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Hair Analysis for Buprenorphine and Its Dealkylated Metabolite by RIA and Confirmation by LC/ECD

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ABSTRACT: Hair samples were obtained from 14 subjects admitted 2 or 3 months previously to a detoxification center. All reported an history of intravenous heroin abuse. After decontamination by two dichloromethane washes, about 50 mg hair were pulverized in a ball mill and incubated at 56°C overnight in 1 mL 0.1 HCl.

After neutralization, buprenorphine analyzed by RIA was in the range of 0.01 to 0.47 ng/mg. To confirm buprenorphine, liquid chromatography was used. After neutralization, drugs were extracted with toluene at pH 8.5 during a 3-step extraction procedure. A portion of the reconstituted residue was injected into a Lichrosorb CN column, with a mobile phase of phosphate buffer (pH 4.0)-acetonitrile-1-heptane sulfonic acid-butylamine (85:17:2:0.01, v/v).

Detection was achieved by coulometry, and the potential of the electrodes was 0.15 and 0.50 V, respectively.

Linear calibration curves were obtained from 0.02 to 2.0 ng/mg with a correlation coefficient $r > 0.99$ for both drugs. The detection limit for the major metabolite was about 0.01 ng/mg and 0.02 ng/mg for buprenorphine, using a 50 mg hair sample. Recovery (at 0.2 ng/mg) was 54 and 62% for norbuprenorphine and buprenorphine, respectively. Drugs concentrations in hair were in the range 0.02–0.59 and not detected—0.15 ng/mg for buprenorphine and norbuprenorphine, respectively. Results suggest that a dose-reponse relationship exists between the concentration of buprenorphine in hair and the administered dose.

KEYWORDS: toxicology, hair, buprenorphine, narcotics

Buprenorphine is a powerful partial agonist analgesic with opiate antagonist properties. The drug is obtained from thebaine after a seven-step chemical procedure, and was initially developed for the treatment of acute and chronic pain [*1*].

Buprenorphine is largely used all over the world under different commercial names, Temgesic in Europe excepted in Spain, (where it is known as Buprex or Prefin) and Buprenex in the United States. Two galenic formulations are available, one injectable form (0.3 or 0.6 mg) and one sublingual form (0.2 mg). The latter presentation represents 79% of the wholesale market.

An addiction potential has been reported and suggestions for the use of buprenorphine in the management of heroin addicts have been made [2].

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Heroin addicts can switch to buprenorphine as the drug is sometimes easier to find, of lower cost and because it can be taken orally it represents less risk of AIDS contamination in comparison with intravenous administration.

The usual recommended doses are 200 to 600 μg by slow intravenous or intramuscular injection, repeated every 6 to 8 h or 400 μg sublingually also every 6 to 8 h, leading to plasma concentrations in the range of 0.1 to 8.0 ng/mL [*3*].

Buprenorphine is metabolized by N-dealkylation to procedure norbuprenorphine and subsequent conjugation with glucuronic acid of both buprenorphine and N-desalkyl metabolite is occuring. Buprenorphine has a long half-life of about 8 h; the elimination of the N-desalkyl metabolite is even slower [*4*].

Determination of chronic drug abusers is generally achieved by identifying the corresponding drug in hair [*5–7*] and all these papers showed that hair analysis and urinalysis were complementary.

Therefore, in this study, a previous published method for the analysis of buprenorphine in urine [*8*] was extended to hair.

Material and Methods

Specimens

Hair samples were obtained from 14 subjects (five male and nine female), aged from 21 to 34 years, who had been admitted 2 or 3 months previously to a detoxification center. All reported an history of intravenous heroin abuse.

Hair Preparation

Hair samples, weighing at least 50 mg, were cut as close as possible to the skin from the posterior vertex.

After decontamination by two dichloromethane washes (5 mL, 2 min), about 50 mg hair were pulverized in a ball mill and incubated at 56°C overnight in 1 mL 0.1N HCl.

RIA Analysis

After neutralization with 1 mL 0.1N NaOH, and centrifugation, the supernatant was assayed by RIA using the DPC Urine Buprenorphine Kit (Los Angeles, CA). Briefly, 25 μL were incubated 60 min at room temperature with 100 μL buprenorphine antiserum and 100 μL ^{125}I-buprenorphine. After the addition of a second antibody, incubation, centrifugation, decantation and count, results are obtained in 60 min. Each point was determined in triplicate.

Mass Spectrometry

Different mass spectrometry procedures were tested to confirm buprenorphine and its metabolite.

Gas chromatography was achieved using the method of Blom and Bonderson [*3*]. Liquid chromatography was evaluated with Hewlett Packard materials using two different interfaces.

The HPLC equipment consisted of an HP 1090 system, with a mobile phase of acetonitrile-water-acetic acid (50:50:1, v/v) and a flow rate of 0.4 mL/min (particle beam) or 2 μL/min (electrospray). The mass spectrometer was a HP 5989 A coupled either with a particle beam interface (HP 5998 B) or an electrospray interface (HP 59987 A).

Liquid Chromatography Coupled with Coulometric Detection

The HPLC equipment used for this assay consisted of a pump (Waters 510), and an automatic sample injection module (Waters Wisp 710B), which were coupled to a coulometric detector (ESA Coulochem II). The separation was performed on a Merck Lichrosorb CN column (5 μm particle size, 250 × 4 mm). The mobile phase was 10 mM phosphate buffer (pH 4.0)-acetonitrile—1-heptane sulfonic acid (Kodak)-butylamine (85:17:2:0.01, v/v). The flow rate was 1 mL/min.

Calibration curves and analytical parameters were realized with homogenates of laboratory personnel drug-free hair spiked with buprenorphine and norbuprenorphine.

Buprenorphine Extraction

After neutralization, drugs were extracted with 10 mL toluene after addition of 1 mL of saturated pH 8.5 phosphate buffer. After agitation and centrifugation, the organic phase was purified by an additional acidic extraction (5 mL 0.1N HCl). Then, the aqueous layer was reextracted after addition of 1 mL ammonia solution, 1 mL of pH 8.5 phosphate buffer and 5 mL toluene. After agitation and centrifugation, the organic phase was taken off and evaporated to dryness. The residue was reconstituted in 100 μL mobile phase and 60 μL were injected into the column.

Results and Discussion

Accuracy and precision of the buprenorphine RIA procedure was assayed with control materials prepared by the addition of buprenorphine to drug free hair. Control samples containing buprenorphine at concentrations of 0.05 and 0.2 ng/mg were tested. Mean ± S.D. determinations were 0.048 ± 0.006 and 0.22 ± 0.04 ng/mg of hair, respectively. Between run (n = 3) coefficients of variation were 12.8 and 6.9%, respectively.

Analysis of control subjects' hair samples provided background measures of buprenorphine, giving concentrations lower than 3 pg/mg. Hair samples from known drug free persons (laboratory personal) were used to establish a mean + 2SD for a background reading (cpm). It was therefore decided that a positive RIA has to be three times higher than the highest value of the control sample.

All the hair samples extracts from treated subjects contained buprenorphine with concentrations in the range of 0.01 to 0.47 ng/mg.

These results indicated that buprenorphine can be detected by RIA in hair after the administration the drug. Cross reactivity of the RIA with the normetabolite was dose dependant (Fig. 1), which can influence the measure of the concentration. Therefore, it was decided to determine buprenorphine and its metabolite using chromatography.

In forensic toxicology, mass spectrometry has been accepted universally as the technique of choice.

In order to determine the most suitable mass spectrometry procedure, gas chromatography and liquid chromatography coupled either with a particle beam interface or an electrospray interface were tested. The major parameter of interest was the sensitivity due to the low concentrations measured by RIA. The limit of detection was evaluated with decreasing concentration of the drug, until a response equivalent to 3 times the background noise was observed. Results are presented Table 1. Both GC and LC mass spectrometry procedures were not sensitive enough to detect buprenorphine in hair. Moreover, it was demonstrated that buprenorphine is thermally unstable in the heated source of the mass spectrometer (Fig. 2). The thermal degradation of buprenorphine was described by Blom et al. [*3*] leading the authors to chemically modify the compound by ring formation between the side-chain and the methoxy group. Due to the very low concentrations in hair, this solution was not retained. Figure 3 represents the mass spectrogram of buprenorphine obtained by LC with

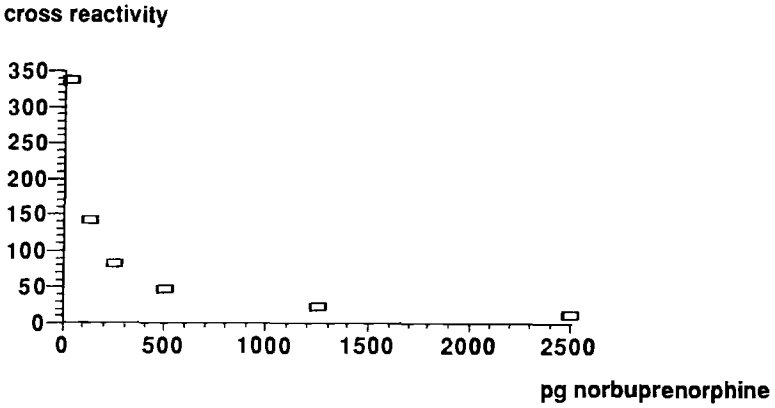

FIG. 1—*Cross-reactivity of norbuprenorphine with the RIA antibody. The metabolite was dissolved in drug-free urine and the samples were measured using the calibration curve of buprenorphine.*

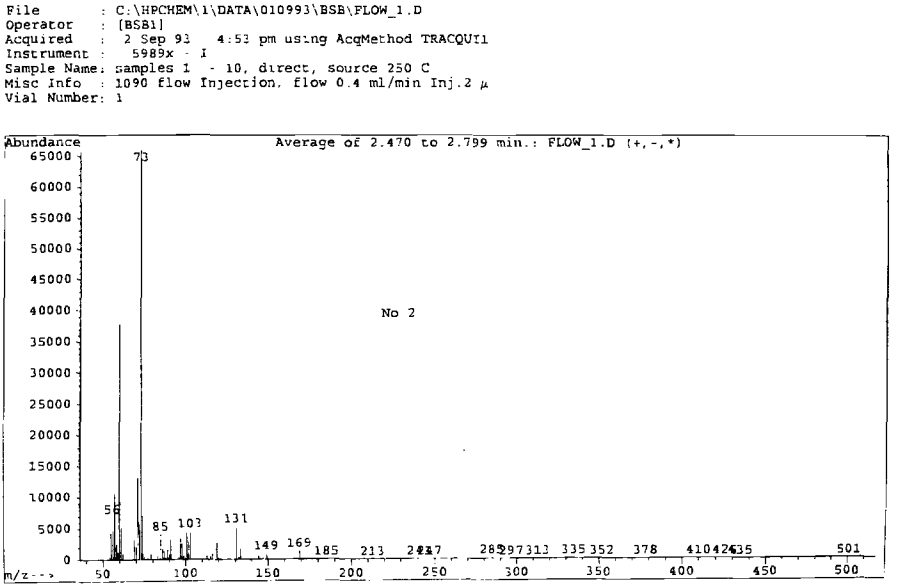

FIG. 2—*Mass spectrogram of buprenorphine obtained by liquid chromatography with a particle beam interface. Note that buprenorphine is thermally unstable.*

TABLE 1—*Limits of detection of buprenorphine.*

Technique	Limit of detection
RIA	40 pg
GC/MS	500 pg
LC/Particle beam	10 ng
LC/Electrospray	5 ng

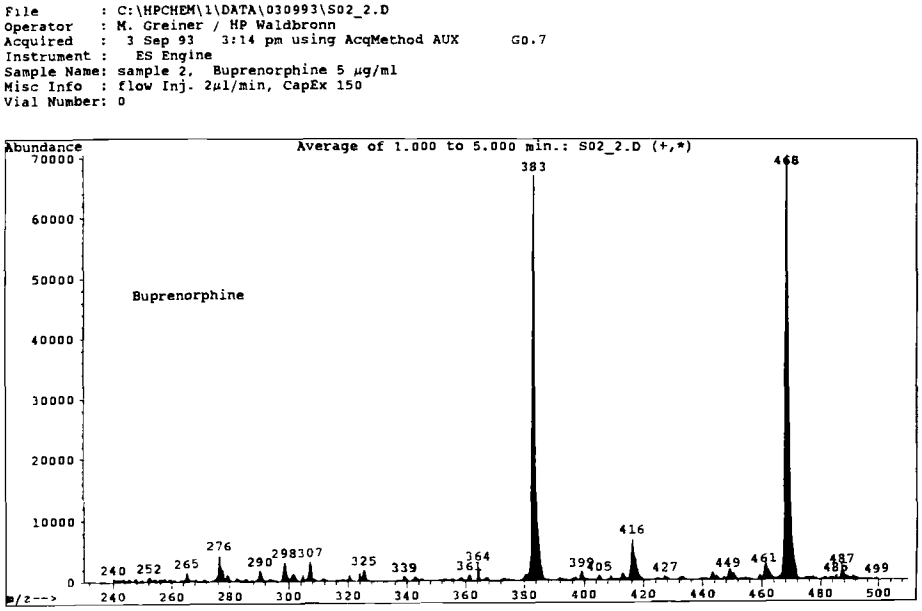

FIG. 3—*Mass spectrogram of buprenorphine obtained by liquid chromatography with an electrospray interface.*

an electrospray interface. Therefore it was decided to determine buprenorphine by liquid chromatography coupled to coulometric detection, which is usually presented as one of the more sensitive of the separative method.

The principal advantage of the coulometric detector, compared with an amperometric detector, is that all of the eluting component can be oxidized at a certain potential. With two electrodes coupled in series, it is thus possible to pre-react the more easily oxidizable components, generally interfering components at a low potential on the first electrode, and then detect the component of interest at a higher potential on the second electrode. This results in a lower background current, and consequently a lower noise level and a cleaner chromatogram. The best detection potential was established by constructing a hydrodynamic voltammogram and plotting the detector response (area under the curve, AUC) versus the applied potential. Analysis of the current–voltage response for buprenorphine and its metabolite showed a plateau indicating full oxidation of the electrochemically active group at a potential of about + 0.50 V (Fig. 4).

Maximum buprenorphine peak height was reached at 0.50 V potential on the second electrode. This was taken as 100% oxidization. The first electrode was set at a potential of 0.15 V, resulting of about 10% oxidization of both drugs.

Linear calibration curves were obtained from 0.02 to 2.0 ng/mg with a correlation coefficient $r > 0.99$ for both drugs. The detection limit for the major metabolite was about 0.01 ng/mg and for buprenorphine 0.02 ng/mg, using a 50 mg hair sample. The interday precision, measured at 0.2 ng/mg, was 6.9 and 8.8% for norbuprenorphine and buprenorphine, respectively. Recovery, studied at 0.2 ng/mg, was 54 and 62% for norbuprenorphine and buprenorphine. No interferences were noted with morphine, and other opiates since their retention times were quite different (Table 2).

Buprenorphine was found in the hair of all 14 subjects and norbuprenorphine was found in eleven subjects. Concentrations were in the range 0.02 to 0.59, and not detected—0.15 ng/mg for buprenorphine, and norbuprenorphine, respectively. Hair concentrations are presented Table 3. As it is generally the case for other drugs, the concentrations of the metabolite

TABLE 2—*Retention times (min) of potential interfering compounds.*

Compound	Retention time (min)
Morphine	3.67
Codeine	Not detected (4.28)
6-acetylmorphine	5.55
Ethyl-morphine	8.36
Norbuprenorphine	12.38
Buprenorphine	31.48
Methadone	>50
Propoxyphene	>50

TABLE 3—*Buprenorphine and norbuprenorphine hair concentrations (ng/mg).*

Subject	Buprenorphine	Norbuprenorphine
1	0.48	0.06
2	0.50	0.15
3	0.59	0.14
4	0.12	0.04
5	0.03	Not detected
6	0.18	0.07
7	0.42	0.12
8	0.53	0.03
9	0.02	Not detected
10	0.11	0.07
11	0.15	0.02
12	0.09	0.04
13	0.19	0.02
14	0.13	Not detected

were lower than those of the parent drug. Buprenorphine was present at concentrations approximately 2 to 18 times higher than norbuprenorphine.

These data provide strong evidence that buprenorphine can be detected in hair following administration.

One critical question about hair analysis that remains controversial is the relationship between intake dose and concentration in hair. Some papers reported a correlation between

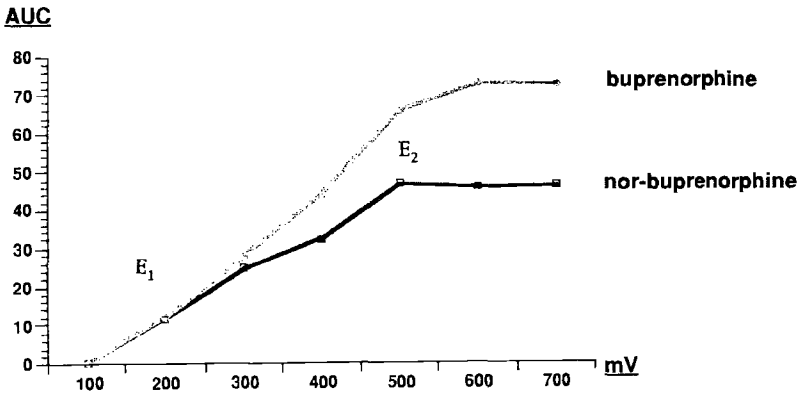

FIG. 4—*Hydrodynamic voltammogram of buprenorphine and its metabolite.*

TABLE 4—*Dose-response relationship.*

Medical reported dose	Buprenorphine (ng/mg)
0.2 to 2.0 mg/week	0.02–0.03
0.4 to 0.6 mg/day	0.09–0.13
1.2 mg/day	0.15–0.19
4.0 then 2.0 mg/day	0.42–0.59

the concentration of drugs in hair and the administered dose, while others have presented conflicting results. Although the limited subject data (14 subjects) in the present study preclude generalization, the results are suggestive that a dose-response relationship exists (Table 4).

The high sensitivity achieved by coulometric detection allows the investigation of buprenorphine content in small hair samples that can be collected without any aesthetic complications. This feature seems important for screening applications of this assay. HPLC was recently used for the analysis of morphine, haloperidol and β-blockers in hair [*9–11*]. In case of doubt, buprenorphine and norbuprenorphine can be accurately determinated by comparing the current-voltage curve obtained by application of different oxidation potentials for the case sample with a standard.

References

[*1*] Bullingham, R. E. S., McQuay, H. J., and Moore, R. A., "Clinical Pharmacokinetics of Narcotic Agonist—Antagonist Drugs," *Clinical Pharmacokinetics*, Vol. 8, 1983, pp. 665–673.

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

[*3*] Blom, Y. and Bonderson, U., "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.

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

[*5*] Cone, E. J., "Testing Human Hair for Drugs of Abuse. I. Individual Dose and Time Profiles of Morphine and Codeine in Plasma, Saliva, Urine and Beard Compared to Drug Induced Effects on Pupils and Behavior," *Journal of Analytical Toxicology*, Vol. 14, 1990, pp. 1–7.

[*6*] Baumgartner, W. A., Hill, V. A., and Blahd, W. H., "Hair Analysis for Drugs of Abuse," *Journal of Forensic Sciences*, Vol. 34, 1989, pp. 1433–1453.

[*7*] Moeller, M. R., "Drug Detection in Hair by Chromatographic Procedures," *Journal of Chromatography*, Vol. 580, 1992, pp. 125–134.

[*8*] Debrabandere, L., Van Boven, M., and Daenens P., "Analysis of Buprenorphine in Urine Specimens," *Journal of Forensic Sciences*, Vol. 37, 1992, pp. 82–89.

[*9*] Staub, C. and Robyn, C., "Analysis of Morphine in Hair by RIA and HPLC with Electrochemical Detection," *Proceedings of the 25th International Meeting TIAFT* Groningen 1988, pp. 220–229.

[*10*] Matsuno, H., Uematsu, T., and Nakashima, M., "The Measurement of Haloperidol and Reduced Haloperidol in Hair As an Index of Dosage History," *British Journal of Clinical Pharmacology*, Vol. 29, 1990, pp. 187–194.

[*11*] Kintz, P., and Mangin, P., "Hair Analysis for Detection of Beta-Blockers in Hypertensive Patients," *European Journal of Pharmacology*, Vol. 42, 1992, pp. 351–352.

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