Lode Debrabandere,[1] *D.Pharm.; Maurits Van Boven,*[1] *D.Sc.; and Paul Daenens,*[1] *D.Sc.*

Analysis of Buprenorphine in Urine Specimens

REFERENCE: Debrabandere, L., Van Boven, M., and Daenens, P., **"Analysis of Buprenorphine in Urine Specimens,"** *Journal of Forensic Sciences,* JFSCA, Vol. 37, No. 1, Jan. 1991, pp. 82–89.

ABSTRACT: The simultaneous determination of buprenorphine (Temgesic®) and its major metabolite, *N*-desalkylbuprenorphine, in urine samples has been studied. By using reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection, therapeutic concentrations of unconjugated buprenorphine down to 0.2 ng/mL, and 0.15 ng/mL for the metabolite, can be detected in urine samples. This method has been applied to a variety of urine samples from drug users. The possible analytical interference from several other regulated drugs has been studied. The results were also compared with those obtained from a commercial radioimmunoassay (RIA) test. This test is only capable of detecting buprenorphine concentrations higher than 1 ng/mL.

KEYWORDS: toxicology, buprenorphine, urine, chromatographic analysis, high-performance liquid chromatography (HPLC), electrochemical detection, radioimmunoassay

Buprenorphine is a powerful partial agonist analgesic, effective in the treatment of acute and chronic pain [*1–2*]. Its molecular structure is similar to that of morphine, and it is administered intramuscularly, intravenously [*3*], or sublingually [*4*] in a dose of 0.3 to 0.6 mg. Therapeutic urine concentrations of the unconjugated drug range in the nanograms to even picograms per millilitre [*5*]. After parenteral or sublingual administration, it is absorbed and metabolized, principally by *N*-dealkylation and conjugation.

A low addiction potential has been reported [*6*], and suggestions for the use of buprenorphine in the management of opiate addicts have been made [*7–11*]. Reports of misuse of the drug have been published [*12–14*], but the frequency of buprenorphine use by drug addicts remains uncertain.

The gas chromatography methods described [*15–17*] require derivatization of the drug prior to analysis. The sensitivity of those methods is not sufficient for the detection of one therapeutic dose of buprenorphine in urine samples. In a single subject, using chemical degradation of the buprenorphine molecule, followed by derivatization with pentafluorpropionyl anhydride, Blom et al. [*18*] were able to detect small amounts of unconjugated buprenorphine (>150 pg/mL) in both urine and plasma samples by gas chromatography/mass spectrometry (GC/MS), using selected-ion monitoring. The high-performance liquid chromatography (HPLC) methods published so far are insufficiently sensitive [*19–21*].

The applicability of a newly developed HPLC method [*5*] has been studied on urine samples, all collected from persons suspected of buprenorphine (Temgesic®) misuse. The

Received for publication 16 April 1991; accepted for publication 7 May 1991.

[1]Pharmacist, chemist, and director, respectively, Laboratory of Toxicology, University of Louvain, Leuven, Belgium.

samples were previously screened by radioimmunoassay (RIA) for buprenorphine. In addition, a study was undertaken to examine possible interferences from a number of other regulated drugs commonly encountered in forensic toxicology.

Materials and Methods

Materials

Buprenorphine and *N*-desalkylbuprenorphine were synthesized in the laboratory according to the method of Kleemann and Engel [*22*]. As an internal standard, the ethyl derivative *N*-ethyl-7-[1-(5)-hydroxyl,2,2-trimethylpropyl]-6,14-endoethano-6,7,8,14-tetra-hydronororipavine was synthesized. All the other chemicals and solvents were of analytical reagent grade.

The DPC buprenorphine double-antibody kit was used as supplied by the manufacturer (DPC-Medico-Service Benelux, Brussels, Belgium). It contains buprenorphine antiserum, iodine-125 (^{125}I) labeled buprenorphine, buprenorphine calibrators (0, 1, 2, 5, 10, and 25 ng of buprenorphine per millilitre in processed human urine), and a precipitating solution (goat anti-rabbit gamma globulin and dilute polyethylene glycol in saline).

Instrumentation

The equipment used for this assay was a Merck-Hitachi L-6200 high-performance liquid chromatograph, equipped with a 20-μL Rheodyne injector (Berkeley, California). A 25-cm by 0.4-cm inside-diameter Lichrosorb CN (5-μm) reversed-phase column was used (Merck, Darmstadt, Germany). The HPLC system was combined with a Chromatofield electrochemical detector (Model Eldec 201, Chateauneuf-les-Martigues, France). The potential was set at 0.75 V versus the reference electrode, and the sensitivity was set at 1 nA full scale [*5*]. In addition, an ultraviolet (UV) detector (Model 440, Waters Associates, Milford, Massachusetts) was used to measure the retention time of several regulated drugs. The response signals were recorded on a Merck-Hitachi Model D-2500 chromatointegrator. The extractions were carried out with a rotary mixer. The extraction tubes were centrifuged with a Heraeus Sepatech Labofuge A centrifuge. The desintegration rate was measured with a gamma counter (Berthold BF 5300, Wildbad, Germany).

Chromatographic Conditions

The optimum mobile phase for separating both buprenorphine and its major metabolite contained 10 mmol of 1-heptane sulfonic acid sodium salt and 0.01% tetrabutylammonium sulfate in 1 L of acetonitrile phosphate buffer, pH 4.0 (13:87). The eluent was filtered through a 0.2-μm filter and completely degassed with helium before use. The flow rate was 1 mL/min.

Extraction Procedure

An extraction with toluene, combined with an acidic cleanup procedure, (Fig. 1) was found to be the most suitable solution for obtaining extracts free of interfering impurities [*5*].

Calibration graphs were constructed by spiking drug-free urine samples with known amounts of buprenorphine and *N*-desalkylbuprenorphine (0.1, 0.5, 1.0, 2.0, 5.0, 10, 50, and 100 ng/mL) and with 100 μL of the internal standard solution (0.1 ng/μL). The samples were extracted as described.

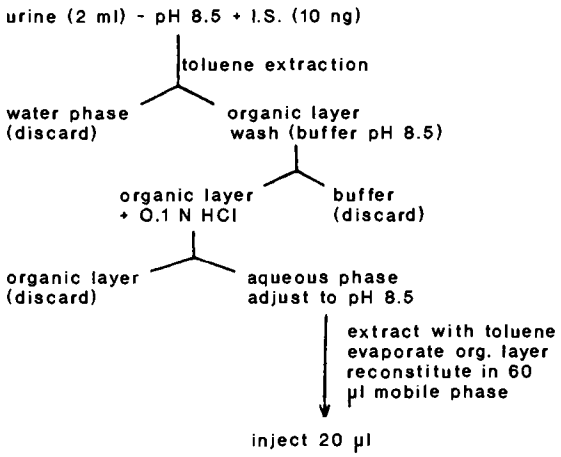

FIG. 1—*Extraction procedure.*

Radioimmunoassay Procedure

For this procedure, all the components except the precipitating solution must be at room temperature before use. Calibration graphs were constructed as follows. Urine samples (25 µL) containing 0 (maximum binding) 1, 2, 5, 10, and 25 ng/mL of buprenorphine were analyzed in duplicate. An amount of 100 µL of ^{125}I buprenorphine was added to each tube, along with 100 µL of buprenorphine antiserum. The tubes were vortexed and incubated for 60 min at room temperature. One millilitre of cold precipitating solution was added to all the tubes and they were again incubated for 10 min at room temperature. After centrifugation for 15 min at 3000 × *g*, the supernatant was decanted. The precipitate was counted on the gamma counter for 1 min. Unknown samples were analyzed following the same procedure.

Study of Interferences

A study was undertaken to examine the possible interference from several narcotic analgesics and other regulated drugs using the HPLC/electrochemical detection (ECD) procedure. In addition to phenolic compounds such as morphine, pentazocine, and naloxone, a variety of other drugs was examined, including structurally related molecules such as etorphine and diprenorphine. Since flunitrazepam (Rohypnol®) recently became a regulated drug in Belgium, the interference of some of its oxidizable metabolites was also investigated. Standard solutions of aminoflunitrazepam and *N*-desmethyl-7-acetaminoflunitrazepam (5 mg of each per 100 mL of methanol) were injected into the chromatograph. The eventual interference from cannabinoid metabolites was studied on urine samples previously analyzed with the AD_x fluorescent immunoassay for cannabinoids (Abbott Laboratories, Chicago, IL) and was shown to be positive by GC/MS for the presence of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid. In addition, a screening program had previously confirmed that these urine samples were free of other drugs. They were extracted and analyzed using the HPLC-ECD method, as described above for buprenorphine.

For measuring the retention time of all the compounds examined, including those that are virtually not oxidizable at 750 mV, a UV detector (280 nm) was put in series with the electrochemical detector. The sensitivity of the UV detector was set at 0.01 full scale,

and the electrochemical detector was set at 20 nA. Standard solutions of different drugs were prepared in a concentration of 5 mg/100 mL of methanol. Ten-microlitre aliquots of these solutions were injected into the chromatograph.

Results and Discussion

A straight calibration graph was obtained for the HPLC method by plotting the area under the curve ratios of both buprenorphine and *N*-desalkylbuprenorphine (concentration range, 0.1 to 100 ng/mL) to the internal standard versus the buprenorphine and *N*-desalkylbuprenorphine concentrations. The graphs were linear up to a concentration of 100 ng/mL (correlation coefficient, $r > 0.99$). The measured concentration of buprenorphine and *N*-desalkylbuprenorphine in spiked urine samples ranged from 86 to 98%. The detection limits were 0.15 ng/mL for the major metabolite and 0.2 ng/mL for buprenorphine.

As for the radioimmunoassay, a standard curve for buprenorphine in the range of 1 to 25 ng/mL was constructed. The mean value obtained for the 5-ng/mL control was 4.6 ng/mL [coefficient of variation (CV), 9.2%] and that for the 15-ng/mL control was 16.1 ng/mL (CV, 8.5%). It is difficult to establish an adequate threshold value for the determination of buprenorphine in urine. Repeated measurements on blank urine samples showed that results below 1.0 ng/mL cannot be distinguished from zero in a reproducible way. This value was chosen as a cutoff value for the presence of buprenorphine in unknown samples. Of 50 urine specimens from persons suspected of buprenorphine misuse, 23 (46%) were found positive with RIA and were further examined by HPLC. For reference purposes, a urine sample was collected from a volunteer 12 h after the intramuscular injection of one single therapeutic dose of 0.3 mg of buprenorphine; the RIA analysis for this sample showed an apparent buprenorphine concentration near the cutoff value and was, therefore, not conclusive. However, using the HPLC-ECD method described, it was still possible to measure the separate concentrations of unchanged buprenorphine and *N*-desalkylbuprenorphine. In Fig. 2*c*, the chromatogram of this sample is shown demonstrating very low concentrations of buprenorphine (± 0.2 ng/mL) and *N*-desalkylbuprenorphine (± 0.6 ng/mL). The chromatograms of a blank urine sample and of a standard solution containing buprenorphine, *N*-desalkylbuprenorphine, and the internal standard are illustrated in Figs. 2*a* and *b*. For the 23 preselected urine samples further examined by the HPLC method, the concentration of unchanged buprenorphine ranged from 0.2 to 15 ng/mL and that of *N*-desalkylbuprenorphine from 0.6 to 25 ng/mL. In some of these samples, concentrations of unchanged buprenorphine and *N*-desalkylbuprenorphine up to 15 ng/mL and 25 ng/mL, respectively, were even found. These high concentrations demonstrate a misuse of the drug.

It was shown in our study that the RIA procedure cannot distinguish buprenorphine from its major metabolite or from structurally similar drugs, including etorphine and diprenorphine. Consequently, it is mandatory that all positive results be confirmed by another method of equal sensitivity. HPLC with electrochemical detection seems to be the method of choice.

Table 1 shows the results of the study of the interference of a variety of regulated drugs on the HPLC-ECD procedure, along with the retention times of the different compounds studied. A strong electrochemical response was observed for all phenolic compounds, including nalorphine, naloxone, morphine, and pentazocine. Except for pentazocine, these substances, being more polar than buprenorphine, elute early in the system (<4 min). Only pentazocine interfered with the *N*-desalkyl metabolite of buprenorphine, at exactly 7.00 min. A weak response was observed for pholcodine (β-morpholinylethylmorphine), a drug used in cough remedies in Europe, because of the enolic function in the molecule. The very potent, structurally related compounds etor-

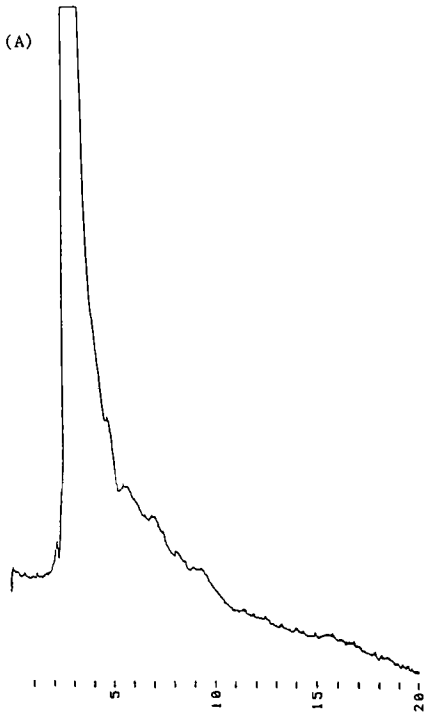

FIG. 2*a*—*Chromatogram of a blank urine sample.*

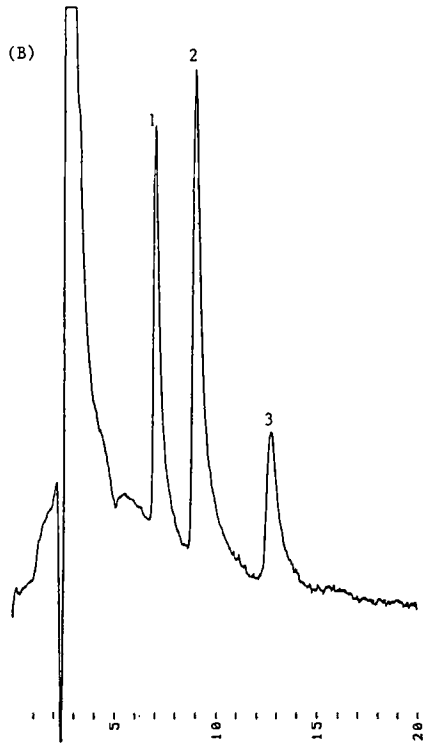

FIG. 2*b*—*Chromatogram of a standard solution containing 5 ng of* N-*desalkylbuprenorphine (1), 5 ng of internal standard (2), and 5 ng of buprenorphine (3).*

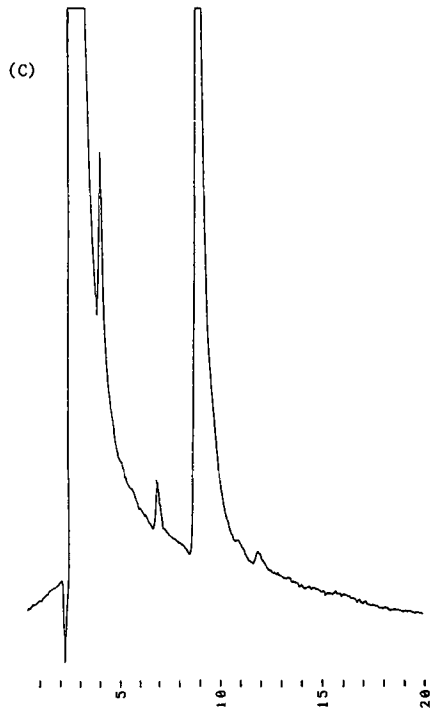

FIG. 2c—*Chromatogram of a urine sample for which the RIA test was not conclusive. (The buprenorphine concentration was around the cutoff value of 1 ng/mL.) Buprenorphine at 0.2 ng/mL and* N*-desalkylbuprenorphine at 0.6 ng/mL can still be detected with HPLC-ECD.*

TABLE 1—*Results of the study of interferences of a variety of regulated drugs by the HPLC-ECD procedure.*

Compound	Electrochemical Response, 0.75 V	Retention Time, min
Morphine	+ + +	3.40
Naloxone	+ + +	4.00
Nalorphine	+ + +	3.80
Pentazocine	+ + +	7.00
Buprenorphine	+ + +	11.91
N-desalkylbuprenorphine	+ + +	7.00
Etorphine	+ + +	7.21
Diprenorphine	+ + +	6.81
Pholcodine	+	3.60
Aminoflunitrazepam	+	7.30
Acetaminoflunitrazepam	+	5.92
Cannabinoid metabolite	+	16.00
Codeine	–	4.00
Cocaine	–	3.95
Heroin	–	5.40
Methadone	–	12.41
Pethidine	–	3.36
Piritramide	–	[a]
Dextromoramide	–	[a]
Propoxyphene	–	[a]
Fentanyl	–	[a]
Alfentanyl	–	[a]

[a]Not eluted within 20 min in the chromatographic system.

phine and diprenorphine also show a strong electrochemical response and elute near the metabolite of buprenorphine, although separate from it. The electrochemical signal for the metabolites of flunitrazepam (aminoflunitrazepam and acetaminoflunitrazepam) is weak, but when urine samples with a high concentration of flunitrazepam (>30 ng/mL) were analyzed, the response of the oxidation of the metabolites became more significant. There was no interference for the acetamido metabolite, but the amino metabolite elutes near *N*-desalkylbuprenorphine at 7.30 min. It was demonstrated that all the urine specimens that were found positive for cannabinoid metabolites showed a peak at 16.00 min. The cannabinoid or metabolite responsible for the electrochemical response is not known and has not been further examined. A number of other regulated drugs do not show an electrochemical response or do not elute in the chromatographic system used.

Conclusions

The high-pressure liquid chromatography method described, combined with electrochemical detection, enables detection in the picogram range for both buprenorphine and its major metabolite in urine specimens. It constitutes a valuable alternative method for confirmation of buprenorphine (Temgesic®) in prescreened urine samples by radioimmunoassay.

References

[1] Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E., and Gilbert, P. E., "The Effects of Morphine- and Nalorphine-like Drugs in the Nondependent and Morphine-Dependent Chronic Spinal Dog," *Journal of Pharmacology and Experimental Therapeutics*, Vol. 197, No. 3, 1976, pp. 517–537.

[2] Bullingham, R. E. S., McQuay, H. J., and Moore, R. A., "Clinical Pharmacokinetics of Narcotic Agonist-Antagonist Drugs," *Clinical Pharmacokinetics*, Vol. 8, 1983, pp. 332–343.

[3] Bullingham, R. E. S., McQuay, H. J., Moore, R. A., and Bennet, M. R. D., "Buprenorphine Kinetics," *Clinical Pharmacology and Therapeutics*, Vol. 28, 1980, pp. 667–672.

[4] Bullingham, R. E. S., McQuay, M. S., Porter, E. J. B., Allen, M. C., and Moore, R. A., "Sublingual Buprenorphine Used Postoperatively: Ten Hour Plasma Drug Concentration Analysis," *British Journal of Clinical Pharmacology*, Vol. 13, 1982, pp. 665–673.

[5] Debrabandere, L., Van Boven, M., and Daenens, P., "High-Performance Liquid Chromatography, with Electrochemical Detection, of Buprenorphine and Its Major Metabolite in Urine," *Journal of Chromatography*, Vol. 564, No. 2, April 1991, pp. 557–566.

[6] Jasinski, D. R., Pevnick, J. S., and Griffith, J. D., "Human Pharmacology and Abuse Potential of the Analgesic Buprenorphine," *Archives of General Psychiatry*, Vol. 35, April 1978, pp. 501–516.

[7] Mello, N. K. and Mendelson, J. H., "Buprenorphine Suppresses Heroin Use by Heroin Addicts," *Science*, Vol. 207, Feb. 1980, pp. 657–659.

[8] Blom, Y., Bonderson, U., and Gunne, L. M., "Effects of Buprenorphine in Heroin Addicts," *Drug and Alcoholic Dependence*, Vol. 20, 1987, pp. 1–7.

[9] Bickel, W. K., Stitzer, M. L., Bigelow, G. E., Liebson, I. A., Jasinski, D. R., and Johnson, R. E., "A Clinical Trial of Buprenorphine: Comparison with Methadone in the Detoxification of Heroin Addicts," *Clinical Pharmacology and Therapeutics*, Vol. 43, No. 1, Jan. 1988, pp. 72–78.

[10] Kosten, T. R., Kleber, H. D., and Morgan, C., "Treatment of Cocaine Abuse with Buprenorphine," *Biological Psychiatry*, Vol. 26, 1989, pp. 637–639.

[11] Kosten, T. R. and Kleber, H. D., "Buprenorphine Detoxification from Opiate Dependence: A Pilot Study," *Life Sciences*, Vol 42, No. 6, 1988, pp. 635–642.

[12] Strang, J., "Abuse of Buprenorphine," *The Lancet*, Sept. 1985, p. 725.

[13] Rainey, H. B., "Abuse of Buprenorphine," *New Zealand Medical Journal*, Feb. 1986, p. 72.

[14] Robertson, J. R. and Bucknall, A. B. V., "Buprenorphine: Dangerous Drug or Overlooked Therapy?" *British Medical Journal*, Vol. 292, 1986, p. 1465.

[15] Cone, E. J., Gorodetzky, C. W., Yousefnejad, D., and Darwin, W. D., "^{63}Ni Electron-Capture Gas Chromatographic Assay for Buprenorphine and Metabolites in Human Urine and Feces," *Journal of Chromatography*, Vol. 337, 1985, pp. 291–300.

[16] Lloyd-Jones, J. G., Robinson, P., and Henson, R., "Plasma Concentration and Disposition of Buprenorphine After Intravenous and Intramuscular Doses to Baboons," *European Journal of Drug Metabolism and Pharmacokinetics*, Vol. 5, No. 4, 1980, pp. 233–239.

[17] Cone, E. J., Gorodetzky, C. W., Yousefnejad, D., and Darwin, W. D., "The Metabolism and Excretion of Buprenorphine in Humans," *Drug Metabolism and Disposition*, Vol. 12, No. 5, 1984, pp. 577–581.

[18] Blom, Y., Bondesson, U., and Ängard, E., "Analysis of Buprenorphine and Its *N*-Dealkylated Metabolite in Plasma and Urine by Selected-Ion Monitoring," *Journal of Chromatography*, Vol. 338, 1985, pp. 89–98.

[19] Tebbett, I. R., "Analysis of Buprenorphine by High-Performance Liquid Chromatography," *Journal of Chromatography*, Vol. 347, 1985, pp. 411–413.

[20] Hackett, L. P., Dusci, L. J., and Ilett, K. F., "Sensitive Screening Method for Buprenorphine in Urine," *Journal of Chromatography*, Vol. 374, 1986, pp. 400–404.

[21] Mullersman, G., Toufflin, S., and Derendorf, H., "HPLC Analysis of Buprenorphine in Plasma and Urine Using Coulometric Detection," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 5, No. 3, 1987, pp. 303–308.

[22] Kleemann, A. and Engel, J., *Pharmaceutische Wirkstoffe; Ergänzungsband 1982–1987*, Georg Thieme Verlag, Stuttgart, Germany, 1987.

Address requests for reprints or additional information to
P. Daenens, Director
Laboratory of Toxicology
University of Louvain
E. Van Evenstraat No. 4
3000 Leuven, Belgium