Determination of Buprenorphine and Norbuprenorphine in Urine and Hair by Gas Chromatography–Mass Spectrometry

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Abstract

Buprenorphine, which is used in France as a substitution drug for opioid addiction, is widely abused, and several fatal cases have been reported. In order to confirm a recent intoxication or to establish retrospectively chronic abuse, a simple and reliable gas chromatographic–mass spectrometric method was developed and validated for quantitation of buprenorphine and its active metabolite norbuprenorphine in urine and hair. Two milliliters of urine or 50 mg of pulverized hair was submitted to a pretreatment (enzymatic hydrolysis for urine and decontamination with dichloromethane followed by incubation in 0.1M HCl for hair). Buprenorphine-d₄ was chosen as the internal standard. Selective solid-phase extraction with Bond Elut Certify™ columns provided recoveries higher than 85% for urine and 43% for hair. By using a mixture of MSTFA/TMSI/TMCS (100:2:5), buprenorphine and norbuprenorphine produced stable silylated derivatives. The detection was carried out with a quadrupole mass detector working in El selected ion monitoring mode. Ions at m/z 450 and 468 were chosen for the quantitation of buprenorphine and norbuprenorphine, respectively (m/z 454 was used for the internal standard). Limits of quantitation were 0.25 and 0.20 ng/mL, respectively, for buprenorphine and norbuprenorphine in urine and 0.005 ng/mg for the two compounds in hair. Calibration curves were linear from 0 to 50 ng/mL in urine and from 0 to 0.4 ng/mg in hair. Between-day and within-day precisions were less than 8.4% in hair and 6.1% in urine for both molecules in all cases. This method was applied to urine and hair samples collected from patients in a withdrawal treatment program and demonstrated its good applicability in routine analysis and its benefit for clinicians. This technique, which requires instruments already available to many toxicology laboratories, offers an attractive alternative to more sophisticated techniques.

Introduction

Buprenorphine, a semisynthetic derivative of thebaine is a narcotic analgesic. It binds to the μ opioid receptors with a high affinity as a partial agonist and to the κ opioid receptors as an antagonist. Consequently, it develops a ceiling effect on respiratory depression (1).

Since 1996, buprenorphine has been used in France as a substitution drug for opioids and is available more easily than methadone. Buprenorphine (high dosage tablets, Subutex®, 0.4, 2, and 8 mg) may be initially prescribed by general practitioners, but methadone must be initially prescribed by a psychiatrist in a “methadone clinic” (“centre de substitution”). More than 40,000 patients are maintained with buprenorphine, whereas only 6000 are maintained with methadone. It has been shown that buprenorphine is widely abused, and 20 fatal cases have been reported recently (2). Moreover, lethal intoxications with buprenorphine are probably underestimated, as few French laboratories have the ability to quantitate buprenorphine and its metabolite in biological fluids (3).

In this paper, a method for screening populations of addicts, for toxicological monitoring of withdrawal therapeutics, or for forensic applications is described.

A poor correlation between blood levels (a few nanograms per milliliter) and clinical effects or toxicity has been described for buprenorphine (2,3). Taking repetitive blood samples is not convenient with drug addicts undergoing withdrawal treatment. Therefore, urine, which is the most common biological sample analyzed in the case of drug addicts, was selected for this study. Buprenorphine was determined together with norbuprenorphine, its N-dealkylated metabolite, which is also present in urine, usually at even higher concentrations.

Information about recent exposure to a drug (2–3 days) acquired by urine analysis can be complemented by hair analysis, which can provide a retrospective view of drug intake over several weeks or months depending on its length (4,5). In order to benefit from this important information, hair analysis was included in this study.

Literature reports several analytical methods for the determination of buprenorphine and norbuprenorphine in biological samples.

Radioimmunoassay methods (RIA) were developed for urine and plasma analysis (6–8). They offer a rapid and very sensitive...
identification of buprenorphine and are well adapted to general screening situations. Unfortunately, two main drawbacks limit their application in toxicology: the legislation in course about the use of radioactive isotopes and the lack of specificity due to cross-reactivity with metabolites. An alternative assay to RIA, substituting a fluorescent marker for the classical radioactive one, led to a less sensitive detection (9).

Up to now, no EMIT or FPIA immunoassay has ever been developed to analyze buprenorphine and its metabolites.

Various modes of detection have been reported for gas chromatographic (GC) methods. The nitrogen phosphorus detector was not sensitive enough to detect buprenorphine concentrations lower than 50 ng/mL (10). The use of an electron capture detector enabled a 10-fold increase in sensitivity (11,12) or even a 100-fold increase after derivatization with heptafluorobutyric anhydride (10). Thermal instability was reported if no derivatization was performed before analysis (8,12,13).

Reversed-phase high-performance liquid chromatographic (HPLC) methods require no derivatization and are less time consuming. Among the classical modes of detection, fluorimetric detection was reported (14). This last method is simple and easy to perform, but it lacks sensitivity and specificity, for the detection of buprenorphine in the nanogram-per-milliliter range. HPLC with electrochemical detection (15–17) offers better sensitivity, allowing a limit of detection (LOD) of 0.02 ng/mg in hair (8,18). Nevertheless, this detection mode remains delicate to operate and is not well suited for routine screening.

Independently of the choice of the chromatographic separation method, mass spectrometric (MS) detection always provides better specificity. LODs in the range of 0.1 to 0.2 ng/mL could be reached using either the GC–MS in electron impact (EI) mode (6,13), or negative (NCI) or positive (PCI) chemical ionization mode (11,19,20).

The use of HPLC combined with MS was reported for the analysis of buprenorphine in hair (8,21) with an LOD of 0.02 ng/mg, in blood (21–23) with an LOD of 0.05 ng/mL, and in homogenates of viscerae (liver, brain, kidney, myocardium) with an LOD of 2 ng/g (3).

Recently, a GC–NCI method (24) and a
liquid chromatography–electrospray ionization (LC–ESI) method (19), both coupled with tandem mass spectrometry (MS–MS), were proposed for buprenorphine blood determination with limits of quantitation (LOQ) of 0.2 and 0.1 ng/mL, respectively.

These last methods (GC–MS–MS, LC–MS, and LC–MS–MS) provide a very good specificity and sensitivity, but the necessary equipment is rarely available in standard toxicology laboratories.

The procedure described in this paper enables the quantitation of buprenorphine and its active metabolite in urine and hair samples. We used the selective properties of the solid-phase extraction (SPE) and produced stable silylated derivatives for both compounds. Good sensitivity and specificity were reached with a GC coupled with a classical benchtop mass spectrometric detector operated in EI selected ion monitoring (SIM) mode.

**Experimental**

**Samples**

Drug-free urine and hair samples spiked with different concentrations of buprenorphine and norbuprenorphine were used for method validation. Positive urine and hair samples were collected from patients who had just been included in a methadone program or who were hospitalized for a withdrawal treatment. All subjects had a long history of buprenorphine abuse. Their age ranged from 24 to 46 years.

**Chemicals and materials**

Drug reference standards of buprenorphine, norbuprenorphine, and buprenorphine-d₄ (100 mg/L in methanol) were obtained from Radian (Austin, TX). Methanol (for pesticides analysis), dichloromethane (for trace analysis), and isopropyl alcohol (HPLC grade) were purchased from SDS (Valdonne-Peypin, France). Glacial acetic acid 100%, potassium hydroxide, potassium dihydrogen phosphate (Pro analysis), and ammonia solution 25% (Suprapur) were purchased from Merck (Darmstadt, Germany). Sodium acetate and hydrochloric acid 36% (RP Normapur) were obtained from Prolabo (Paris, France). B-Glucuronidase from Helix pomatia was supplied by Sigma (St. Louis, MO). N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was provided by Pierce Chemical Company (Rockford, IL). Trimethylchlorosilane (TMCS) and 1-(trimethylsilyl)imidazole (TMSIM) were from Fluka (Buchs, Switzerland). The GC column was a fused-silica capillary column (HP-5MS 5% phenyl methyl siloxane, 30 m × 0.25-mm i.d., 0.25-µm film thickness) from Hewlett-Packard (Wilmington, DE). SPE was performed with Bond Elut Certify cartridges for rapid extraction of drugs of abuse (type LRC, size 10 mL/130 mg of sorbent phase, Varian Sample Preparation Products, Harbor City, CA). SPE columns were positioned on a Vac Elut Vacuum manifold (Varian). Urine and hair were spiked with standards using a Hamilton Microliter Syringe 7105 (Hamilton Bonaduz AG, Bonaduz, Switzerland). Creatinine urinary dosages were conducted on an Hitachi 917® automatic analyzer (Boehringer Mannheim, Mannheim, Germany).

Working solutions of the drugs were prepared by diluting the standard solutions in methanol to obtain concentrations of 10 and 1 mg/L for buprenorphine and norbuprenorphine and of 10 mg/L for the internal standard. The solutions were kept in the dark at ~18°C for one month. HCl (0.1M) and 1M KOH were prepared by diluting concentrated solutions with deionized water obtained on a MilliQ water purification system (Millipore, Bedford, MA). Phosphate buffer (0.1M, pH 6.0) was made up of potassium dihydrogen phosphate (13.61 g) dissolved in deionized water and was adjusted to pH 6.0 with 1M KOH. The total volume was brought up to 1 L. Acetate buffer (2M, pH 5.0) was obtained by mixing 100 mL of a 2M sodium acetate solution (16.4 g sodium acetate/100 mL deionized water) with 32.2 mL of a 2M acetic acid solution (28.6 mL glacial acetic acid/250 mL deionized water). Buffers were stored at 4°C for one month. The working solution of β-glucuronidase was prepared daily with 15 mg of β-glucuronidase in 1 mL of 2M acetate buffer (pH 5.0). The silylating reagent was prepared by mixing 1000 µL of MSTFA, 50 µL of TMCS, and 20 µL of TMSIM; it was stored at 4°C for two weeks.

**GC–MS method**

**Sample pretreatment.** To prepare urinary calibration, seven aliquots of drug-free urine were spiked with buprenorphine and norbuprenorphine at concentrations of 0, 1, 2, 5, 10, 20, and 50 ng/mL. After addition of the internal standard (2 µL of buprenorphine-d₄ at 10 mg/L) and 250 µL of β-glucuronidase

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Compound</th>
<th>Concentration range</th>
<th>No. of curves</th>
<th>Slope†</th>
<th>Intercept†</th>
<th>Correlation coefficient (r²)†</th>
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<tbody>
<tr>
<td>Urine</td>
<td>buprenorphine</td>
<td>0-50 ng/mL</td>
<td>7</td>
<td>1.06 ± 0.05</td>
<td>-0.02 ± 0.03</td>
<td>0.999 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>norbuprenorphine</td>
<td>0-50 ng/mL</td>
<td>7</td>
<td>2.8 ± 0.1</td>
<td>-0.07 ± 0.07</td>
<td>0.998 ± 0.001</td>
</tr>
<tr>
<td>Hair</td>
<td>buprenorphine</td>
<td>0-0.4 ng/mg</td>
<td>10</td>
<td>0.84 ± 0.08</td>
<td>0.08 ± 0.05</td>
<td>0.998 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>norbuprenorphine</td>
<td>0-0.4 ng/mg</td>
<td>10</td>
<td>1.95 ± 0.07</td>
<td>0.07 ± 0.09</td>
<td>0.998 ± 0.001</td>
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</tbody>
</table>

*Calibration curves are expressed as y = b + ax. y = area ratio; x = concentration of analyte/concentration of internal standard. v = area ratio; peak area of analyte/peak area of internal standard.

† Mean ± SD
working solution, the calibration points and patient samples (2 mL) were incubated at 37°C for 20 h. Finally, 4 mL of 0.1M phosphate buffer (pH 6.0) were added before extraction.

Hair strands were cut into sections (usually 1 cm). Decontamination was achieved with two dichloromethane washes: hair was mechanically shaken by inversion in a 20-mL stoppered glass tube with 5 mL of dichloromethane for 5 min. After drying, hair samples were pulverized in a ball mill (Retsch MM 2000) for 15 min. Samples for calibration (50 mg) were prepared by spiking pulverized drug-free hair with buprenorphine and norbuprenorphine at concentrations of 0, 0.02, 0.04, 0.1, 0.2, 0.3, and 0.4 ng/mg. After addition of the internal standard (1 µL of buprenorphine-d4 at 10 mg/L), calibration samples were incubated at 56°C overnight in 1 mL of 0.1M HCl. Patient samples were spiked with internal standard and incubated similarly. After neutralization with 1 mL of 0.1M KOH and addition of 4 mL of 0.1M phosphate buffer (pH 6.0), samples were centrifuged for 10 min at 3500 rpm before extraction.

SPE and derivatization procedures. This extraction procedure was closely related to the one recommended by the manufacturer for the extraction of cocaine and benzoylecgonine.

After being positioned on the Vac Elut Vacuum manifold, the Bond Elut Certify LRC columns were conditioned with 2 mL of methanol followed by 2 mL of 0.1M phosphate buffer (pH 6.0). Then the samples were poured into the cartridge reservoirs and passed through the column at a flow rate of 0.5 mL/min. After that, the columns were washed with 6 mL of deionized water, dried under vacuum for 5 min, rinsed with 3 mL of 0.1M HCl, dried again under vacuum for 5 min, and washed with 5 mL of methanol. After drying under vacuum for 5 min, all of the compounds of interest were eluted successively by 2 mL and 1 mL of dichloromethane/isopropyl alcohol (80:20, v/v) with 2% ammonia solution (prepared daily) at a flow rate of 0.5 mL/min. The eluates were collected in a borosilicated glass tube. Samples were evaporated under nitrogen flow at room temperature. Residues were reconstituted in 0.5 mL of dichloromethane, transferred to a vial, and again evaporated under nitrogen at room temperature. To the dried extracts, 30 µL of silylating reagent was added, and the vials were heated at 65°C for 30 min. A 1-µL aliquot was injected into the chromatographic system.

GC-MS conditions. The quantitative analysis was performed using a Hewlett-Packard GC-MS system consisting of an HP 5973 mass selective detector (MSD), an HP 6890 series GC, and an HP 6890 series automatic liquid sampler. HP ChemStation software was used for data acquisition and processing. The initial oven temperature of 100°C was maintained for 1 min and then increased at a rate of 20°C/min to reach a maximum temperature of 300°C, which was held for 9 min. There was a final isotherm at 310°C for 2 min to purge the column. The injector system mode was splitless (45 s). The carrier gas was helium at a constant flow rate of 1 mL/min. GC-MS temperatures were as follows: injector 250°C, interface 300°C, source 220°C, and quadrupole 100°C. The MS was operated in EI mode at 70 eV. The electron multiplier voltage was set at +250 V above the autotune voltage. The MSD was autotuned daily with PFTBA. Ions at m/z 450, 482, and 506 served for buprenorphine identification; m/z 468, 500, and 524 served for norbuprenorphine identification; and m/z 454, 486, and 510 served for buprenorphine-d4 identification. Ions at m/z 450, 468, and 454 were selected for quantitation. The dwell time for the different ions was set at 30 ms. Identification was established by taking into account both retention times and relative abundance of qualifier ions. Concentrations were evaluated in patient samples with calibration curves calculated using peak-area ratios (analyte/internal standard) plotted versus concentration ratios.

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Concentration</th>
<th>n</th>
<th>Coefficient of variation (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Buprenorphine</td>
<td></td>
<td>Norbuprenorphine</td>
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<tr>
<td>Urine</td>
<td>2 ng/mL</td>
<td>10</td>
<td>2.9</td>
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<tr>
<td></td>
<td>10 ng/mL</td>
<td>10</td>
<td>3.7</td>
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<td></td>
<td>50 ng/mL</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Hair</td>
<td>0.04 ng/mg</td>
<td>10</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>0.2 ng/mg</td>
<td>10</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>0.4 ng/mg</td>
<td>10</td>
<td>3.4</td>
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<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Concentration</th>
<th>n</th>
<th>Coefficient of variation (%)</th>
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<tbody>
<tr>
<td></td>
<td>Buprenorphine</td>
<td></td>
<td>Norbuprenorphine</td>
</tr>
<tr>
<td>Urine</td>
<td>2 ng/mL</td>
<td>10</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>10 ng/mL</td>
<td>10</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>50 ng/mL</td>
<td>10</td>
<td>3.2</td>
</tr>
<tr>
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<td>0.02 ng/mg</td>
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</tr>
<tr>
<td></td>
<td>0.4 ng/mg</td>
<td>10</td>
<td>5.4</td>
</tr>
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</table>

Results

Chromatogram and mass spectra of buprenorphine-1TMS and norbuprenorphine-2TMS are presented in Figure 1. The two compounds appear well separated: their mean retention times were 17.70 and 15.38 min, respectively (Figure 1A).

Linearity

Linear correlation was found for buprenorphine and norbuprenorphine in the ranges 0 to 50 ng/mL for urine and 0 to 0.4 ng/mg for hair. The slopes, the intercepts and the average linear correlation coefficients (r2) for urine and hair are presented in Table I.

Limit of linearity

The limit of linearity was determined by extracting drug-free urine and hair samples.
spiked with increasing concentrations of buprenorphine and norbuprenorphine. The calibration curves of buprenorphine and norbuprenorphine were found to be linear over the range 0–500 ng/mL for urine and 0–10 ng/mg for hair.

Within-day and between-day precision
Within-day precision was calculated from repeated analysis of urine and hair, during one working day, by the same operator. It was calculated for three concentrations of buprenorphine and norbuprenorphine. Results are given in Table II.

Between-day precision was calculated from the analysis of samples at the same concentrations of buprenorphine and norbuprenorphine. One analysis was performed per day; results are given in Table III.

**LOD**

Figure 2 shows typical selected ion monitoring chromatograms, recorded from a urine extract, spiked with 2 ng/mL of buprenorphine and 2 ng/mL of norbuprenorphine. The LOD was estimated by extracting drug-free urine or hair samples spiked with decreasing concentrations of buprenorphine and norbuprenorphine until a response equivalent to three times the mean background noise registered on blank samples at the retention time of drugs tested was obtained. LOD was 0.10 ng/mL in urine and 0.002 ng/mg in hair for the two compounds.

**LOQ**

Similarly, the LOQ was estimated by testing decreasing concentrations of buprenorphine and norbuprenorphine until a response equivalent to 10 times the mean background noise was obtained. In urine, LOQ was 0.25 and 0.20 ng/mL for buprenorphine and norbuprenorphine, respectively. LOQ was 0.005 ng/mg in hair for the two compounds.

**Extraction recovery**

Extraction recovery, expressed as a percentage, was defined as the ratio of calibration curve slope of extracted analyte to calibration curve slope of non-extracted analytes. In all cases, buprenorphine-d₄ was added just before derivatization. The recoveries were 88% and 43% for buprenorphine in urine and hair; for norbuprenorphine, they were 85% and 51%, respectively.

**Discussion**

**Choice of the analyzed molecules**

Although norbuprenorphine shows a notably reduced analgesic effect compared with its parent compound (25), its analysis provides useful information. Norbuprenorphine respiratory depressant activity is estimated to be 10 times higher than that of buprenorphine (26). The study of parent compound/metabolite ratio can also give interesting information about the time of drug intake and patient biotransformation capacity. Presence of norbuprenorphine in biological samples brings the proof of drug intake. This positive result excludes an external addition of the drug to urine or an environmental contamination of hair.

**Sample collection and pretreatment methods**

As patients were hospitalized, urine was easily collected. In order to limit errors linked to daily variation in urinary dilution, especially when daily quantitative analysis had to be done, sampling was standardized by collecting the first morning urine. When this was not possible, creatinine dosages were achieved and concentrations were expressed as nanograms of buprenorphine per milligrams of creatinine.
Buprenorphine is metabolized to norbuprenorphine by N-dealkylation. Both molecules appear in urine mainly in the form of glucuronide conjugates (11). Before analysis, urine samples must be hydrolyzed to determine the total amount of buprenorphine and norbuprenorphine. A classical enzymatic method (27) that has already been used for other opiate derivatives was performed.

Collection of hair samples was standardized by cutting them in the region of the vertex posterior. This area presents less variability in hair growth rate (4,28–30). Hair analysis first requires a washing to remove external contamination, then an extraction of the drugs from the hair matrix. Various methods were used to liberate drugs of abuse; the most commonly used were HCl extractions, enzyme digestions, methanol extractions, and NaOH extractions. According to the results of Welch et al. (31,32), all techniques gave comparable results. The preparation method used here was described by Kintz et al. (8,33). It involves two washes with dichloromethane, pulverization in a ball mill, and incubation at 56°C overnight in 1 mL of 0.1M HCl.

Choice of internal standard

Buprenorphine-d₄ has been recently used for the determination of both buprenorphine and its metabolite, as norbuprenorphine-d₄ was not yet available (19,21–23). Norcodeine has been used as the internal standard for the determination of norbuprenorphine (24). Norcodeine was tested but not selected because of the lack of stability of its 2TMS derivative. Therefore, buprenorphine-d₄ was the only internal standard.

Choice of extraction method

Both liquid–liquid extraction (LLE) and SPE methods are reported in literature. Cleanliness of extracts is a determining factor in overall sensitivity. The important background noise observed after a single-step liquid extraction can be overcome by using a MS–MS detection method (19), but this technology is not yet widely used. Most authors proceed to multiple step LLE to limit the interfering peaks (11,13,21,34). These methods are delicate, lengthy, and lead to decreased recoveries.

In 1996, Kuhlman et al. (24), investigating different extraction methods, concluded that SPE was more rapid, reproducible, and efficient than LLE. They described an SPE method using Clean Screen (ZCDAU020) columns; the columns' phase combines both hydrophobic and cation exchange functional groups. Similar columns (Bond Elut Certify columns) had already been found satisfactory in our laboratory for the analysis of other morphinic drugs and dextropropoxyphene (35); their use was extended to buprenorphine analysis. Chromatograms of urine and hair extracts from two drug addicts are presented in Figure 3.

Recoveries obtained in urine were at least as satisfactory as those obtained with a multiple-step LLE method for buprenorphine and even higher for norbuprenorphine (21,22). The extraction with hair proved to be less efficient, which is consis-

![Figure 3](image-url)
tent with similar observations made with LLE (18). This result seems to be better explained by difficulties in releasing drugs from the hair matrix due to a holding-back phenomenon (31) rather than by real difficulties with the SPE method, which gives good results with other biological samples.

Choice of detection and derivatization methods

GC–MS was chosen for its specificity and sensitivity. When GC is used, it is necessary to derivatize polar functions of buprenorphine and norbuprenorphine in order to improve their chromatographic behavior. Several derivatizing agents have been proposed; many authors have used fluorinated reagents such as pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) (12,13,19,20,24); some were working in PCI mode (20) or in NCI mode (24).

Few authors have studied silylating reagents. Lloyd-Jones et al. (34) prepared monosilyl derivative with BSA after heating at 40°C for 15 min for GC–MS analysis. In 1993, Debrabandere et al. (6) chose MSTFA at 70°C for 15 min to measure buprenorphine in horse urine. As opiates are usually analyzed as their silylated derivatives, a first series of trials was conducted with bis(trimethylsilyl)trifluoroacetamide (BSTFA). Buprenorphine could be easily silylated on its hydroxy phenolic function, resulting in buprenorphine-1TMS. However, norbuprenorphine-2TMS was not produced in a reproducible manner because of the difficulty of silylating the amine function. Both norbuprenorphine-1TMS and -2TMS were present in an unpredictable ratio regardless of the temperature and the duration of the reaction. No improvement was observed with the addition of a catalyst such as TMCS. N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) possesses a high silylating power which can be increased by adding a catalyst, such as TMCS and/or 1-(trimethylsilylimidazole) (TMSIM). A mixture of MSTFA, TMCS, and TMSIM constitutes a powerful silylating reagent used to determine free steroids (36) or to silylate indolic amine functions (36). To detect nalbuphine, another morphine compound structurally related to buprenorphine, a mixture of MSTFA/TMSIM/TMCS (100:2:5) allowed 100% conversion to nalbuphine-3TMS, whereas the use of BSTFA even associated with TMCS led to both nalbuphine-2TMS and nalbuphine-3TMS (37). Using the same mixture, norbuprenorphine was quantitatively converted into norbuprenorphine-2TMS. After testing the influence of temperature and reaction time, optimal parameters were found to be 65°C and 30 min. Although thermal instability of buprenorphine has been re-

<table>
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<tr>
<th>Patient</th>
<th>Buprenorphine (ng/mg creatinine)</th>
<th>Norbuprenorphine (ng/mg creatinine)</th>
<th>Buprenorphine / Norbuprenorphine</th>
<th>Buprenorphine (ng/mg)</th>
<th>Norbuprenorphine (ng/mg)</th>
<th>Buprenorphine / Norbuprenorphine</th>
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<td>A</td>
<td>1041</td>
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<td>0.361</td>
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<td>B</td>
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<td>0.266</td>
<td>0.785</td>
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</tr>
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<td>0.470</td>
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</table>

Table IV. Buprenorphine and Norbuprenorphine Concentrations in Hair and Urine of Drug Addicts

ported in literature (8,12,13), we have never experimented any problem after derivatization of this molecule. Moreover the derivatives of both molecules remained stable during several days. Nevertheless, in the case of norbuprenorphine, deactivated glass liners were very important to preserve the integrity of the derivative-2TMS.

Quantitation method

Convenient performances in terms of both sensitivity and specificity were achieved with the use of the SIM method. In Figure 1B, the peak at m/z 539 corresponds to the molecular ion of buprenorphine-1TMS (C21H39NO4Si); other significant ion peaks are at m/z 450 (M−89; loss of CH3OH and C4H9) and m/z 482 (M−57; loss of C4H9) (6). The base peak (m/z 450) was chosen for the quantitation of buprenorphine, and m/z 482 and 506 were selected as qualifier ions.

Figure 1C shows the full scan mass spectrum of norbuprenorphine-2TMS. The molecular ion peak is at m/z 557 (C31H51NO4Si2). Buprenorphine-1TMS and norbuprenorphine-2TMS undergo a similar fragmentation. The base peak at m/z 468 was selected for quantitation and ions at m/z 500 and 524 as qualifier ions.

Validation parameters

Calibration curves were always linear through the examined concentration range. Given that concentrations can be very high in the urine of addicts, it was often necessary to dilute the patient samples. In the case of hair, limit of linearity was much higher than concentrations usually found in human samples.

Between-day and within-day precisions were systematically less than 10%, which is appropriate in terms of quality. Because SPE provides clean extracts, detection and quantitation limits were almost equivalent to values obtained through GC–MS–MS (24), LC–MS (21,23), or LC–MS–MS (19) analysis. In the case of hair, it should be noted that optimal instrument conditions were needed to achieve these values.

Clinical applications

Results presented in Table IV refer to urine and hair samples collected at the same time from five different patients. All of them had a long history of buprenorphine use at high doses and were candidate for a withdrawal treatment. Urinary concentrations of total buprenorphine and norbuprenorphine reach several hundreds or even thousands of
nanograms per milliliter or nanograms per milligram of creatinine. The buprenorphine/norbuprenorphine concentration ratio showed a great interindividual variability.

Buprenorphine and norbuprenorphine were found in the hair of the five subjects. Buprenorphine concentrations ranged from 0.060 to 0.360 ng/mg; norbuprenorphine was often found in even more abundant quantity, ranging from 0.029 to 0.785 ng/mg. Few reports are related to buprenorphine in human hair. Kintz et al. (8) determined buprenorphine concentrations in the range 0.020 to 0.590 ng/mg in the hair of 14 young drug addicts admitted to a withdrawal program; norbuprenorphine concentrations ranged from not detected to 0.150 ng/mg in the same subjects. For Tracqui et al. (21), concentrations measured in the hair of six addicts under substitutive therapy ranged from 0.004 to 0.140 ng/mg and from undetectable to 0.067 ng/mg for buprenorphine and norbuprenorphine, respectively. More recently, Valdez et al. (38) presented hair analysis of samples from four subjects admitted to a buprenorphine-treatment program. The authors observed a gradual trend of increasing hair concentrations over time and noted that in all cases norbuprenorphine metabolite hair concentrations were greater than those of the parent compound, as we obtained in many cases. It should be noted that this result is somewhat unusual as drugs are generally incorporated into hair according to their lipophilicity; this fact is well known for cocaine and 6-monoacetylmorphine (5).

Figure 4A displays the daily urinary elimination of buprenorphine and of its major metabolite concerning a drug addict hospitalized for voluntary withdrawal treatment. This patient had been consuming buprenorphine for one year at an average daily intake of 8 mg by the sublingual route. During his 8-day stay in the hospital, this patient suffered two relapses in buprenorphine intake that caused his eviction from the program. In this case, the buprenorphine/norbuprenorphine concentration ratio was systematically lower than 0.1. Variations in the metabolite concentrations were more significant than those in the parent compound and brought the proof of the patient moral contract breach.

Figure 4B shows the toxicological monitoring of another drug addict, taking 16 mg of buprenorphine per day by the intravenous route. He was hospitalized as the previous subject, but he did behave as agreed during the withdrawal treatment. His buprenorphine and norbuprenorphine urinary concentrations regularly decreased, reaching very low levels (nanograms per milliliter) after about 8 days, as generally observed after stopping the intake. This patient benefited, of course, from the whole withdrawal treatment.

In this case, as in other cases of withdrawal treatment without relapse, even if norbuprenorphine concentrations were initially higher than buprenorphine, norbuprenorphine could not be detected any longer than buprenorphine.

Figure 5 presents the toxicological hair monitoring of a third patient who had received 16 mg of buprenorphine per day by the intravenous route for 3 years, then was admitted to a withdrawal treatment. Concentrations of buprenorphine and norbuprenorphine were measured in 1-cm hair segments. During the period of chronic buprenorphine intake, hair concentrations were relatively constant. The buprenorphine/norbuprenorphine concentration ratio was close to 0.2, indicating
that norbuprenorphine was more incorporated than buprenorphine as previously observed. Then, the withdrawal treatment induced a decrease in hair concentrations until the levels were undetectable. Segmental hair analysis requires a lot of care in result interpretation until a precise knowledge about the dynamics of appearance and disappearance is acquired, especially for buprenorphine. A delay occurs between drug intake and drug incorporation into the hair (4, 5, 28–30). Valdez et al. (38) showed that there was a delay of several weeks after beginning buprenorphine treatment before buprenorphine and norbuprenorphine could be detected in hair. Hair is also known to grow approximately by 1 cm per month, but to define precisely the time of a particular drug exposure, it may be necessary to establish an accurate growth rate of hair for each patient (5, 28). Hair pigmentation also appears to be an important factor in drug incorporation.

After the withdrawal period, only four random urinary controls were conducted once each month. All were negative, but they could reflect the abstinence over the last few days only. Hair analysis, adjunct to urinalysis, brought a reliable proof of nonrecidivism.

Conclusions

An analytical procedure combining SPE and GC–MS analysis was developed to demonstrate a buprenorphine use or a drug abstinence. This method is sensitive enough for essaying buprenorphine and norbuprenorphine in urine and hair samples. A wide interindividual variability is observed in urine and hair samples. Norbuprenorphine appeared as a marker of buprenorphine intake in both matrices; however, the precise buprenorphine/norbuprenorphine ratio could not be defined. Norbuprenorphine appeared to be more incorporated in hair than its parent compound. Historical records constituted through hair segmentation coupled with another technique such as urinalysis can be very useful to monitor recidivism or abstinence in withdrawal treatments. This method, even if somewhat time consuming, can be applied to a series of samples and can be wholly automated. Its practice does not require too sophisticated or expensive equipment. As no immunoassay is yet available, it can be used as a “routine” method in clinical toxicology and forensic medicine laboratories.

References


Manuscript received May 26, 1998; revision received September 8, 1998.