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TECHNICAL NOTE

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Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis

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Abstract A 44-year-old female nurse in a department of anesthesiology was found dead at home. An empty bottle of Hypnovel (midazolam 5 mg/5 ml) and a hypodermic syringe were found near the corpse. The nurse was a known abuser of anaesthetic agents for many years. A complete screening for general unknown substances by FPIA, GC/MS, head space GC/MS and HPLC/DAD revealed the simultaneous presence of midazolam, propofol and ethanol in femoral blood. Segmental analysis of a 6-cm-long hair strand revealed the presence of midazolam and propofol in each 2-cm-long segment. Repetitive consumption of the two anaesthetic agents during the last 6 months before the death was therefore demonstrated. These compounds were also detected in pubic and axillary hairs. Self-administration of midazolam and propofol without respiratory assistance and medical control certainly contributed to the death.

Keywords Midazolam · Propofol · Abuse · Hair · Mass spectrometry · Intoxication

Introduction

Midazolam (Hypnovel) and propofol (Diprivan) are two short-acting anaesthetic agents which are administered parenterally to induce anaesthesia. Midazolam, or 8-chloro-6-(*o*-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine, is metabolised in the liver to its major metabolite 1-hydroxy-midazolam (1-OH-M), which is less active than the parent drug [1]. The volatile anaesthetic propofol (2,6-diisopropylphenol) with sedative-hypnotic properties, is rapidly conjugated to form inactive metabolites and excreted in urine as 1- and 4-glucuronides [2].

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In recent years, reports in the literature have described deaths from midazolam or propofol intoxication [3, 4, 5, 6, 7, 8] and assays for the quantification of these two anaesthetic agents in biological fluids and tissues have been published. Blood and urine samples are usually employed to document drug consumption, however, the elimination of such drugs occurs within a few days. In contrast, hair is known to allow a drug administration to be traced back for months or even years and therefore offers the possibility of determining long-term drug exposure [9]. The international literature is very poor in reports dealing with midazolam or propofol determination in hair [8, 10] and only one paper reported midazolam excretion in beard hair from 4 patients receiving this drug over a relatively long period of time [10]. More recently, propofol was detected in head hair using a routine method for general unknown substances [8]. In the present case, chronic abuse of both anaesthetic agents midazolam and propofol was demonstrated by hair analysis.

The established methods involved head-space gas chromatography/mass spectrometry (HS-GC/MS) technology and GC/MS in the negative chemical ionization (NCI) mode of detection for the determination of propofol and midazolam, respectively.

Case history

A 44-year-old female nurse in a department of anesthesiology was found dead at home. An empty bottle of Hypnovel (midazolam 5 mg/5 ml) and a hypodermic syringe were found near the corpse. The body showed no signs of violence but several needle marks on the arms and a congestion of the lungs were noticed at autopsy but otherwise there were no remarkable findings. Femoral blood and different types of hair were collected for toxicological investigations. Head hair strands (6 cm long) were cut as close as possible to the skin with small scissors in the vertex posterior region and stored in dry plastic tubes at room temperature. No cosmetic treatment (e.g. perming, bleaching, dying etc.) of the hair was noticed. Axillary and pubic hair samples were also cut as close as possible to the skin with small scissors and stored in dry tubes at room temperature. The colour of all hair specimens was brown.

Toxicological analyses

Screening of blood was performed by immunoassay, liquid chromatography coupled with diode array detection (HPLC-DAD) and GC/MS either with NCI detection (midazolam) or head space (HS) preparation (propofol).

Two new procedures were developed for the identification of midazolam and propofol in hair.

Midazolam and 1-OH-midazolam in hair

After two successive washes in methylene chloride, hair was pulverised in a ball mill and 50 mg of powdered hair was incubated in 1 ml Soerensen buffer pH 7.6 overnight at 40°C in the presence of deuterated diazepam used as internal standard (50 ng). After extraction of the homogenate with 5 ml of diethylether/chloroform (80:20 v/v), the organic phase was evaporated and the dry extract derivatised with 35 µl of N,O-bis(trimethylsilyl)trifluoroacetamide and 1% trimethylchlorosilane. Analyses were performed on a GC/MS system operating in NCI mode. A 1.5 -µl aliquot of the derivatised extract was injected (injector temperature 250 °C) into a HP-5MS capillary column (30 m × 0.25 mm I.D. × 0.25 µm film thickness). Separation was achieved at a constant carrier gas flowrate of 1.0 ml/min (helium, purity grade N55) using an initial oven temperature of 60 °C maintained for 1 min, then 30 °C/min to 295 °C and the final temperature was maintained for 6 min. The ion source and quadrupole temperatures of the Hewlett Packard 5989 mass selective detector were 200 °C and 100 °C, respectively. Methane was used as reactant gas at a pressure of 1.3 Torr in the ion source. Mass spectra were recorded in full scan mode from 250 to 450 amu. Analytes were identified on the basis of their relative retention times and specific mass spectra, i.e. midazolam R_t 10.08 min, m/z 325-327, 1-OH-midazolam R_t 10.64 min, m/z 325-413-415, diazepam-d_5 R_t 9.55 min, m/z 289. Quantification was made after determination of the response factor against diazepam-d_5.

Propofol in hair

After two successive washes in methylene chloride, the hairs were cut into small segments of 1 mm with scissors and 50 mg was transferred to a 20 ml head-space glass tubes with tetrahydrofurane (THF) as internal standard and 1 ml of Soerensen buffer, pH 7.6. The tubes were sealed and incubated overnight at 40 °C. The homogenate was analysed directly with a HS-GC/MS system operating in electronic impact mode. For volatilisation, the tubes were pressurised for 15 min at 80°C in a HS 40 (Perkin Elmer) and an aliquot was transferred to the gas chromatograph (Hewlett Packard 5890) under a helium pressure of 100 kPa. The split ratio was 1:1 in a injector warmed at 180 °C. Chromatography was operated on a HP wax capillary column (30 m × 0.25 mm I. D. × 0.25 mm film thickness) using the following oven temperature: 45 °C for 3 min, 10 °C/min to 180 °C and 30 °C/min to 240 °C. Detection was realised on a Hewlett Packard 5971 mass selective detector used in the selected ion monitoring (SIM) mode. Propofol was identified on the basis of its retention time and the relative abundance of three confirming ions: propofol R_t 18.34 min, m/z 117-163-178, THF R_t 2.13 min, m/z 72. Propofol quantification was made after determination of its response factor against THF.

Results and discussion

Ethanol was detected in the blood sample at 0.38 g/l. The analytical results for the two anaesthetic agents in femoral blood were midazolam 45 ng/ml, 1-OH-midazolam 1 ng/ml and propofol 39 ng/ml. The concentrations of the anaesthetic agents determined are lower than the therapeutic ranges [1, 2], but the presence of ethanol, the absence of respiratory assistance and medical control and a rapid injection might have contributed to the death.

Table 1 Analytical results for midazolam, 1-OH-midazolam (1-OH-M) and propofol in the different types of hair from the victim

Hair specimens	Midazolam (ng/mg)	1-OH-midazolam (ng/mg)	Propofol (ng/mg)
Head hair (0–2 cm)	0.76	0.04	1.39
Head hair (2–4 cm)	0.71	0.03	1.86
Head hair (4–6 cm)	0.59	0.01	0.89
Pubic hair	0.46	0.08	19.68
Axillary hair	1.34	0.06	Insufficient sample

The procedures developed for the identification and quantification of midazolam, 1-OH-midazolam and propofol in human hair were validated. For midazolam and 1-OH-midazolam, linearity was observed for concentrations ranging from 0.05 to 5.00 ng/mg (r = 0.97 and 0.96, respectively). Within-run precision and extraction recovery at 0.5 ng/mg were found to be acceptable (midazolam 6.3 and 93.8%, 1-OH-midazolam 8.1 and 90.2%). At the lowest calibration point (0.05 ng/mg), the signal-to-noise ratio for midazolam was 35. For propofol, linearity was observed for concentrations ranging from 0.1 to 10.00 ng/mg (r = 1.00). Within-run precision and extraction recovery at 0.5 ng/mg were found to be acceptable (6.0 and 27%, respectively). At the lowest calibration point (0.1 ng/mg), the signal-to-noise ratio for propofol was 12. Recently, the solid-phase microextraction (SPME) technology has been proposed as another way to detect volatile or semi-volatile drugs from human hair [11] but the first results for the local anaesthetic lidocaine by HS-SPME-GC/MS reported extraction recoveries (1.8–2.4%) more than 10 times less than our direct HS-GC/MS for propofol (27%).

Segmental analysis of a 6 -cm hair strand revealed the presence of midazolam in each of the 2-cm-long segments. Concentrations were 0.76, 0.71 and 0.59 ng/mg from the root to the end of the strand (Table 1). As is generally the case for other drugs in hair [9], the parent drug concentrations were higher than the concentrations of the metabolite 1-OH-midazolam. For the full length pubic and axillary hair specimens, midazolam and 1-OH-midazolam concentrations are reported in Table 1. In contrast to the previous paper [12], it was observed that midazolam is incorporated more into axillary hair than pubic and head hair. Figure 1 shows the extracted ion chromatograms for the axillary hair extract positive for midazolam (m/z 325) and its metabolite (m/z 413).

Segmental analysis of a 6 -cm hair strand revealed the presence of propofol in each of the 2-cm-long segments (Table 1). The concentrations determined are in the range of those (1.05–3.5 ng/mg) previously published [8]. Propofol concentrations in pubic hair samples were higher than in head hair (Table 1). This result is in accordance with a review reporting higher concentrations for other substances in pubic hair than in head hair [12]. Figure 2 shows the SIM chromatogram obtained from the second head hair segment (2–4 cm).

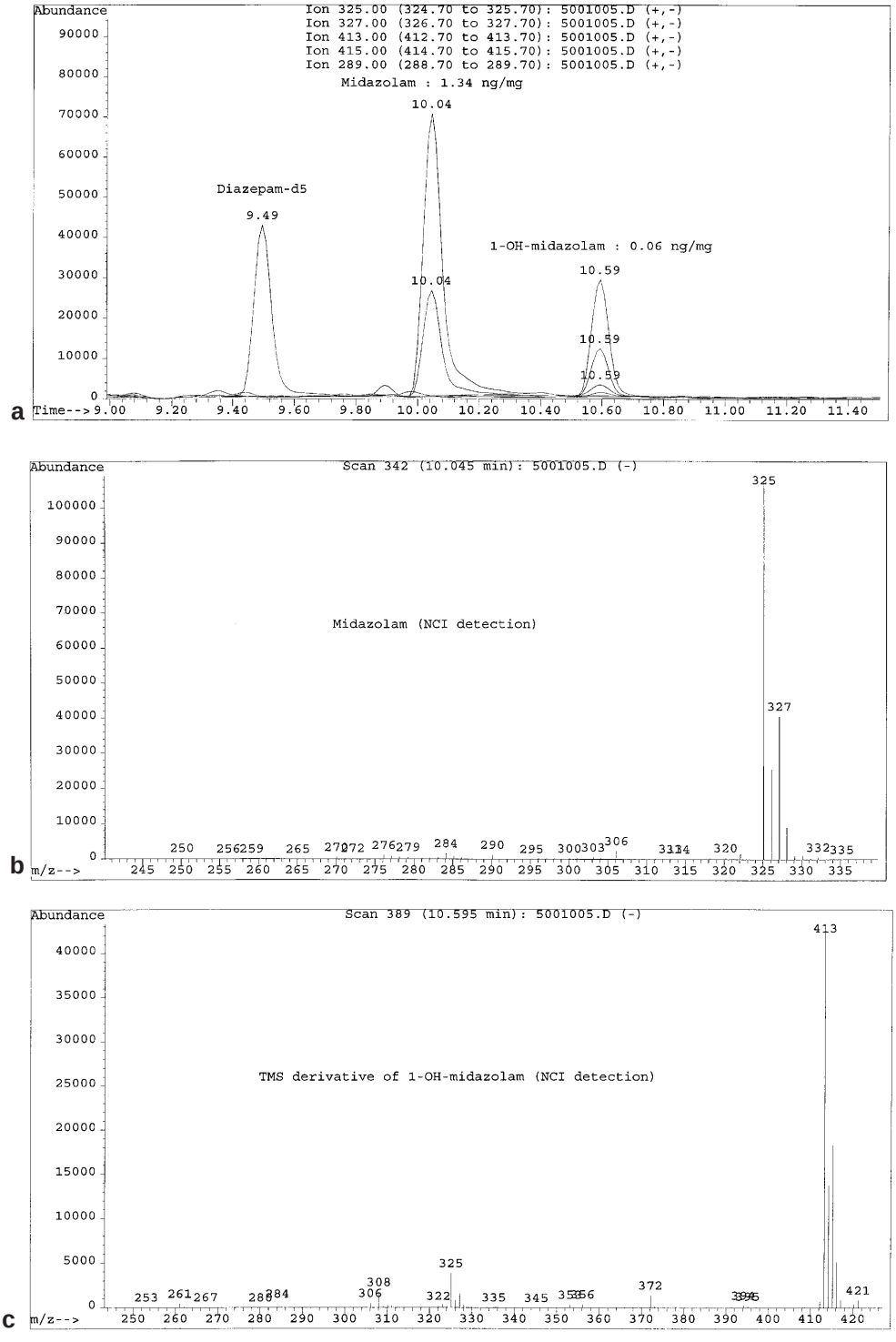

Fig. 1 **a**) Extracted ion chromatograms for midazolam (m/z 325-327), 1-OH-midazolam (m/z 425-413-415) and the internal standard (m/z 289) of the axillary hair extract. The underlined ions were used for the quantification. **b**) Mass spectra of midazolam observed in NCI mode of detection. **c**) Mass spectra of the TMS derivative of 1-OH-midazolam observed in NCI mode of detection

The presence of midazolam and propofol in the 6-cm-long hair strands of the victim clearly indicate chronic abuse of these two anesthetic agents over the months preceeding death (Table 1).

Conclusion

Chronic abuse of anesthetic agents (e.g. halothane, nitrous oxide, lidocaine, fentanyl and fentanyl derivatives such as sufentanyl and alfentanyl etc.) by medical staff for recreational purposes is well known and described in the literature but analytical proof is often lacking. In these situa-

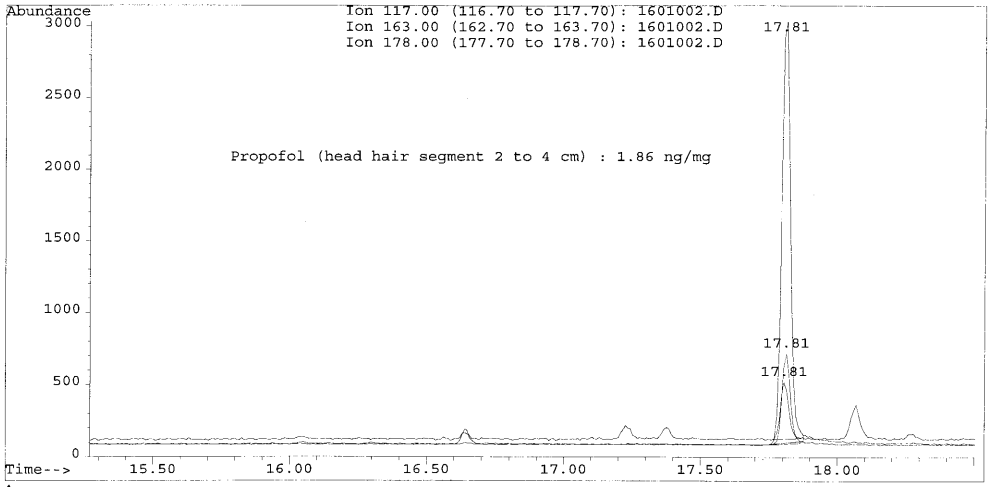

Fig. 2 SIM chromatogram of propofol (m/z 117-163-178) obtained after head hair extraction. The underlined ion was used for the quantification

tions, hair analysis can be of particular interest [13, 14, 15, 16, 17].

This new observation confirms that GC/MS-NCI represents the technique of choice to detect benzodiazepines in human hair [18].

This paper reports for the first time the detection of an obvious chronic exposure to propofol by hair analysis. The physico-chemical properties of propofol permit the headspace preparation of the hair specimen and the development of an original method which does not require tedious extraction procedures.

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