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ORIGINAL ARTICLE

Characterization of metizolam, a designer benzodiazepine, in alternative biological specimens

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Sweat

Summary Designer benzodiazepines provide an attractive alternative to prescribed benzodiazepines for abuse purposes as they are readily available over the Internet at a low price. Although these compounds have been developed by pharmaceutical companies, at this time, they do not have a legal status. Their detection in biological fluids is challenging owing to the very few available data. For instance, there is no information about metizolam (also known as desmethyletizolam) analysis, distribution and metabolism in the scientific literature. Metizolam was ordered via Internet and a 2 mg blue tablet was orally administered to a 54-year-old healthy man (85 kg). Oral fluid (saliva) was collected over 8 hours using the NeoSal™ device. Sweat was collected with the PharmChek™ sweat patch technology over 72 hours. Exhaled breath was collected with the ExaBreath® DrugTrap device over 15 hours. Beard hair was collected 4 and 10 days after administration. Finally, head hair was collected 3 weeks after administration. Metizolam was detectable in saliva during 8 hours, with concentrations lower than 1 ng/mL (max at 0.77 ng/mL after 2 hours). Increasing concentrations up to 186 pg/patch (after 72 hours) of metizolam were detected in the sweat patches. Metizolam produced for 4 hours a very low but significant chromatographic signal in exhaled breath, with concentrations always lower than 10 pg/filter. Finally, metizolam tested positive in beard hair (0.73 and 0.28 pg/mg after 4 and 10 days, respectively) and head hair (0.27 pg/mg). From the data obtained from one subject,

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metizolam showed very low concentrations in various alternative matrices, attesting that the potential forensic implications (drug-facilitated crime, addiction, DUID...) of metizolam abuse must be considered as a new challenge for toxicologists.

Introduction

Benzodiazepines are central nervous system depressants commonly prescribed to treat anxiety, insomnia, and seizures. Benzodiazepines are considered safer alternatives to barbiturates because they have fewer side effects and have a reduced risk of causing an overdose. However, benzodiazepine abuse is often associated with people taking benzodiazepines in combination with other controlled substances such as opiates, methadone, and cocaine. Benzodiazepines are taken in combination with opiates in particular because they enhance the euphoric effects of opiates; for other drugs, such as stimulants, benzodiazepines may temper the negative side effects (e.g., restlessness, agitation).

Although not approved for use, new benzodiazepines have been observed in forensic cases (death, acute intoxication, drug-facilitated crime, driving under the influence of drug). This includes phenazepam [1,2], etizolam and pyrazolam [3], flubromazolam [4], or clonazolam, meclonazepam and nifoxipam [5].

The detection of designer benzodiazepines in biological fluids is challenging owing to the very few available data. Some of these drugs have been detected using immunoassays with high cross-reactivity [3,6], demonstrating that designer benzodiazepines can be detected in standard blood or urine immunoassay drug screening. Other authors have developed liquid chromatography mass spectrometry methods, mostly to study the metabolism of specific drugs, including clonazolam, meclonazepam and nifoxipam [5], pyrazolam [7], flubromazepam [8], diclazepam [9], and clonazolam, deschloroetizolam, flubromazolam and meclonazepam [10]. Among the designer benzodiazepines group, the thienodiazepines, obtained after replacement of the benzene ring by a thiophene ring, are represented by etizolam (licenced for use in Japan, Italy and India), and more recently by metizolam (Fig. 1), also known as desmethyletizolam. Metizolam differs structurally from etizolam through removal of the methyl group on the triazole ring. Although etizolam has already been described in overdose [11] and death cases [12], there is no citation for metizolam (PubMed, consultation on 24 July 2016). For instance, there is no data about metizolam analysis in alternative biological specimens, such as hair, saliva or exhaled breath. This compound was patented by a Japanese company in 1995, as an anti-anxiety medication. It has no history of human usage prior to its availability by online vendors in September 2015.

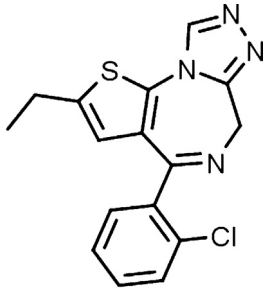

Figure 1. Chemical structure of metizolam.

A UPLC-MS/MS technique were used to detect metizolam in saliva, hair, sweat and exhaled breath after administration of an oral dose of 2 mg to a human volunteer.

Material and method

Self-administration study

To gain preliminary data on the detectability of metizolam in various alternative specimens, one of the authors (male, 54-year-old, 85 kg) ingested one blue tablet (declared 2 mg metizolam) with 200 mL of tap water. Oral fluid was collected over 8 hours using the NeoSal™ (Neogen) device and used as recommended by the manufacturer, by handling the pad in the mouth of the subject for 2 min. Immediately thereafter, the pad was placed into the diluent, present in a plastic tube. Sweat was collected with the PharmChek™ (Sudormed) sweat patch technology over 72 hours. Four patches were placed over the skin of the right arm at T0 and periodically removed. Exhaled breath was collected with the ExaBreath® DrugTrap Sensa Bues™ device (Sensa Bues™) over 15 hours. This device consists of a filter that traps the aerosols (that contain the drugs) from breath. The test was complete after 20 breaths through the device, which approximately corresponds to 20—30 litres of breath going through the filter (about 2 min). No mouth rinsing was performed prior to breath sampling. Beard hair was collected 4 and 10 days after administration. Finally, head hair was collected 3 weeks after administration. Except hair, all specimens were stored at +4 °C until analysis.

In France, approval by an ethics committee is not required for self-experiments (except for drugs of abuse, that is not the case for metizolam).