Hair Analysis by LC–MS as Evidence of Nalbuphine Abuse by a Nurse

F. Klinzig, E. Vinner*, C. Brassart, E. Houdain, L. Humbert, and M. Lhermitte
Laboratoire de Toxicologie & Génopathies, Hôpital Calmette, CHRU Lille, Av du Pr. J. Leclercq, 59037 Lille, France

Abstract

Individuals in any profession can succumb to chemical abuse. Among the healthcare profession, nurses represent a specific group because of their ease of access to drugs, particularly narcotics. Opioids, potentially highly addictive agents, are usually their drug of choice. Nalbuphine, a synthetic opioid analgesic, is prescribed for moderate-to-severe acute pain, for chronic pain syndromes, and in obstetrics to decrease the adverse respiratory effect of opioid epidural administration. The case of a nurse who was suspected of drug misuse after the disappearance of two nalbuphine ampules in an obstetrics service is described. Because of discrepancies in the results of her blood and urine samples, a sample of head hair was subsequently collected from the nurse. A hair analysis of nalbuphine by liquid chromatography–mass spectrometry has not been previously described. Following decontamination and grinding, hair was mixed with a Söerensen buffer, then subjected to ultrasonic treatment (1 h), and extracted with ethyl acetate. A quantitative analysis was performed with two channels (30 and 45 V), and it is based on a m/z 358 for nalbuphine and a m/z 330 for methylclonazepam as an internal standard. The method was linear from 0.020 to 12 ng/mg of hair ($R^2 = 0.972$), and the limit of detection and limit of quantitation are 0.020 ng/mg. Accuracy (CV), assessed at 0.4 and 1.6 ng/mg of hair, was 6.18% and 5.77%, respectively, for intraday assays and 4.5% and 10.9% for interday assays. Recovery efficiency at 1.6 ng/mg and 8 ng/mg of hair was 100% and 97.4%, respectively. The hair specimen from the nurse (6 cm) was cut into three equal lengths. Nalbuphine, venlafaxine, and nordiazepam were detected. The concentration of nalbuphine was similar in the three hair locks: 5.07, 7.06, and 5.70 ng/mg of hair. A hair analysis revealed the repeated intake of nalbuphine by the nurse. This person was treated for depression for several months with Effexor® (venlafaxine) and Nordaz® (nordiazepam) prior to the investigation. Hair appears to be a unique matrix to provide evidence for chronic drug exposure by establishing a historic record that is not possible by blood or urine analysis.

Case History

The disappearance of two ampules of nalbuphine was noted in the stock of an obstetrics service in the north of France, and after investigation, a nurse affirmed that they were administered to two patients. Urine samples from these two women were then collected to determine the presence of nalbuphine and to confirm the statement of the nurse. Nalbuphine was screened for by LC–MS and was not detected in any sample. Consequently, the nurse was summoned to attend a formal meeting with the Occupational Preventive Medicine Panel. At this time, and as required, she brought with her a urine sample, and a blood sample was also collected during the consultation. The two matrices were subsequently screened by LC–MS and nalbuphine was neither detected in serum nor in urine. However, results of the analyses were contradictory in that nordiazepam was detected in...
the serum, and quinidine was detected in the urine. This raised the suspicion that the urine came from another person, and to verify this hypothesis, a head hair specimen from the nurse was therefore collected after an informed consent was obtained.

Experimental

Chemicals

Ethyl acetate, ammonium formate, methanol, and acetonitrile were purchased from Carlo-Erba (Italy), sodium hydroxide (NaOH) was from Riedel-de Haeı (Germany), formic acid was from Scharlau (Spain), and potassium dihydrogen phosphate (KH2PO4), di-sodium hydrogen phosphate (Na2HPO4), and dichloromethane were from Prolabo (France). Methylclonazepam and nalbuphine were generously provided by Roche (France) and Renaudin laboratory (France), respectively.

Solutions

Sørensen buffer. KH2PO4 (38.8 mL) (9.07 g/L) was mixed with 61.2 mL of Na2HPO4 (11.87 g/L) and adjusted to pH 7.6 with 1M NaOH. The buffer was stored for one month at ambient temperature in a dark flask.

Internal standard. A stock solution of methylclonazepam was made up in methanol at a concentration of 100 mg/L and stored at -20°C. The working solution (2.5 mg/L) was stored at 4°C for 1 month.

Nalbuphine stock solutions. A stock solution was prepared in methanol at a concentration of 400 mg/L and stored at -20°C. Two working solutions (I: 400 ng/mL, and II: 40 ng/mL), stored for 1 month at 4°C, were obtained by appropriate dilutions in methanol.

Mobile phase. The mobile phase was a mixture of 50 mM ammonium formate, pH 3.0, (A) and acetonitrile containing 0.05% of formic acid (B).

Head hair preparation

Head hair was collected in accordance with the Society of Hair Testing recommendations (9). To establish the validation criteria of the method, head hair without drug was used as the blank specimen. The nurse hair was cut into three lengths: root to 2 cm (40 mg); 2 to 4 cm (65 mg); and 4 to 6 cm (49 mg). Each hair specimen was decontaminated by two washes with CH2Cl2 (5 mL, 2 min) and then dried. Grinding was performed with a Retsch® MM 200 ball mill (Haan, Germany) at a frequency of 27 Hz (30 min). The powder obtained was stored in glass flasks at room temperature prior to analysis.

Calibration curve

Internal standard (IS) (50 μL) and appropriate volumes of solutions I or II of nalbuphine, to cover the anticipated concentration ranges of nalbuphine in hair from 0.020 ng to 12 ng/mg, were added to 15-mL glass tubes and evaporated to dryness over a slight stream of nitrogen at 60°C. Blank hair (50 mg) and 1 mL of Sørensen buffer were then added, and homogenization was conducted with an ultrasonic Branson® 3210 apparatus (Danbury, CT) for 1 h.

Analysis of nurse hair

Sørensen buffer (1 mL) was added to the entire quantity of each powdered length of the hair. An ultrasonic treatment was then applied as previously described.

Extraction procedure

Liquid-liquid nalbuphine extraction was performed with 2 mL of ethyl acetate. Tubes were rotated for 15 min and then centrifuged at 3500 rpm for 15 min. The upper organic phase was transferred to a glass tube and dried over a stream of nitrogen at 60°C. After cooling, the residue was dissolved in 100 μL of the mobile phase, and 20 μL was injected onto the LC column.

Chromatographic specifications

LC. The chromatograph used was a system Alliance, fitted with an X Terra®. MSC18, 2.1 x 150 mm, 3.5 μm column (Waters, Saint-Quentin en Yvelines, France). The column was conditioned with the mobile phase mixture of A/B (95:5, v/v) for 2 min. A gradient, at a flow rate of 0.2 mL/min, was then applied over a 16-min period to achieve an A/B mixture of 10:90 (v/v), and over a 4-min period to get a mixture of A/B 95:5 (v/v). This last specification was maintained for 6 min.

MS. The detector was a “Micromass ZQ” (Waters, Saint-Quentin en Yvelines, France). The capillary voltage was fixed to 3 kV. The desolvation and the cone gas flows were 300 and 150 L/h, respectively. The temperature of desolvation was fixed at 250°C, and the source temperature was 120°C. For the quantitative analysis, fragmentation was specifically performed in electron spray positive mode (ES+), acquisition was achieved in full scan mode (m/z 80 to 450) using two channels (30 and 45 V), and it was based on a mass-to-charge ratio of 358 for nalbuphine and a mass-to-charge ratio of 330 for methylclonazepam. The qualification of nalbuphine was based on a mass-to-charge ratio of 340. At 30- and 45-V channels, only two nalbuphine fragment ions (m/z 358 and 340) and one IS
fragment ion (m/z 330) were produced and used as quantifying and identifying ions. For the identification analysis of each length of nurse hair, specimen fragmentation was carried out in full scan mode (m/z 100 to 650) using seven channels (−30 V to 90 V).

Results

Validation data
The linearity was studied from 0.020 to 12 ng/mg of hair, and the linear regression equation was $Y = 25042 X$ ($R^2 = 0.972$).

Intra- and interday accuracy ($n = 6$) was assessed at concentrations of 0.4 and 1.6 ng/mg of hair. A CV of 6.2% and 5.8% was achieved for intraday assays, respectively, and a CV of 4.5% and 10.9% was achieved for interday assays, respectively. The recovery was studied at two concentrations and was 100% at a concentration of 1.6 ng/mg and 97.4% at 8.0 ng/mg. The estimated limit of detection (LOD) [signal-to-noise (S/N): 3/1] and limit of quantitation (LOQ) (S/N: 10/1) were 0.0024 ng/mg and 0.008 ng/mg, respectively, and the first concentration studied was 0.020 ng/mg, this concentration was chosen as the LOD and LOQ. Specificity was studied opposite to standard, IS, matrix, and matrix plus standard (± IS). No interference was noted at the retention time of nalbuphine. The stability of stock solutions was estimated to be 6 months at the storage conditions previously described.

Analysis of nurse head hair
Identification analysis by spectra library revealed the presence of nalbuphine, venlafaxine, and nordiazepam in each of the three hair locks. Figure 1 shows an example chromatogram obtained with the 2–4 cm length of hair (middle of the lock). Figure 2 presents the IS (m/z 330) (A) and nalbuphine (m/z 358) (B) chromatograms, with their respective retention times of 16.97 and 11.07 min when quantitative analyses were assessed in single-ion recording. Figure 3 confirms the presence of nalbuphine, showing its characteristic fragmentation spectra. The concentrations of nalbuphine quantified in the hair locks from root to 2 cm, 2 to 4 cm, and 4 to 6 cm were, respectively, 5.07, 7.06, and 5.70 ng/mg of hair.

Discussion
The identification and quantitation criteria of nalbuphine in head hair by this new LC–MS method were well satisfied and the technique could be applied to the nurse hair specimen. Nalbuphine concentrations detected in the three lengths of locks were approximately the same. These findings suggested the repeated intake of nalbuphine by the nurse for some months prior to the analysis (the length of the lock was 6 cm). Moreover, the presence of venlafaxine and nordiazepam was in accordance with the medical file of the nurse because she had been treated for depression with Effexor® (venlafaxine) and Nordaz® (nordiazepam) for several months. In contrast to nordiazepam, nalbuphine was not detected in the blood sample of the nurse because of its short half-life of 3 h. In urine, the presence of quinidine and the lack of venlafaxine, nordiazepam, and nalbuphine confirmed the hypothesis that the nurse did not provide her own urinary sample to the Occupational Preventive Medicine Panel.

Conclusions
The new LC–MS method described satisfied the criteria of nalbuphine identification and quantitation in head hair. Hair
appears to be the unique matrix to provide evidence of chronic drug exposure by establishing a historical record that is not possible by blood or urine analysis and to prove drug use in an individual.

References


Manuscript received June 26, 2006; revision received August 21, 2006.