ORIGINAL ARTICLE

Unusual pattern in hair after prazepam exposure

Julie Maublanc a, Sylvain Dulaurent a, Laurent Imbert a, Pascal Kintz b, Jean-Michel Gaulier a,∗

a Laboratoire de pharmacologie et toxicologie, CHU Dupuytren, 2, avenue Martin-Luther-King, 87042 Limoges cedex, France
b X’Pertise consulting, 67205 Oberhausbergen, France

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Summary  Prazepam is known to be totally and quickly metabolized in nordiazepam, oxazepam, and 3-hydroxyprazepam after oral intake, and consequently to be undetectable in blood or in urine. The authors reported a case of positive findings of prazepam in hair. After a drug-facilitated sexual assault (DFSA), a large screening for benzodiazepines, hypnotics and other psychotropic drugs was performed in the victim’s hair sample, collected one month after the aggression. The positive analytical findings were firstly, zolpidem presence in the hair segment corresponding to the DFSA period (zolpidem was afterwards identified as the used weapon for the DFSA), and secondly, prazepam (together with nordiazepam) presence in all the analysed hair segments. This last result was in connection with the regular prazepam treatment of the victim (LYSANXIA, at a dose of 3 × 10 mg tablets per day). The presence in hair of a parent compound, which is not usually detected in blood or in urine is not incongruous, as attested by the presence of heroin in addicts’ hair. However, the possibility of prazepam presence in hair raises questions about the analytical methods that do not look for this benzodiazepine in hair, and worse still about those using prazepam as an internal standard. Indeed, in case of the presence of prazepam in hair, these analytical methods will not only be incapable of detecting prazepam, but, they will also underestimate the concentrations of other benzodiazepines sought at the same time.

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Introduction

Prazepam, 7-chloro-1-(cyclopropylmethyl)-1,3-dihydro-5-phenyl-1,4-benzodiazepin-2-one, is a benzodiazepine orally used for the treatment of symptoms associated with anxiety disorders and for short-term improvement of the symptoms of anxiety or anxiety

∗ Corresponding author.
E-mail address: jm-gaulier@unilim.fr (J.-M. Gaulier).

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associated with depression. After oral intake, prazepam (commercialized in France under the name LYSANXIA) is known to be totally and quickly metabolized ("first-pass" dealkylation or C3-hydroxylation by the liver) in nordiazepam, oxazepam, and 3-hydroxyprazepam. As a result, prazepam itself is considered to be usually not detectable in blood or in urine, with the exception of particular physiopathological contexts [1]. Consequently, prazepam is rarely included in toxicological assays in blood, urine or hair [2–4], and on the opposite, this benzodiazepine is used as an internal standard in analytical screening methods for benzodiazepines and/or specific determination methods of benzodiazepines in blood or urine [5–7] as well as in hair [8,9]. However, prazepam was recently detected in the hair of a 10-year-old child [10]. Nevertheless, in this case, the possibility of an external contamination to explain this finding was not completely excluded by the authors.

In order to assert this possibility of prazepam presence in hair, we report here a positive case of prazepam finding in hair.

**Case history**

A 25-year-old woman, who was experiencing marital problems, went to the police to report a drug-facilitated sexual assault (DFSAs) by her husband. Three weeks previously, after dining at home with him, she began to feel drowsy and, in spite of memory lapses in her account, she remembered having been sexually assaulted. In addition, she declared that she was taking a regular prescribed treatment with LYSANXIA, at a dose of 3 × 10 mg tablets per day.

Because of the long delay, no blood or urine samples were collected, and, in order to document this case, we were requested by the court to analyze a hair specimen of the victim, collected one month after the DFSAs.

**Experimental**

A large screening for benzodiazepines, hypnotics and other psychotropic drugs (alimemazine, alprazolam, bromazepam, buprenorphine, clonazepam, clonazepam, 7-aminoclonazepam, cyamemazine, diazepam, diphenhydramine, doxylamine, flunitrazepam, 7-amino-flunitrazepam, halo-peridol, hydroxyzine, ketamine, levomepromazine, loprazolam, lorazepam, lorazepam, lorazepam, LSD, methadone, midazolam, niaprazine, nordiazepam, oxazepam, prazepam, propoxyphene, scopolamine, temazepam, tetrazepam, tramadol, triazolam, zolpidem, et zopiclone) was performed in the hair specimen using a chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) method, according to a previously established method dedicated to LSD determinations in hair [11]. Briefly, hair strands (black hair, 8 cm length) were decontaminated using two 2-min baths in water, followed by two 1-min baths in dichloromethane. The last dichloromethane bath was kept to be tested for psychoactive drugs. After drying, the victim’s hair shafts were segmented from root to tip as follows: 0–2 cm (corresponding to the period of DFSAs), 2–4 cm, 4–6 cm and 6–8 cm (corresponding to several months before DFSAs). Each segment was then cut into small pieces (< 1 mm length). A 50 mg sample of each segment was used for the analysis. At the same time, the calibration curve was achieved in the 0.5 to 500 pg/mg range using free hair samples. After internal standards addition (buprenorphine-d₄, flunitrazepam-d₇, methadone-d₃, LSD-d₃ and scopolamine-d₃), hair and spiked hair were incubated in 3 mL of a phosphate buffer pH 5 and were submitted to gentle shaking for one night (16 h) at room temperature without sonication. After centrifugation (3000 rpm for 5 min), the supernatants were collected and 1 mL of 0.25 N NaOH, 5 mL of dichloromethane/ether (70:30, v/v) were added. Samples were shaken vigorously for 15 min and centrifuged (3000 rpm for 5 min). The organic phase was collected and subsequently evaporated to dryness at 30 °C under a gentle stream of nitrogen. The dry residue was reconstituted with 70 μL of formate buffer (2 mM, pH 3)/acetonitrile (90:10, v/v): 20 μL were injected into the LC-ESI-MS/MS system. The chromatographic system consisted of a Perkin-Elmer Series 200LC pumping system, and a Series 200 auto-sampler and the separation was performed on a C18 Atlantis T3 150 × 2.1 mm, 3 μm column (Waters™) at 200 μL/min flow rate. 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, 10% B; 1–9 min, 10 to 40% B; 9–14 min, 40% B; 14–20 min, 40 to 95% B; 20–24 min 95% B, 24–25.5 decrease from 95 to 10% B; column equilibration with 10% B during 7.5 min. A triple quadrupole mass spectrometer (Api 3200 QTRAP, AB Sciex™) in positive electrospray ionization mode was used for detection. The source parameters were a temperature of 600 °C, a nebulizer gas flow of 30 psi, and an auxiliary gas flow of 40 psi. Detection was performed using selected multiple reaction-monitoring mode in accordance with international recommendations [12] using one transition for quantification (i.e. m/z 325 → 271, for prazepam; m/z 321 → 275 for flunitrazepam-d₇, used internal standard for prazepam) and one transition for confirmation (i.e. m/z 325 → 140 for prazepam). In these conditions, the detection limits were between 0.5 and 10 pg/mg according to psychoactive drugs (i.e. 0.5 pg/mg for prazepam), and inter-assay precision CVs and relative bias were less than 20% over the calibrating range (i.e. 1.0 to 500 pg/mg for prazepam).

**Results and discussion**

The positive analytical findings in the victim’s hair are presented in Table 1.

Zolpidem was found only in the first hair segment, and at a low concentration, which is consistent with a single exposure to zolpidem [13]. In this DFSAs case, this result was reported to the officer in charge of the investigation, and the suspect (the victim’s husband) subsequently admitted the aggression and the oral administration of zolpidem (which was one of his own medications) to his wife.

In accordance with other authors [4], the screening method used for psychoactive drugs in hair includes the search for prazepam. The presence of prazepam and nor-diazepam in significant concentrations was highlighted in all...
segments of hair (no prazepam was detected in the second dichloromethane decontamination bath): that is in connection with the regular treatment (LYSANXIA) of the victim. It is of note that:

- if the first metabolite of prazepam (nordiazepam) was detected, oxazepam (which is both the metabolite of nordiazepam by C3-hydroxylation, and the metabolite of 3-hydroxyprazepam by dealkylation) was no;
- the nordiazepam/prazepam ratio decreased from the scalp to the end of the hair strand.

This last observation could be the result of an instability of nordiazepam and/or the result of normal hair hygiene that might progressively wash out this relatively more polar metabolite from the hair.

**Conclusion**

The presence in hair of a parent compound, which is not usually detected in blood, or in urine, is not incongruous, as attested by the presence of heroin in addicts’ hair in spite of its very fast degradation (<5 min) in monoacetyl-6 morphine in blood [14]. This finding raises questions about the methods that do not look for this benzodiazepine in hair, and in particular about those which use prazepam as an internal standard. Indeed, not only these last analytical methods will not detect prazepam, but in the case of the presence of prazepam in hair, they will also widely underestimate the concentrations of other benzodiazepines sought at the same time.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**References**