Rolf, A. Gralow, C.C. Kaufmann, M. Unterhalt, W. Hiddemann, J. Chromatogr. 614 (1993) 275.
[11] A. Salem, T.L. Pierce, W. Hope, J. Pharmacol. Toxicol. Meth. 37 (1997) 75.
[12] S.Y. Liu, K.S. Liu, C.H. Kuei, J.I. Tzeng, S.T. Ho, J.J. Wang, J. Chromatogr. B 818 (April (2)) (2005) 233.
[13] M. Ohtani, F. Shibuya, H. Kotaki, K. Uchino, Y. Saitoh, F. Nakagawa, J. Chromatogr. 487 (1989) 469.
[14] S. George, C. George, M. Chauhan, Forensic Sci. Int. 143 (2004) 121.
[15] M.A. Elsohly, W. Gul, S. Feng, T.P. Murphy, J. Anal. Toxicol. 29 (September) (2005) 570.
[16] S. Pirnay, S. Bouchonnet, F. Herve, D. Libong, N. Milan, P. D'Athis, F. Baud, I. Ricordel, J. Chromatogr. B 807 (August (2)) (2004) 335.
[17] S. Kintz, Clin. Biochem. 35 (October (7)) (2002) 513.
[18] M.J. Bogusz, J. Chromatogr. B 748 (October (1)) (2000) 3.
[19] H. Hoja, P. Marquet, B. Verneuil, H. Lotfi, J.L. Dupuy, G. Lachatre, J. Anal. Toxicol. 21 (March–April (2)) (1997) 160.
[20] A. Tracqui, P. Kintz, P. Mangin, J. Forensic Sci. 42 (January (1)) (1997) 111.
[21] D.E. Moody, M.H. Slawson, E.C. Strain, J.D. Laycock, A.C. Spanbauer, R.L. Foltz, Anal. Biochem. 306 (2002) 31.
[22] A. Polettini, M.A. Huestis, J. Chromatogr. B 754 (2001) 447.
[23] C.M. Murphy, M.A. Huestis, J. Mass Spectrom. 40 (January (1)) (2005) 70.
[24] A. Ceccato, R. Klinkenberg, P. Hubert, B. Streel, J. Pharm. Biomed. Anal. 32 (August (4–5)) (2003) 619.
[25] D. Favretto, G. Frison, S. Vogliardi, S.D. Ferrara, Rapid Commun. Mass Spectrom. 20 (8) (2006) 1257.
[26] R.S. Schottenfeld, M.C. Chawarski, J.R. Pakes, M.V. Pantalon, K.M. Caroll, T.R. Kosten, Am. J. Psychiatry 162 (February (2)) (2005) 340.
[27] M.K. Greenwald, C.E. Johanson, D.E. Moody, J.H. Woods, M.R. Kilbourn, R.A. Koeppe, C.R. Schuster, J.K. Zubieta, Neuropsychopharmacology 28 (November (11)) (2003) 2000.
[28] R. Ortner, R. Jagsch, S.D. Schindler, A. Primorac, G. Fischer, Eur. Addict. Res. 10 (2004) 105.
[29] G. Gonzalez, A. Oliveto, T.R. Kosten, Curr. Opin. Pharmacother. 5 (2004) 713.
[30] M.E. Rodriguez-Rosas, K.L. Preston, D.H. Epstein, E.T. Moolchan, I.W. Wainer, J. Chromatogr. B 796 (2003) 355.
[31] M.E. Rodriguez-Rosas, J.G. Medrano, D.H. Epstein, E.T. Moolchan, K.L. Preston, I.W. Wainer, J. Chromatogr. A 1073 (2005) 237.
[32] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[33] Guidance for Industry. Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, May 2001.