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CASE REPORT

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Detection and quantification of lorazepam in human hair by GC-MS/NCI in a case of traffic accident

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Abstract A traffic accident caused by a man who declared that he was driving under influence of drugs (Temesta), led our laboratory to develop a procedure for the detection and the quantification of lorazepam in human hair. The method involves decontamination of hair with dichloromethane, incubation in Soerensen buffer (pH 7.6) in the presence of lorazepam-d_4, liquid-liquid extraction with diethylether-chloroform (80:20, v/v) at pH 8.4, derivatization by silylation and detection by GC-MS/NCI. The increasing concentrations of lorazepam from the end to the roots of a 16-cm-long hair strand (i.e. 31 pg/mg, 40 pg/mg and 49 pg/mg) proved that the driver had taken the drug over a long period of time.

Key words Lorazepam · Hair · GC-MS/NCI · Traffic accident · Drug influence

Introduction

Lorazepam (7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1,4-benzodiazepin-2-one) is used as an anxiolytic and tranquillizer drug. Benzodiazepines such as lorazepam are now widely prescribed for various reasons: as an anxiolytic, as centrally acting muscle relaxants, as hypnotics, tranquillizers, and anticonvulsive drugs, and in the treatment of psychiatric disorders. Therefore, members of the benzodiazepine family, alone or in conjunction with alcohol, morphinomimetics (analgesics and antitussives), antidepressants, sedatives or neuroleptics, could frequently be involved in traffic accidents. Benzodiazepines have frequently been reported in cases of driving under the influence of drugs [1, 2], as have cannabis [3, 4], cocaine and amphetamines [5, 6].

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This report presents the results of an investigation based on hair analysis for lorazepam in a case of a traffic accident caused by a man under treatment with Temesta.

Case report

A 57-year-old man who was under treatment with Temesta (lorazepam, 1 mg) declared to the justice that he was currently taking large amounts of the drug because of persistent anxiety. According to his statement to the police he had been taking increasingly large amounts for several months. He had no memory of the accident and declared that he was driving under the influence of the drug.

Materials and methods

Head hair strands of 16 cm length were cut as close as possible to the skin and stored dry at room temperature. Before toxicological analyses, the hairs were twice decontaminated in methylene chloride (2 min at room temperature) and pulverized in a ball mill (Retsch MM2 type, Haan, Germany). Lorazepam was extracted using the Kintz et al. [7] procedure for nordiazepam. Approximately 50 mg of powdered hair were incubated in Soerensen phosphate buffer (pH 7.6), for 2 h at 40° C in the presence of 5 ng deuterated lorazepam (LOR-d_4). The homogenate was directly extracted with 5 ml of diethylether-chloroform (80:20, v/v) at pH 8.4. After agitation and centrifugation, the organic phase was removed and evaporated to dryness. The residue was derivatizated by silylation at 60° C for 20 min using 35 µl BSTFA + 1% TMCS.

A 1-µl portion of the derivatizated extract was injected onto the column of a Hewlett Packard (HP) 5890 gas chromatograph via a HP 7673 autosampler. The flow of carrier gas (helium, purity grade N55) through the column (HP-5MS capillary column, 5% phenyl-95% methylsiloxane, 30 m × 0.25 mm i.d.) was 1 ml/min. Injector temperature was 250° C and splitless injection was used with a split-valve off-time of 1 min. Temperature column was programmed to rise from an initial temperature of 60° C, kept for 1 min, to 295° C at 30° C/min and kept at 295° C for the final 5 min. The detector used was a HP 5989 B Engine operating in negative chemical ionization (NCI) mode. The ion source temperature was 200° C and 100° C for the quadrupole. The electron multiplier voltage was set at + 500 V above the NCI tune voltage. Methane was used as gas reactant at an apparent pressure of 1.4 torr in the ion source.

Mass spectra were recorded in the mass range 100–580. Quantitative results were obtained in single ion monitoring (SIM) mode by comparison of the retention time (t_R) and confirming ion (m/z 302) with those of LOR-d_4 (m/z 306).

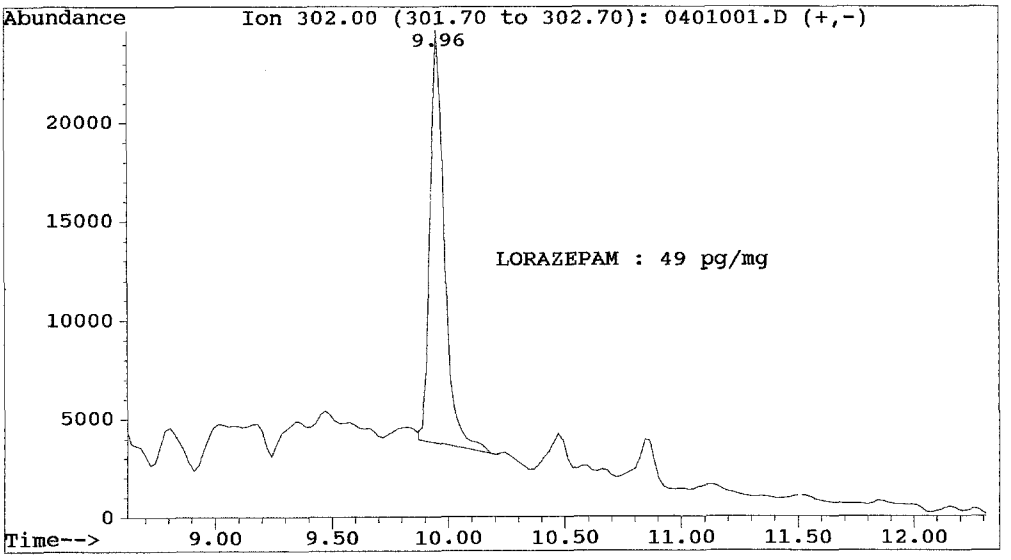

Fig. 1 SIM chromatogram (m/z 302) of the proximal hair segment positive for lorazepam. The concentration determined was 49 pg/mg of hair using LOR-d_4 (m/z 306) as the standard

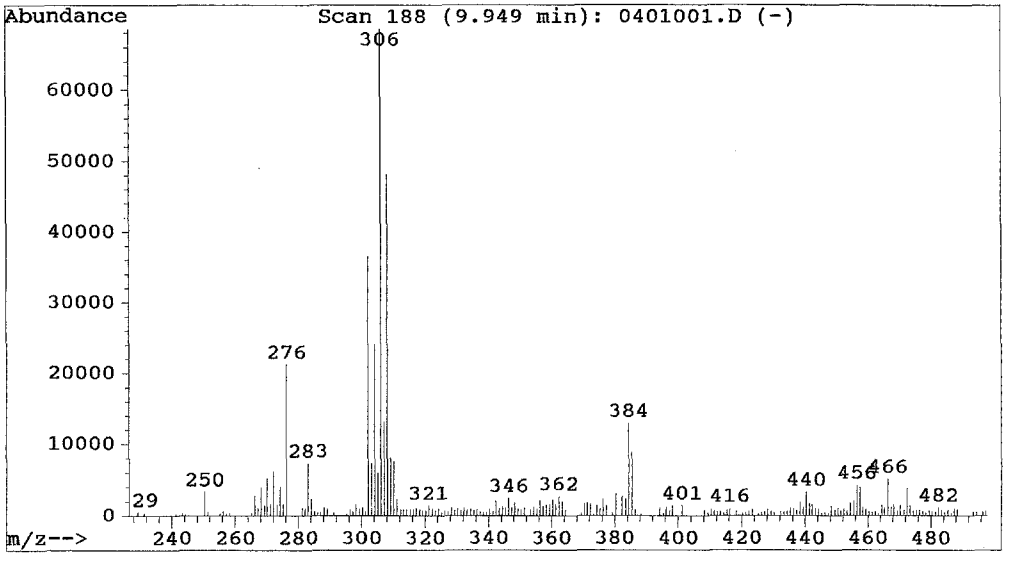

Fig. 2 Electron impact mass spectrum of lorazepam and LOR-d_4 at the t_R 9.96 min

Results and discussion

Under the chromatographic conditions used, there was no interference with the drug and LOR-d_4 by any extractable endogenous materials present in hair. The SIM chromatogram of lorazepam (m/z 302) is shown in Fig. 1. At the t_R 9.96 min, a concentration of 49 pg/mg of lorazepam was determined. The electron impact mass spectrum of lorazepam and LOR-d_4, obtained from the Fig. 1, is presented Fig. 2.

Standard calibration curves were obtained by adding 5, 25, 50 µl (0.1 mg/l), 10, 25, and 50 µl (1 mg/l) of lorazepam to 50 mg of pulverized blank control hair (drug free) and 5 ng LOR-d_4, resulting in final lorazepam concentrations of 10, 50, 100, 200, 500 and 1000 pg/mg of hair. The correlation coefficient of the calibration curve was 0.98, showing linearity between 10 and 1000 pg/mg.

Recovery and day-to-day precision were determined by adding 5 µl of lorazepam (1 mg/l) to 50 mg of powdered blank control hair (drug free) and 5 ng of LOR-d_4 (n = 8), corresponding to a final concentration of 100 pg/mg. Recovery was higher than 80% and day-to-day precision was 7.4%. The minimum detectable lorazepam quantitity, calculated for a signal-to-noise ratio (S/N) = 3, was 2 pg/mg.

The 16-cm-long hair strand was cut into four pieces from the roots to the ends, and the concentrations of lorazepam found in the different segments are summarized in Table 1. In accordance with the declaration to the justice, these results were suggestive of an increased use of Temesta during the test period. It is the first time that lorazepam has been detected in hair of a chronic abuser. No comparison with the literature was possible owing to the lack of reported data for lorazepam in hair. To date, only one paper [8] reports detection of benzodiazepines by radioimmunoassay. Diazepam was readily detected, but lorazepam and alprazolam were not found in subjects receiving therapeutic dosages. This was certainly due to a lack of sensitivity of the method used.

Table 1 Lorazepam concentrations found in the four hair segments corresponding to approximately 16 months of drug exposure

Segments (roots to end)	Lorazepam (pg/mg)
0– 2 cm	49
2– 5 cm	40
5– 9 cm	40
9–16 cm	31

In this study, the GC-MS apparatus represents the state of the art for the analysis of human hair for drugs of abuse. For lorazepam and for all benzodiazepines, derivatizated by silylation, the detector sensitivity was increased using NCI by comparison with other modes of ionization (electronic impact or positive chemical ionization). These compounds possess halogen groups (Cl = electronegative functional group) located on aromatic rings with high negative density that will give more stability to the complex (stabilization of the anions) and give a very good signal to noise ratio in NCI mode [9].

In conclusion, the first report on lorazepam detection and quantification in hair from a chronic benzodiazepine abuser shows the interest of the GC-MS system operating in the negative chemical ionization mode.

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