TECHNICAL NOTE

Antoine Tracqui,[1] *M.D., Ph.D.; Pascal Kintz,*[1] *Ph.D.; and Patrice Mangin,*[1] *M.D., Ph.D.*

HPLC/MS Determination of Buprenorphine and Norbuprenorphine in Biological Fluids and Hair Samples

REFERENCE: Tracqui A, Kintz P, Mangin P. HPLC/MS determination of buprenorphine and norbuprenorphine in biological fluids and hair samples. J Forensic Sci 1997;42(1):111–4.

ABSTRACT: An original method, based upon HPLC (high performance liquid chromatography)/Ionspray-MS, has been developed for the identification of buprenorphine (BUP) and norbuprenorphine (norBUP) in biological fluids and hair samples. Biological fluids (2 mL) are extracted at pH 8.4 by $CHCl_3$/2-propanol/*n*-heptane (25:10:65, v/v) after addition of deuterated BUP (BUP-d4, 10 ng). Hair samples (40 mg) are extracted in the same conditions after decontamination by CH_2Cl_2, mechanical pulverization, addition of BUP-d4 (1 ng), acidic incubation (1 mL 0.1 N HCl, 56°C overnight), then neutralization by NaOH. Analytes are separated on a 4-μm NovaPak C18 (Waters) column (150 by 2.0 mm, ID) with a mobile phase of acetonitrile/2 mM NH_4COOH buffer, pH 3.0 (80:20, v/v; flow rate 200 μL/min; post column split 1:3). Detection is done by a Perkin-Elmer Sciex API-100 mass analyzer equipped with an ISP interface (nebulizing and curtain gas:99.95-% N_2; main settings: orifice + 50 V, electron multiplier + 2400 V). The mean retention times for BUP, BUP-d4, and norBUP are 5.84, 5.79, and 4.42 min, respectively. For all compounds, mass spectra exhibit a unique, protonated molecular ion [M + H]+ at *m/z* 414 (norBUP), 468 (BUP), and 472 (BUP-d4), without any significant fragmentation. The lower limits of detection are 0.10 and 0.05 ng/mL blood, and 4 and 2 pg/mg hair for BUP and norBUP, respectively. BUP and norBUP concentrations measured in hair from six addicts under substitutive therapy by BUP ranged from 4 to 140 pg/mg, and from nondetected to 67 pg/mg, respectively. The good performances of this method in terms of both sensitivity and specificity make it a convenient alternative to HPLC/coulometry and GC/MS for the separate analysis of BUP and norBUP in biological samples.

KEYWORDS: forensic science, buprenorphine, norbuprenorphine, high performance liquid chromatography (HPLC), mass spectrometry, forensic toxicology, hair

Buprenorphine (BUP) is an hemi-synthetic opioid derivative, closely related to morphine and congener alkaloids (Fig. 1), which is obtained from thebaine after a 7-step chemical procedure. BUP is a powerful analgesic (25 to 40 times more potent than morphine) that exhibits both partial agonist activity at the μ-opiate receptor and antagonist activity at the κ-opiate receptor (1,2). This drug has been initially developed for the treatment of acute and chronic pain, especially of surgical or neoplastic origin. Its main advantages over morphine are a poor respiratory depressant activity and a lack of significant withdrawal symptoms, however, it presents some addiction potential and cases of abuse have been reported in France and other countries. BUP is available in France (Temgesic®) under two forms, one injectable (0.3 and 0.6 mg, 1987) for hospital use only, and one sublingual (0.2 mg, 1990); usual therapeutic doses are 0.3 to 0.6 mg parenterally or 0.2 to 0.4 mg sublingually, every 6 to 8 h.

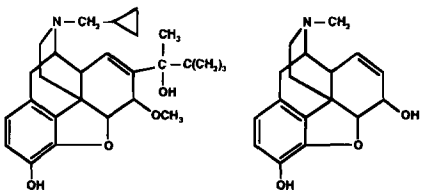

FIG. 1—*Chemical structures of buprenorphine and morphine.*

Suggestions have also been made for the use of BUP in the management of heroin addicts (3,4), and very recently (February 1996) a specific sublingual formulation (Subutex®) got the approval of the French Ministry of Health to be delivered to addicts under physician's prescription, as an alternative to the methadone substitution previously organized in detoxication centers.

As a consequence, it has become necessary for every forensic laboratory to be able to assay BUP in biological samples; this determination is, however, difficult, due to: 1) the very low therapeutic plasma levels (peak plasma concentrations of 0.45 to 0.84 ng/mL after a single sublingual administration of 0.4 mg; steady-state concentrations in the range 0.1 to 8.0 ng/mL (1)), 2) the existence of a N-dealkylated active metabolite, norbuprenorphine (norBUP), that requires to be separately quantified.

Among numerous procedures, the coupling of HPLC to mass spectrometry has been recently presented in some preliminary reports as a promising tool for this analytical challenge (5–7). This paper describes an improved procedure based upon HPLC hyphenated to ionspray-mass spectrometry (HPLC/ISP-MS) for the sensitive and specific determination of BUP and norBUP in various biological materials including hair.

[1]Chief toxicologist and medical examiner, chief toxicologist, chief medical examiner and director of the Institute, respectively, Institut de Médecine Légale, 11 rue Humann, 67085, Strasbourg Cedex, France.

Received 25 March 1996; accepted 10 May 1996.

Material and Methods

Liquid Chromatography

The HPLC separations were performed at ambient temperature on a 4-μm NovaPak (Waters) C18 column (150 by 2.0 mm, ID) protected by a 5-μm Opti-Guard™ (Interchim) C18 guard cartridge (15 by 1.0 mm ID). The mobile phase was acetonitrile/2 mM NH_4COOH buffer, pH 3.0 (80:20, v/v). A 20-mL, dual syringe HPLC pump (Applied Biosystems 140 B) was used to deliver a continuous flow of 200 μL/min. A post column split of 1:3 was used to reduce at 50 μL/min the flow rate infused into the HPLC/MS interface.

Mass Spectrometry

The MS detection was carried out on a Perkin-Elmer Sciex API-100 apparatus equipped with an Ionspray™ (= pneumatically assisted electrospray) interface. Nitrogen (99.95%, 40 psi) was used as both the nebulizing gas (flow rate 1.16 L/min) and the curtain gas (flow rate 1.08 L/min) that prevents solvent vapors and solid contaminents from entering the vacuum chamber. The ion sampling orifice was held at a potential of + 50 V, and the electron multiplier at + 2400 V. MS data were collected as either: 1) total ion chromatograms (TIC) by monitoring the signal over the mass range *m/z* 260–475 for drug identification, or 2) multiple ion monitoring (MIM) at *m/z* 414 (norBUP), 468 (BUP), and 472 (BUP-d4).

Specimens

Blood (2 mL taken after informed, written consent), urine (2 mL) and hair samples (at least 50 mg cut close to the scalp at the posterior vertex) were obtained from six male subjects (aged 19 to 31 years) freely participating in an experimental detoxication program using oral BUP. Calibration curves and parameter optimization were obtained using drug-free whole blood, plasma, urine, and homogenates of hair from our laboratory personnel, these materials being subsequently loaded with BUP and norBUP at various concentrations. According to Kintz et al. (8), hair preparation involved an initial decontamination by two CH_2Cl_2 washes (5 mL, 2 min), then a mechanical pulverization (Retsch® MM2 ball mill, 5 to 10 min); 40 mg of the resulting powder were then incubated overnight at 56°C in 1 mL of 0.1 N HCl, after addition of 1 ng of tetradeuterated BUP (BUP-d4, Radian); BUP-d4 was used for internal standardization of both BUP and norBUP because deuterated norBUP is presently not available on the market.

BUP/norBUP Extraction Procedure

To 2 mL blood, plasma, or urine were added 10 ng of BUP-d4 (Radian), 1.5 mL of a saturated, $(NH_4)_2HPO_4$ buffer, pH 8.4, and 5 mL of $CHCl_3$/2-propanol/*n*-heptane (25:10:65, v/v). After agitation and centrifugation (3500 g, 10 min), the organic phase was evaporated (Speed Vac Concentrator, 45°C, 30 min); the dry extract was resuspended in 20 μL of the mobile phase, and after a final centrifugation (10,000 g, 5 min), 12 μL of the supernatant were removed, from which 2 μL were injected onto the column at each chromatographic run using a Rheodyne mod. 8125 low-dispersion manual valve.

Acidic hair homogenates (1 mL) were extracted in the same way after neutralization (using 0.1 N NaOH, 1 mL).

Results and Discussion

The ISP mass spectra of BUP, norBUP, and BUP-d4 (Fig. 2) are quite simple because they exhibit one unique peak corresponding to the protonated molecular ion [M + H]+ at *m/z* 468, 414, and 472,

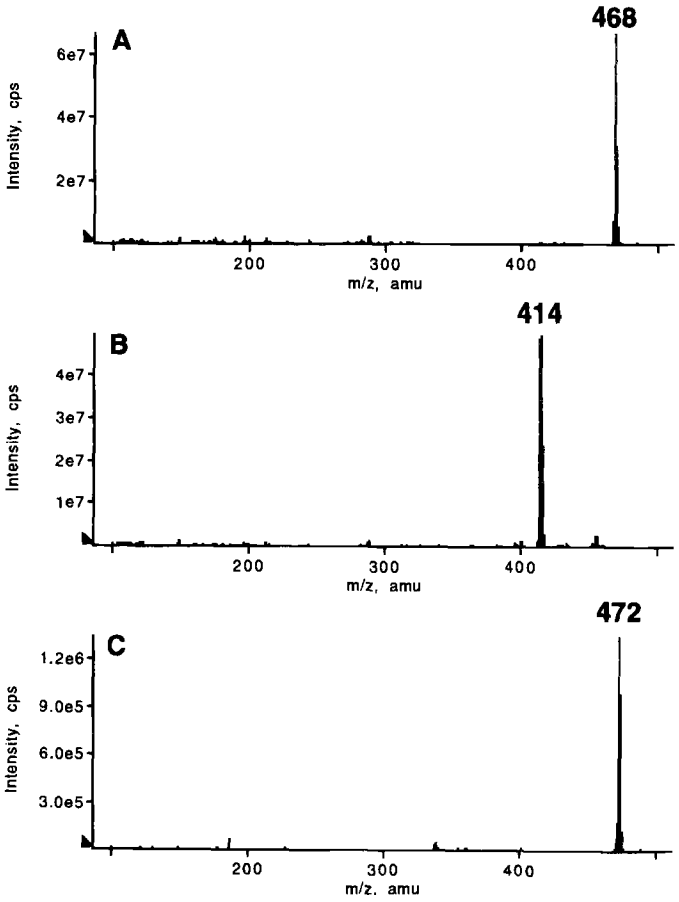

FIG. 2—*Positive-ion, ISP mass spectra of (A) buprenorphine, (B) norbuprenorphine, and (C) buprenorphine-d4. Conditions : Infusion (5 μL/min) of a 10.0-μg/mL solution of each drug in acetonitrile/2 mM NH_4COOH, pH 3.0 buffer (80:20, v/v).*

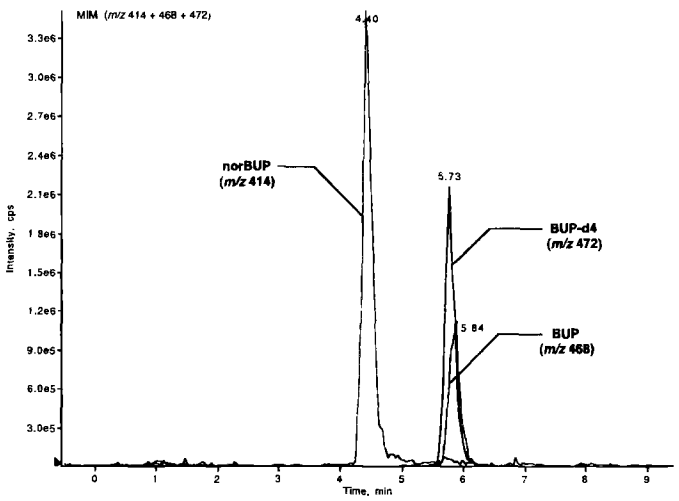

FIG. 3—*Chromatogram of a plasma extract from a 23-year-old subject under BUP therapy (data recorded in MIM mode at m/z 414 + 468 + 472); concentrations measured: BUP 2.7 ng/mL, norBUP 16.9 ng/mL.*

TABLE 1a—*Within-Run Accuracy and Precision (plasma spiked).*

	Concentration added (ng/mL)	Concentration found (ng/mL)	Accuracy	Precision
Buprenorphine	1 (n = 10)	1.07 ± 0.14	6.9%	13.3%
	10 (n = 10)	10.15 ± 0.53	1.5%	5.2%
Norbuprenorphine	1 (n = 10)	0.99 ± 0.15	1.1%	13.9%
	10 (n = 10)	10.19 ± 0.50	1.9%	4.9%

TABLE 1b—*Day-to-Day Accuracy and Precision (plasma spiked).*

	Concentration added (ng/mL)	Concentration found (ng/mL)	Accuracy	Precision
Buprenorphine	10 (10 days)	10.24 ± 0.83	2.4%	8.1%

respectively. This low abundance or absence of fragmentation is a typical character common to mass spectra generated by the different atmospheric pressure ionization (API) HPLC/MS interfaces (9).

Under our chromatographic conditions, the average retention times for BUP, BUP-d4, and norBUP were 5.84, 5.79, and 4.42 min, respectively. As an example, Fig. 3 shows the MIM chromatogram (recorded at *m/z* 414 + 468 + 472) obtained by extracting and assaying a plasma sample from a 23-year-old addict under BUP therapy (2.4 mg/d); BUP and norBUP concentrations were 2.7 and 16.9 ng/mL, respectively.

Extraction recoveries were estimated using drug-free plasma samples loaded with BUP and norBUP at 5 and 50 ng/mL (six replicates at each concentration). Results were found excellent for BUP (87.5 ± 4.5% and 94.5 ± 5.2%) and acceptable for norBUP (65.8 ± 7.2% and 61.4 ± 6.9%). The within-run and day-to-day variability of the method was determined by assaying blank plasma spiked with BUP and norBUP at 1 or 10 ng/mL. Results (Table 1 (a) and (b)) indicated a significant improvement by comparison to our preliminary study (precision for BUP:21.1% at 1 ng/mL; 11.3% at 10 ng/mL) where prazepam was used as the internal standard instead of deuterated BUP (5).

A wide array of techniques have been proposed for the determination of BUP in biological fluids, including RIA (1,8,10), GC with electron-capture (11,12), or MS detection (8,13,14), as well as HPLC with UV (15), electrochemical (8,16–18) or fluorometric detection (19,20), or combined TLC/HPLC (21). RIA offers a rapid and extremly sensitive identification of BUP which is well-adapted to general-unknown screening situations; however, the technique may suffer from interferences and does not allow the separate quantitation of BUP and norBUP (30 to 330% cross-reactivity of norBUP with the BUP antibody, depending on the concentration (8). Former HPLC (15,16,21) and GC (12) procedures were insufficiently sensitive for assaying BUP in blood samples; in addition, GC methods require tedious derivatizations

TABLE 2—*Detection Limits for Buprenorphine (from Ref 8).*

Technique	Detection Limit (ng)
RIA	0.04
HPLC/Electrochemical detection	0.2
HPLC/ISP-MS	0.2
GC/MS	0.5
HPLC/Fluorometry	1
HPLC/Electrospray-MS	5
HPLC/Particle beam-MS	10
HPLC/UV	40

TABLE 3—*Hair Concentrations for Buprenorphine and Norbuprenorphine Measured By HPLC/ISP-MS.*

Subjects	Buprenorphine (pg/mg)	Norbuprenorphine (pg/mg)
1	140	29
2	72	67
3	52	31
4	19	ND
5	135	14
6	4	ND

(n = 6 addicts under buprenorphine substitutive therapy. ND = not detected).

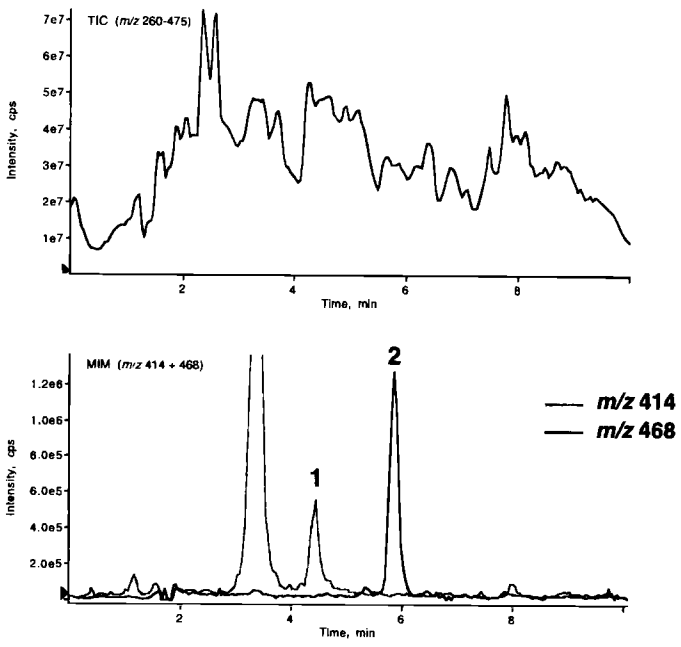

FIG. 4—*Chromatogram of a hair extract from a 27-year-old subject under BUP therapy;* Upper graph: *TIC recording (m/z 260–475);* Lower graph: *MIM recording (m/z 414 + 468); concentrations measured: BUP 140 pg/mg (Peak 2), norBUP 29 pg/mg (Peak 1).*

prior to analysis, and are hampered by the major thermal instability of BUP (8,13). HPLC with fluorometry or electrochemical detection provides a good sensitivity but is not specific enough (especially in forensic situations) because the retention behavior of compounds remains the only criterion available for their identification. The coupling of HPLC to mass spectrometry via API interfaces, that has been already presented as a complement of choice to GC/MS for the determination of nonvolatile and/or thermolabile substances with high sensitivity and specificity (6,9,22,23), thereby appears as an interesting alternative in the case of BUP and norBUP.

The lower limits of detection (LODs), estimated by extracting and assaying in MIM mode drug-free blood samples spiked with decreasing concentrations of the drugs tested until a response equivalent to 3 times the background noise was obtained, were 0.10 and 0.05 ng/mL for BUP and norBUP, respectively. This good sensitivity may be compared with that of other techniques previously described (Table 2); it makes our method convenient for both forensic investigations and clinical studies.

BUP and norBUP have been also reported to be detectable in hair from treated subjects by means of RIA or HPLC with coulometric detection (8). For this application, HPLC/ISP-MS appeared at least as sensitive (LODs in drug-free, spiked hair powder about 4 pg/mg for BUP, and 2 pg/mg for norBUP), and much more specific due to the selected-ion detection. As an illustration, Table 3 presents the drug concentrations measured in hair from six addicts under substitutive therapy by BUP. BUP and norBUP ranged from 4 to 140 pg/mg, and from nondetected to 67 pg/mg, respectively; as previously emphasized (8), the concentrations of the metabolite were much lower than those of the parent drug. Figure 4 shows the TIC (*m/z* 260–475) and MIM (*m/z* 414 + 468) chromatograms of Subject 1 (measured concentrations: BUP 140 pg/mg, norBUP 29 pg/mg).

In conclusion, the present method is the first HPLC/MS procedure described for the analysis of BUP and its dealkylated metabolite in biological samples including hair. It is simple, rapid (owing to the single-step, liquid-liquid extraction, quantitative results may be obtained less than 60 min after receipt of blood or urine samples), highly sensitive and specific. It provides a new illustration of the great capabilities of HPLC/MS with API interfaces in the case of difficult compounds that cannot be conveniently assayed using conventional techniques such as GC/MS.

References

1. Buprenorphine. In: Baselt RC, Cravey RH, editors. Disposition of toxic drugs and chemicals in man. Foster City: Chemical Toxicology Institute, 1995;93–4.
2. Takemori AE, Ikeda M, Portoghese PS. The mu, kappa, and delta properties of various opioid agonists. Eur J Pharmacol 1986; 123:357–61.
3. Reisinger M. Buprenorphine as new treatment for heroin dependance. Drug Alcohol Depend 1985;16:257–62.
4. Blom Y, Bondesson U, Gunne LM. Effects of buprenorphine in heroin addicts. Drug Alcohol Depend 1987;20:1–7.
5. Tracqui A, Kintz P, Cirimele V, Mangin P. Determination of buprenorphine and norbuprenorphine in human blood, urine, and hair using HPLC/Ion-spray-MS. In: Proceedings of the 1995 Fall Meeting of the Northwest Association of Forensic Scientists; Oct. 16–20, 1995; Ashland (OR): 1995;28.
6. Marquet P, Hoja H, Verneuil B, Lotfi H, Dreyfuss MF, Lachâtre G. Applications du couplage LC/MS avec interface à pression atmosphérique à quelques problèmes analytiques en toxicologie: Résultats préliminaires. In: Tracqui A, editor. Proceedings of the Symposium STRATOX II; May 18–19, 1995; Strasbourg (France): Institut de Médecine Légale, 1995;71–6.
7. Hoja H, Marquet P, Verneuil B, Lotfi H, Dreyfuss MF, Lachâtre G. Dosage de buprénorphine et de norbuprénorphine dans l'urine et le sérum par chromatographie liquide couplée à la spectrométrie de masse avec ionisation de type "électrospray" (LC/ES/MS) sur API 100 SCIEX. Analusis. In press.
8. Kintz P, Cirimele V, Edel Y, Jamey C, Mangin P. Hair analysis for buprenorphine and its dealkylated metabolite by RIA and confirmation by LC/ECD. J Forensic Sci 1994;39:1497–503.
9. Wachs T, Conboy JC, Garcia F, Henion JD. Liquid chromatography—mass spectrometry and related techniques via atmospheric pressure ionization. J Chromatogr Sci 1991;29:357–66.
10. Bullingham RES, McQuay HJ, Moore A, Bennett MRD. Buprenorphine kinetics. Clin Pharmacol Ther 1980;28:667–72.
11. Martinez D, Jurado MC, Repetto M. Analysis of buprenorphine in plasma and urine by gas chromatography. J Chromatogr 1990;528:459–63.
12. Cone EJ, Gorodetzky CW, Yousefnejad D, Darwin WD. ^{63}Ni-electron-capture gas chromatographic assay for buprenorphine and metabolites in human urine and feces. J Chromatogr 1985; 337:291–300.
13. Blom Y, Bondesson U. Analysis of buprenorphine and its N-dealkylated metabolite in plasma and urine by selected-ion monitoring. J Chromatogr 1985;338:89–98.
14. Ohtani M, Shibuya F, Kotaki H, Uchino K, Saitoh Y, Nakagawa F. Quantitative determination of buprenorphine and its active metabolite, norbuprenorphine in human plasma by gas chromatography-chemical ionization mass spectrometry. J Chromatogr 1989; 487:469–75.
15. Tebbett IR. Analysis of buprenorphine by high performance liquid chromatography. J Chromatogr 1985;347:411–3.
16. Mullersman G, Toufflin S, Derendorf H. HPLC analysis of buprenorphine in plasma and urine using coulometric detection. J Pharm Biomed Anal 1987;5:303–8.
17. Debrabandere L, Van Boven M, Daenens P. High performance liquid chromatography with electrochemical detection of buprenorphine and its major metabolite in urine. J Chromatogr 1991; 564:557–66.
18. Debrabandere L, Van Boven M, Daenens P. Analysis of buprenorphine in urine specimens. J Forensic Sci 1991;37:82–9.
19. Garrett ER, Chandran VR. Pharmacokinetics of morphine and its surrogates VI: Bioanalysis, solvolysis kinetics, solubility, pKa values, and protein binding of buprenorphine. J Pharm Sci 1985;74:515–24.
20. Ho ST, Wang JJ, Ho W, Hu OYP. Determination of buprenorphine by high performance liquid chromatography with fluorescence detection: Application to human and rabbit pharmacokinetic studies. J Chromatogr 1991;570:339–50.
21. Hackett LP, Dusci LP, Ilett KF, Seow SS, Quigley AJ. Sensitive screening method for buprenorphine in urine. J Chromatogr 1986;374:400–4.
22. Tracqui A, Kintz P, Ludes B, Rougé C, Douibi H, Mangin P. High performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. J Chromatogr B 1996;675:235–42.
23. Tracqui A, Kintz P, Ludes B, Mangin P. HPLC/Ionspray-MS for the specific determination of digoxin and some related cardiac glycosides in human plasma. J Anal Toxicol. In press.

Additional information and reprint requests:
Antoine Tracqui, Ph.D.
Institut de Médecine Légale
11 rue Humann
67085 Strasbourg Cedex, France