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Detection of diazepam in urine, hair and preserved oral fluid samples with LC-MS-MS after single and repeated administration of Myolastan® and Valium®

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Sedative agents are used to facilitate sexual assault due to their ability to render the victim passive, submissive and unable to resist (Drug Facilitated Sexual Assault; DFSA). Most of these substances possess anterograde amnesic properties and can rapidly impair an individual. Benzodiazepines and related hypnotics are the most frequently observed compounds in these cases. Urine is almost exclusively used as the sample of choice in DFSA situations, however, it has recently been reported that hair may also be a valuable matrix for detecting drugs that have been ingested more than several days prior to sample collection.

AIMS: Following the analysis of a hair sample from a person using Myolastan® (tetrazepam) in therapeutic doses, significant concentrations of diazepam (ranging from 1.0 to 8.2 pg/mg) and traces of its metabolite, nordiazepam, were noted. However, diazepam and nordiazepam are not known metabolites of tetrazepam. Analysis of one tablet of Myolastan® also showed the presence of diazepam (0.17% of the amount of tetrazepam). In a study involving the controlled administration of a single dose of Myolastan® and Valium® (containing diazepam only), urine and hair samples (after 3 weeks) were collected. The study included four persons receiving either one tablet of Myolastan® (50 mg) or Valium® (10 mg). In the same study, oral fluid samples were collected (Intercept® device) at regular time intervals.

METHODS: Urine and hair samples were analyzed using a recently published LC-MS-MS method for the quantification of 26 benzodiazepines and metabolites, zolpidem and zopiclone in blood, urine and hair 1. Briefly, drugs were extracted from all matrices by liquid-liquid extraction with 1-chlorobutane after the addition of internal standards. Hair samples (20 mg) were pulverized and incubated with methanol (2h at 45°C) prior to sample clean-up. Analytes were separated on a Gemini C18 column (150 × 2.0 mm, 5 µm) (Phenomenex), using a gradient elution with 0.1% formic acid (A) and methanol (B).

RESULTS: After a single administration of Myolastan®, tetrazepam could be detected for more than 280 hours in urine (n = 2). Diazepam could be detected in the same urine samples for 168 and 228 hours respectively. Only traces of its metabolite, nordiazepam, were observed. However, after the administration of a single dose of Valium®, no diazepam could be detected in the urine. Its metabolites, nordiazepam, oxazepam and temazepam were noted in the urine samples for more than 280 hours.
Unlike the situation with chronic therapeutic intake of Myolastan®, the analysis of hair samples after a single dose showed the presence of only tetrazepam (17.3 and 59.7 pg/mg respectively in the first segment of both persons) and no diazepam was detected. After a single dose of diazepam, both diazepam and nordiazepam were noted in the first two segments of both persons in this group. The concentrations were 5.3 and 5.6 pg/mg diazepam and 3.8 and 3.9 pg/mg nordiazepam for the first segment for both persons respectively.
The analyses of the preserved oral fluid samples of these individuals are ongoing and will be presented.

CONCLUSIONS: These results show that caution should be made in cases of DFSA were diazepam is detected. The results of this study indicate that after a single dose of Myolastan®, diazepam could also be detected in urine over at least 168 hours.
However, the metabolisation pattern is different compared to a single diazepam administration. In addition, hair analysis can be an important complement to urine analysis to document exposure to Myolastan® or Valium®.

REFERENCE:

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