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CASE REPORT

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Identification of Acepromazine in Hair: An Illustration of the Difficulties Encountered in Investigating Drug-facilitated Crimes

ABSTRACT: After a drug-facilitated sexual assault (DFSA), a woman was found in a drowsy state at home. She remembered having drunk an unknown beverage by the accused. Blood samples (collected 8 hours after the DFSA), two glasses, and a teaspoon seized by the police were analyzed. Acepromazine, a phenothiazine tranquilizer used in human and veterinary medicine, was detected in the residue of one of the glasses. In spite of acepromazine absence in the victim's blood, the possible use of acepromazine in the DFSA was reported to the police. Two weeks later, a suspect admitted having orally administered acepromazine to the victim. Using a liquid chromatography-tandem mass spectrometry method, this compound was subsequently detected (31 pg/mg) in a sample of the victim's hair collected a month and a half after the DFSA. A potential short elimination half-life in humans and/or the well-known *in vitro* degradation of acepromazine could explain the negative blood result. DFSA toxicological investigations are challenging and can be complicated when a rather unusual substance is concerned. In particular, special care should be taken when interpreting the results, taking into account elimination and/or instability data, when available.

KEYWORDS: forensic science, forensic toxicology, drug-facilitated sexual assault, acepromazine, hair analysis, liquid chromatography-tandem mass spectrometry

In recent years, there has been an increase in the number of reports of suspected drug-facilitated crimes, including drug-facilitated sexual assaults (DFSA), in France as well as in other countries. However, only a few well-documented identified cases have been reported in the toxicology literature, owing to the difficulties of such investigations (1–5).

The diagnosis of DFSA is sometimes difficult because of the often long delays between the attack and medical examination, and the difficulties of toxicological investigations owing to the numerous and diverse compounds or therapeutic classes of drugs potentially used. The effects expected by criminals can be obtained by impacting on many neuropharmacological mechanisms or combinations of mechanisms. However, some drugs (i.e., several benzodiazepines) seem to be the ideal candidates owing to their pharmacological properties (i.e., low blood concentrations, short elimination half-life) and practical (i.e. availability, formulations) properties. Recently, the Society of Forensic Toxicologists set up a committee to address the issue of DFSA in the toxicology field. This committee prepared a list of drugs that could be, or have been, used in DFSAs (6).

In our practice and in accordance with the guidelines published in the United States (7) as well as in France (8,9), it appears that the quality and promptness of biological specimen collection, the use of specific and sensitive analytical techniques, and the collaboration between the clinician and the toxicologist, are essential keys for successful toxicological investigations when a case of DFSA is suspected.

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Nevertheless, as a result of the criminal's imagination, this list of substances is not exhaustive and when unusual substances are involved in a DFSA case, extra care should be taken in the interpretation of the results, taking into account elimination and/or instability data (10). We report a DFSA case involving a veterinary drug and illustrating such interpretation difficulties.

Case History

A 29-year-old woman, living in a small village in the countryside, was found tied to the kitchen table in her house at 11:00 am by a neighbor. It was hard for her to make her statement as she was drowsy. After a few minutes, she related that in the morning when she was asleep in her bed, she was woken up by a man wearing a hood. He made her go to the kitchen where he forced her to drink an unknown brewage (≈ 8:00 am). In spite of memory lapses in her account, she remembered having her wrists and ankles bound and being subjected to sexual caresses.

Peripheral blood samples (4 × 5 mL collected under EDTA) were sampled at 4:00 pm and sent to our laboratory for toxicological investigations, together with two glasses and a teaspoon seized by the police in the kitchen. At the laboratory's request, two hair strands (brown hairs, 17-cm length) were collected one and a half months after the attack in the posterior vertex region of the victim's scalp.

Material and Methods

On receipt at the laboratory, the blood samples, the two glasses, and the teaspoon were frozen at −20°C and kept frozen until analysis. The hair sample collected a month and a half later was kept in a dry place at room temperature.

Screening of other drugs and toxicants was performed in the blood using both high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS).

Fourier transform infrared spectrometry was employed to directly analyze a part of the dry residues present on the two glasses and on the teaspoon. After addition of a methanol/water mixture (50:50, v/v) to the remains of the residues in the glasses and teaspoon, screening of drugs and toxicants was performed, after a purification step, using both HPLC-DAD and GC-MS.

Specific Blood Analyses

More selective analyses for several classes of therapeutic drugs or drugs of abuse of interest for DFSA cases were carried out in blood with various *ad hoc* dedicated methods: benzodiazepines with HPLC-DAD and liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS); gammahydroxy-butyrate, amphetamines, atropine and scopolamine, cannabinoides, opiates, ketamine, trihexyphenidyle and volatiles with GC-MS; neuroleptics with HPLC-DAD and GC-MS; LSD and meprobamate with liquid chromatography–electrospray–mass spectrometry (LC-ESI-MS).

A specific assay for acepromazine was performed in blood using an adaptation of a previously reported GC-MS method (11). Briefly, after the addition of cyproheptadine as internal standard (IS), 1 mL of blood was added to 1 mL of 0.25 M NaOH and extracted with 8 mL of a heptane/isoamylic alcohol mixture (98.5;1.5, v/v). 1 mL of HCl 0.1 M was added to the organic layer and back extraction of the aqueous layer was performed using 1 mL of 1 M carbonate buffer at pH 9.7 and 7 mL of the heptane/isoamylic alcohol mixture. After evaporation, the residue was reconstituted with 50 μL of ethyl acetate and 3 μL were injected in the chromatograph. Chromatographic separation and detection were achieved using a QP5000 (Shimadzu, France) GC-MS system equipped with a 30 m SPB 5 column, 0.32 mm i.d. (Supelco, France). The mass spectrometer was operated in the single ion monitoring mode using the following *m/z* for quantification (and confirmation): 326 (241, 58) for acepromazine and 287 (215,96) for cyproheptadine (IS). The limit of detection for acepromazine was 2 μg/L, and the within-batch relative standard deviations (RSD) and mean relative errors (MRE) were respectively < 10.2% and 8.8% over the calibrating range (5–200 μg/L).

Hair Analysis for Acepromazine

Chemicals and Reagents—Acepromazine as pure substance was supplied by Sanofi-France and chlorpromazine-D3 (internal standard) by Ceriliant (LGC Promochem, Molsheim, France). Propanol-2, dichloromethane, methylene chloride, and ammonia (Normapur) were purchased from Prolabo (Paris, France). HPLC-grade acetonitrile (Normapur) and methanol (Normapur) were obtained from Carlo-Erba (Milan, Italy). Formic acid and ammonium formate (>99% pure for both) were purchased from Sigma (St Louis, MO). Deionized water was prepared on a Direct-Q laboratory plant (Millipore, Bedford, MA). For sample extraction, Waters Oasis® HLB (3 cc/60 mg) extraction cartridges (Milford, MA) were used.

Stock solutions of acepromazine (1 g/L) and chlorpromazine-D3 (0.1 mg/L) were prepared in methanol and kept at −20°C in the dark: their stability was checked to be at least 3 months. Working solutions of acepromazine (at concentration of 0.5, 1, 5, 10, 25, 50, and 100 μg/L) were freshly prepared.

Sample Preparation and Solid Phase Extraction—Hair strands (from the victim's sample and blank hair samples obtained from volunteers) were decontaminated using a 5-min bath in water, followed by two 2-min baths in methylene chloride. After drying, the victim's hair shafts were segmented from root to top as follows: 0.5–2.5 cm (corresponding to the period of DFSA), 3–17 cm (corresponding to the months before DFSA). Each segment was then cut into small pieces (<1 mm length), as were the blank hair samples.

Calibrating standards were prepared by fortified blank hair samples with appropriate volumes of the different acepromazine working solutions in order to obtain the following levels: 0, 0.5, 1, 5, 10, 25, 50, and 100 pg/mg. About 50 mg of prepared blank or victim's hair were placed in a 15 mL glass vial and precisely weighed. Then the glass vials were incubated for 30 min at 50°C after addition of 1 mL of 1 N NaOH and 50 μL of the chlorpromazine-D3 solution (IS). After 5 min of vigorous shaking, each hair sample was directly applied on an Oasis® HLB cartridge, previously conditioned with 2 mL methanol followed by 2 mL water. Then the column was washed twice: first with 3 mL of methanol/water (40:60, v/v) and then with 3 mL of deionized water. After 15 min drying, the analytes were eluted with 3 mL of dichloromethane/propane-2l (75:25, v/v). The solvent was evaporated at 45°C with a gentle stream of nitrogen using a Zymark Turbo Vap (Hopkinton, MA) evaporator. The residue was reconstituted by adding 50 μL of 2 mM, pH 3.0 ammonium formate: 10 μL of the extract were injected in the LC-ESI-MS/MS system.

Chromatography and Detection—The chromatographic system consisted of a Shimadzu LC 10 ADvp pump with a SIL-Ht auto sampled, and a CTO-10ASvp column-oven. Chromatographic separation was performed using a XTerra MS C18, 5 μm (50 × 2.1 mm i.d.) reversed-phase column (Waters, Milford, MA) maintained at 25°C. The mobile phase was a gradient mixture of 2 mM, pH 3.0 ammonium formate (A) in 2 mM, pH 3.0 ammonium formate/acetonitrile (10:90, v/v) (B), programmed as follows: 0–1 min, 15% B; 1–6 min, 15–30% B; 6–7 min, 30–90% B; 7–8 min, decrease from 90% to 15% B; column equilibration with 10% B for 2 min.

Detection was carried out with a TSQ Quantum Discovery MS/MS system (Thermo-Electron, Les Ulis, France) equipped with an orthogonal electrospray ionization source and controlled by the Xcalibur computer program. Ionization was achieved using electrospray in the positive ionization mode. The following conditions were found to be optimal for the analysis of the compounds: spray voltage, 4 kV; capillary temperature, 200°C; sheath gas pressure (N2), 30 mbar; auxiliary gas pressure (N2) 10 mbar. One quantification (*m/z* 327 → *m/z* 86) and two confirmation (*m/z* 327 → *m/z* 58 and *m/z* 327 → *m/z* 254) transitions were chosen for acepromazine. Only one (quantification) transition *m/z* 322 → *m/z* 89 was chosen for chlorpromazine-D3 (Figs. 1a and 1f). The limits of detection and quantitation for acepromazine were respectively 5 and 10 pg/mg of hair. The within-batch RSD and MRE were respectively < 10.8% and 7.4% over the calibrating range (from 5 to 100 pg/mg).

Results and Discussion

Acepromazine was detected in the residue of one of the glasses by using our usual GC-MS general unknown screening method. No other drug was detected in this sample. No drugs were detected in blood. In particular, the absence of acepromazine in blood was verified. This compound was subsequently detected at the

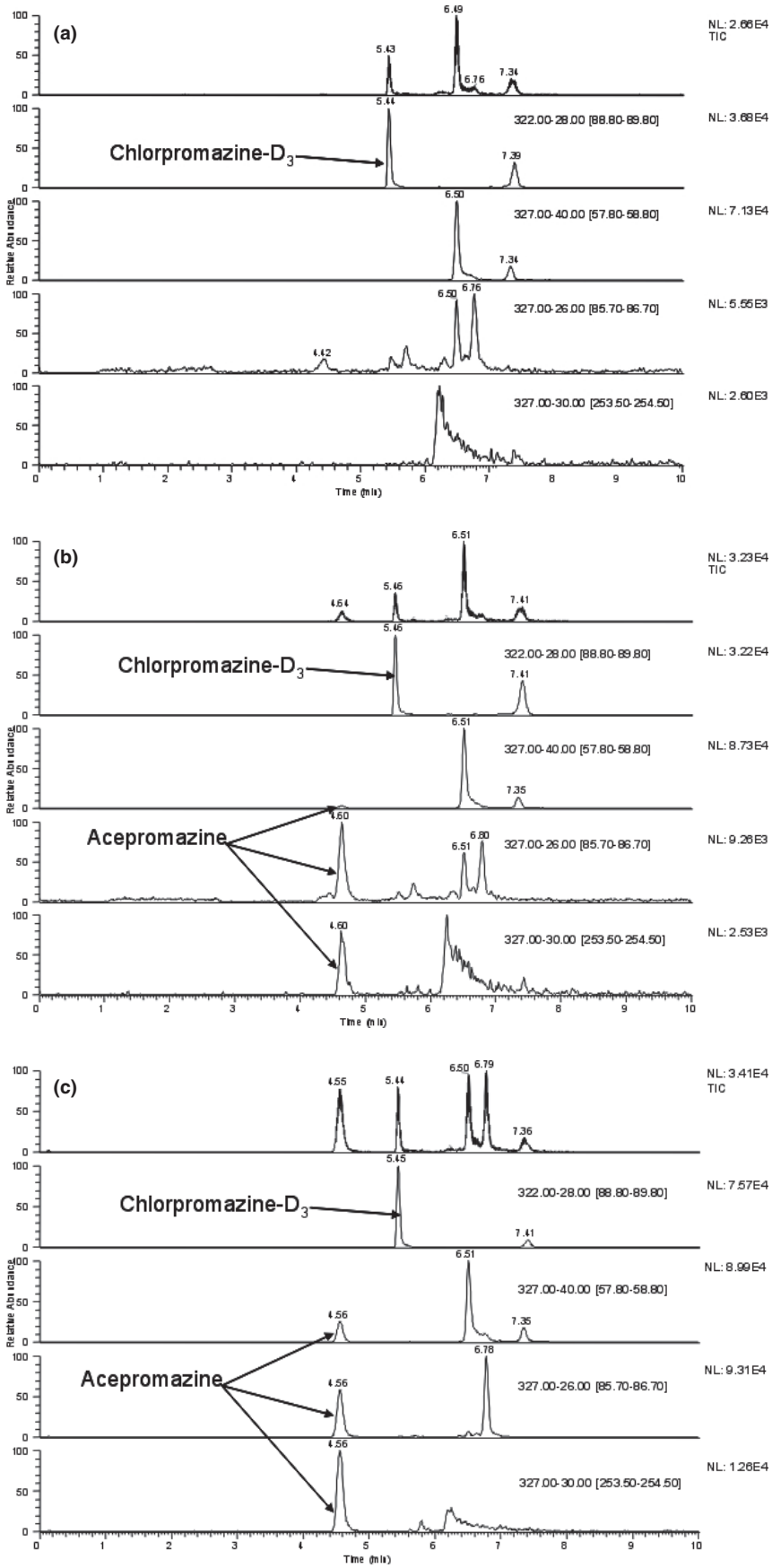

FIG. 1—*LC-ESI-MS/MS chromatogram of (a) a drug-free hair sample, (b) a hair sample containing acepromazine at LOQ concentration (10 pg/mg) and (c) the victim's hair extract (acepromazine concentration is 31 pg/mg).*

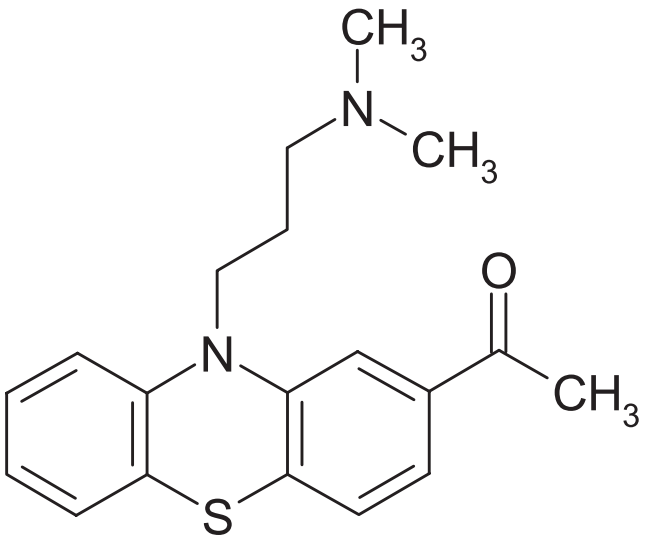

FIG. 2—*Chemical structure of acepromazine.*

concentration of 31 pg/mg in the 0.5–2.5 cm segment of the victim's hair collected one and a half months after the DFSA (corresponding to the period of the DFSA) (Fig. 1*c*).

Acepromazine is a phenothiazine-derivative (PHD) approved as a tranquilizer (Fig. 2). Its mode of action is only partially understood but it involves blockage of dopamine nerve receptors in the brain. It is a tranquilizer and also has an anti-emetic effect. Acepromazine is also classified as an antihistamine; however, because of its tranquilizing properties it would not be used as such. In intoxication cases (sometimes fatal), the main clinical effects observed are sedation and sinusal tachycardia (12–15).

In France, acepromazine is one of the constituents of a drug, Noctran®, usually prescribed in human medicine as a sedative. The tablets contain 0.75 mg acepromazine, 7.5 mg aceprometazine (another PHD), and 10 mg clorazepate (a benzodiazepine). In the present case, only acepromazine was found in the glass residue, while aceprometazine and clorazepate were not. Furthermore, this result was not consistent with the hypothesis of Noctran® administration, since aceprometazine and clorazepate doses are more than 10 times higher than that of acepromazine in the tablets and should have been detected by the analyses performed. Otherwise, acepromazine is one of the most commonly used tranquilizers for anesthetic premedication or immobilization, or for preventing vomiting because of motion sickness in veterinary medicine (16). It is available in France under the brand names of Vetoquinol® and Vetranquil® in tablet, granule, or injection forms. Therefore, the presence of acepromazine only in the residue was consistent with the use of a veterinary drug in the DFSA.

This last hypothesis also appeared compatible with the absence of acepromazine in the victim's blood sampled 8 h after the DFSA. Indeed, a potential (not documented) short elimination half-life and/or the well-known *in vitro* instability of acepromazine could explain this negative result.

There are very few human pharmacokinetic data for PHDs in the literature, and to the best of our knowledge, none for acepromazine. Generally, PHDs are characterized by high oral bioavailability and low therapeutic concentrations, owing to their high lipid solubility: i.e., brain concentrations can be 10 times higher than blood concentrations. As a consequence, blood concentrations verified and clinical effects are usually not correlated. The metabolism of PHDs is mainly hepatic (glucuronidation, sulfoxidation, demethylation, and hydroxylation) and some of the metabolites are pharmacologically active. Elimination is through urine, mainly as glucuronconjugated metabolites (17,18).

As there are no published data regarding acepromazine metabolism in humans, some veterinary data (i.e., in horses) can be taken into account (17,19). The acepromazine onset of action varies from 12 min to 1 h for oral acepromazine. The effects of acepromazine last from 1 to 6 h, but this varies significantly according to the dose and the individual horse. Acepromazine metabolism in animals seems to be extensive. Oxidation reactions lead to sulfoxide derivatives, which are further demethylated. The *N*-desmethylsulfoxide products can undergo hydroxylation on the aryl cycle before glucuronidation. The various metabolites obtained (i.e., 2-(1-hydroxyethyl)promazine) are mainly excreted in urine. Acepromazine elimination half-life in horses averages 2 h, with a high interindividual variability. A study in several horses following a 0.15 mg/kg i.v. dosage showed a biphasic elimination with an elimination half-life of the beta phase ranging between 10 and 149 min (20). Such data in animals cannot readily be extrapolated to humans. Nevertheless, the hypothesis of a short elimination half-life leading to a negative result of the blood analysis (<LOD of 2 μg/L) in a sample collected 8 h after the DFSA can be considered.

In vitro instability of acepromazine in blood has been reported by Elliott and Hale in 1999 (21). This spontaneous event was observed at 20°C in *postmortem* blood samples and lead to a nearly total degradation of acepromazine after 24 h: the degradation product was thought to be 2-(1-hydroxyethyl)promazine. In the present case, the storage conditions throughout the 2 days between blood sample collection and receipt at the laboratory are unknown. Therefore, *in vitro* instability might also explain, or have contributed to, the absence of acepromazine in the blood sample.

Furthermore, in the last 10 years of our forensic practice, we have noted 11 fatalities involving the intake of Noctran® tablets: acepromazine was detected in blood in only four cases, whereas very high concentrations of aceprometazine and clorazepate metabolites (nordazepam and oxazepam) suggested Noctran® intoxication. This observation is consistent with the hypothesis that acepromazine exhibits a short elimination half-life in human and is instable *in vitro*.

In the present case, the preliminary results (presence of acepromazine in the residue of glass, possible use of a veterinary drug in the DFSA in spite of acepromazine absence in blood sampled at 8 h) were reported on day 7 to the officer in charge of the investigations. About 2 weeks later, a suspect admitted the attack and the oral administration of acepromazine (previously prepared using Ventranquil, dose not specified). One of the consequences of his confession was the toxicologist's recommendation of acepromazine analysis in the victim's hair to document the case (22).

There is no specific data in the literature about the potential presence and/or concentration of acepromazine in hair following administration of this drug. Nevertheless, owing to their lipophilicity, antipsychotics including some PHDs (haloperidol, chlorpromazine, thioridazine, and cyamemazine) can generally be found in patients' hair (23–25). For instance, the hair concentrations of chlorpromazine (a phenothiazine with a molecular structure close to that of acepromazine) observed in 23 patients given 30–300 mg of chlorpromazine daily ranged from 1,600 to 27,500 pg/mg (26). Consequently, the presence of acepromazine (31 pg/mg) in the 0.5–2.5 cm segment of the victim's hair sample collected 1 and a half months after the DFSA is consistent with a single dose of acepromazine.

Following an authentic DFSA, unfruitful toxicological investigations in spite of the promptness of biological specimen collection, and the use of specific and sensitive analytical techniques, are so not rare. As a consequence, analyzing the paraphernalia and victims' alternative specimen increase the odds of detecting the drug(s) potentially involved, as has been the case with the glass residue and hair sample in the present DFSA case.

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