Hair Analysis Interpretation of an Unusual Case of Alleged Scopolamine Facilitated Sexual Assault

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Abstract

This case report describes an alleged scopolamine facilitated sexual assault case in which the victim requested a toxicological hair analysis. Hair decontamination wash solvents were evaporated and stored until analysis. Hair was incubated with 2 mL 0.01M phosphate buffer pH 8.4, and liquid-liquid extracted. LC-MS/MS analysis of hair extracts revealed the presence of scopolamine at lower concentrations than the limit of quantification. However, scopolamine was also present in the last wash solvents, with wash/hair ratios >4, suggesting hair external contamination rather than drug use. These data emphasize the usefulness of analyzing wash solvents for the correct interpretation of hair results.

Keywords: scopolamine, DFSA, hair, wash/hair ratio, decontamination

Introduction

Drug facilitated sexual assault (DFSA) has been defined as sexual activity occurring while the victim is incapacitated or unconscious due to the effects of alcohol and/or other drugs and is, therefore, prevented from resisting or unable to consent [1]. The use of a drug to modify a person's behavior for criminal purposes has occurred for centuries. Nevertheless, the number of reported DFSA cases has increased over the past few years, and it has also generated widespread media interest and caused alarm in the society [2-5]. In Spain, few DFSA cases have been toxicologically documented, probably due to a lack of information about this type of crimes within health care professionals and the general population [6].

Typical drugs used in drug facilitated crimes are likely to have one or more of the following properties: cause sedation; cause amnesia; colorless, odorless and tasteless; dissolve readily in alcohol (or other beverage); rapidly absorbed after oral administration; active at small doses; rapidly eliminated and, therefore, difficult to be detected [7-9]. An ideal drug also will render the victim submissive or will decrease her/his inhibitions, resulting in their participation in activities that they normally would not [7]. Common substances typically found in casework include alcohol, cannabis, benzodiazepines and other sedating drugs, or stimulants such as cocaine and ecstasy [7]. Scopolamine, an anti-muscarinic agent that blocks short-term memories formation, has been used to rob or kidnap for decades in South America [10]. In Spain, although the media reported several DFSA cases with scopolamine, literature search did not reveal any extensive illegal use in our country.

The toxicological investigation of a DFSA case is a challenge because of the scarce information about the incident due to the victim's unconsciousness or anterograde

amnesia, the delay in reporting the incident, and the low drug concentrations that should be detected [8,11]. Blood and urine are the classical biological matrices used to perform the toxicological analysis, and they should be collected at the earliest opportunity [7]. However, if the delay between the incident and sample collection is too long, the drug will already be eliminated from these matrices and, therefore, they will not allow to prove the exposure to the drug [7]. Hair analysis represents a useful biological matrix in drug-facilitated cases because of the much larger window of drug detection compared to blood or urine [12]. Moreover, most of the DFSA cases are due to a single drug exposure, which could be discriminated from a chronic exposure by segmental hair analysis. Some DFSA cases have already been documented using hair [2,13-19].

In this case report, hair analysis results from an unusual case of chronic alleged DFSA with scopolamine are presented, emphasizing the importance of the decontamination procedure and the analysis of the wash solvents in the interpretation of the results.

Case history

A middle aged-married woman pleaded in her defense that her lover had surreptitiously drugged her when her husband discovered that she had been having an affair for the last two years. In order to probe her statement, the married couple came to the lab to perform a toxicological investigation two months after the affaire finished. In the lab, they were informed that hair was the only biological matrix that could confirm their suspicions after the time elapsed since the last possible exposure to the drug. Therefore, they agreed to perform the hair analysis, and suggested to look for scopolamine. The married couple and the lover were health care professionals, and had easy access to scopolamine. She did not refer drug abuse or medical drug use in the last year.

Materials and Methods

Chemicals and Reagents

Standard of scopolamine (powder) was purchased from Sigma-Aldrich (Steinheim, Germany) and benzoylecgonine-d_3 (BE-d_3) (0.1 mg/mL in methanol) from Cerilliant™ (Round Rock, TX, USA). LC-MS Chromasolv® acetonitrile (≥ 99.9% pure), dichloromethane (≥ 99.8% pure) and 2-propanol (≥ 99.9% pure) were obtained from Sigma-Aldrich). HPLC grade methanol (≥ 99.9% pure) was from Panreac Química (Castellar del Vallès, Spain), and reagent grade n-heptane, sodium hydroxide, sodium dihydrogen phosphate monohydrate and potassium dihydrogen phosphate were from Merck (Darmstadt, Germany). Reagent grade formic acid (98-100%) and ammonium formate for LC-MS were from Scharlau (Sentmenat, Spain). Purified water was obtained in the laboratory using a Milli-Q water system (Le Mont-sur-Lausanne, Switzerland).

Specimen

One hair strand of approximately 400 mg weight and 15 cm length was collected from the vertex posterior of the head, as close as possible from the scalp. Hair was cosmetically altered (bleached), except for the roots (approximately, 5 cm). The specimen was stored in a paper envelope at room temperature until analysis. Hair specimens from other locations were not collected. Segmental hair analysis was performed to chronologically study the possible exposure to scopolamine.

Toxicological analysis

Hair decontamination, incubation and extraction

Hair specimen was divided in three 4.5-5 cm segments (S1, S2 and S3, being S1 the proximal and S3 the distal segments), which were individually decontaminated twice using 2 mL dichloromethane for 2 min. Wash solvents were transferred into a clean tube, evaporated to dryness with nitrogen in a Turbovap LV evaporator (Zymark, Hopkinton, MA) at 35ºC and refrigerated at 4ºC until analysis.

Hair segments were subsequently dried, weighted (50±2 mg each segment) and cut into small pieces. After fortifying each segment with 25 µL BE-d_3 at 0.1 µg/mL as internal standard, hair specimens were incubated with 2 mL 0.01 M phosphate buffer pH 8.4 at room temperature for 12 hours, following Kintz et al. procedure [20]. Samples were centrifuged at 4000 rpm for 10 min at 4ºC, and the supernatants were liquid-liquid extracted with 5 mL dichloromethane:2-propanol:n-heptane (50:17:33) [20]. After mechanical shaking for 30 min and centrifugation, the organic layer was evaporated to dryness. Extracted hair samples and dichloromethane washes were reconstituted in 100 µL of 2 mM ammonium formate with formic acid 0.1%:acetonitrile (95:5), and 10 µL injected onto the LC-MS/MS.

Preparation of the calibration curve

A stock solution of scopolamine at 1 mg/mL was prepared from the powder. Scopolamine and BE-d_3 stock solutions were individually diluted in methanol to prepare working solutions at 0.1 µg/mL and 0.01 µg/mL for scopolamine, and 0.1 µg/mL for BE-d_3. A calibration curve from 10 to 100 pg/mg was prepared by cutting and fortifying blank hair samples (50 mg) with the appropriate scopolamine working solution and 25 µL BE-d3 at 0.1 µg/mL. The same protocol as described in 2.3.1 section was applied to calibrators.

LC-MS/MS

The HPLC was a Waters Alliance 2795 Separation Module with a Waters Alliance series column heater/cooler (Waters Corp., Mildford, MA). Chromatographic separation was achieved with an Atlantis T3 column (2.1 mm x 50 mm, 3 μm) maintained at 26ºC. Gradient elution with 2 mM ammonium formate with formic acid 0.1% (A) and acetonitrile (B) at a flow rate of 0.3 mL/min was as follows: 5% B for 0.8 min; increased to 45% over 6.2 min; decreased to 5% B over 0.5 min and re-equilibrated for 2.5 min.

A Quattro Micro™ tandem mass spectrometer (Waters Corp.) working in electrospray in the positive mode was used for the detection. The following conditions were found to be optimal: capillary voltage, 3 kV; source and desolvation temperatures, 125 and 350ºC, respectively; desolvation and cone gas (nitrogen) flow, 700 and 50 L/h, respectively. Collision gas (argon) pressure was maintained at 3×10^{-6} Bar. Multiple reaction monitoring (MRM) transitions and optimal conditions for scopolamine and BE-d_3 are summarized in Table 1.

Results and discussion

The present case report describes results and their interpretation after the analysis of a hair specimen from a married woman who alleged that her lover had chronically intoxicated her during their affair, probably with scopolamine. The secret affair finished two months before the analysis request and, therefore, hair was the only biological sample collected. Usually, a single exposure to the drug is used in DFSA cases, which makes hair toxicological analysis very challenging due to the low concentrations expected in this matrix. On the other hand, chronic drug facilitated crimes are unusual,

and only few cases have been described in children and elderly people [21-23]. However, in this case report a chronic intoxication was suspected and, therefore, hair analysis seemed simple.

To perform a chronological study of the possible exposure to scopolamine, hair specimen was divided in three segments of 4.5-5 cm (S1, S2 and S3), which were individually washed, incubated and analyzed. Table 2 shows the peak areas of scopolamine in the incubated hair and in the wash solvents of segments S1, S2 and S3. Criteria for analyte identification included retention time and the presence of the three selected transitions with the appropriate ion ratio (±20% of ion ratio values in the calibrators).

LC-MS/MS analysis of the incubated hair revealed the presence of scopolamine in the three segments, with higher concentrations in S1, indicating a presumptive exposure to scopolamine. However, the concentrations found in all cases were below the limit of quantification (10 pg/mg).

The Society of Hair Testing (SOHT) recommends the analysis of hair washes as one of the criteria to evaluate the possibility of hair contamination [24]. In the method applied to the present case report, hair decontamination was carried out with two 2 mL dichloromethane washes, which were subsequently evaporated and analyzed. Scopolamine was detected in both washes; the lower amount in the second wash solvent compared to the first one proved the efficacy of the decontamination procedure. However, scopolamine peak areas in the washes were much higher than in the incubated hair, with wash/hair (W/H) ratios ranging from 74 to 709 for the first wash, and 4.6 to 42 for the second wash. Figure 1 shows scopolamine comparative chromatograms in the two wash solvents and inside the hair for S1, S2 and S3. The amount of scopolamine in

the wash solvent due to extraction of accumulated scopolamine in hair is unknown. However, the applied wash procedure has been previously employed for the determination of scopolamine [20]. In addition, this common wash procedure is also used in our laboratory for the determination of 35 common drugs in hair [25], including scopolamine, and the calculated W/H was <0.1 for all detected drugs in the analyzed real specimens (n= 85). Therefore, it is very likely that most of scopolamine found in the wash solvent in the present case report came from external contamination. On the other hand, although fully extraction of scopolamine from the inner hair with the selected incubation procedure could not be guaranteed, its usefulness to detect scopolamine has been previously probed by Kintz et al. [20], who detected this analyte in a hair specimen from a subject who declared drinking Datura Inoxia infusion, which contains scopolamine.

Different methods have been proposed to differentiate systemic exposure from external contamination of hair, such as the detection of appropriate levels of metabolites, but the difficulty in the interpretation arises when metabolites are not detected. Tsanaclis et al. [26] proposed the analysis of the wash residue, and the comparison of the concentrations found with those in hair. They analyzed 216 hair specimens from police officers dealing with drug related cases, where hair external contamination could be expected, and established three groups according to the wash/hair (W/H) ratio: W/H ratio 0 and <0.1, would indicate drug use; W/H ratio >0.1 and <0.5 is not conclusive, but indicates possible drug use with a degree of external contamination; and finally they concluded that W/H ratio >0.5 is likely to indicate that the source of most of the drug in the wash residue stems from external contamination. W/H ratios found in this case report were >4.6 in all cases, strongly suggesting external scopolamine hair contamination.

Few data on hair concentrations after exposure to scopolamine have been published so far. Kintz et al. reported hair concentrations of scopolamine from 14 to 48 ng/mL in the three analyzed segments of a young male after repeatedly drinking an infusion of Datura inoxia flowers [20]. However, in this case, wash solvents employed to eliminate external contamination were not analyzed and, therefore, it was not possible to verified the absence of external contamination, or to compare scopolamine amounts in the outer and inner hair. Pujol et al. [22] analyzed hair specimens from three children allegedly exposed to FEMINAX, a pharmaceutical that contains 100 μg scopolamine per tablet, among other active ingredients. Scopolamine hair concentrations ranged from 0.2 to 1.1 pg/mg but, again, wash solvents were not analyzed.

After discussing the analytical results with the marriage couple, they refused to perform a second analysis of, for instance, pubic hair. This fact, in addition to the orientation of the toxicological analysis to look for scopolamine, make us suspect about the possible deliberate contamination of the hair by the victim herself to justify her marital infidelity.

Conclusions

In the present case report, a positive hair result to scopolamine would have been reported in the absence of data related to the amount of scopolamine in the external hair layers, highlighting the importance of evaluating analytes' presence in the incubated hair together with the wash solvents.

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229 Economía e Industria), Galicia, Spain, respectively.

230 **References**

231 1. Hall JA, Moore CB (2008) Drug facilitated sexual assault--a review. J Forensic Leg
232 Med 15:291-297. doi: 10.1016/j.jflm.2007.12.005

233 2. Villain M, Cheze M, Dumestre V, Ludes B, Kintz P (2004) Hair to document drug-
234 facilitated crimes: four cases involving bromazepam. J Anal Toxicol 28:516-519.

235 3. Scott-Ham M, Burton FC (2005) Toxicological findings in cases of alleged drug-
236 facilitated sexual assault in the United Kingdom over a 3-year period. J Clin
237 Forensic Med 12:175-186. doi: 10.1016/j.jcfm.2005.03.009

238 4. Hurley M, Parker H, Wells DL (2006) The epidemiology of drug facilitated sexual
239 assault. J Clin Forensic Med 13:181-185. doi: 10.1016/j.jcfm.2006.02.005

240 5. European Monitoring Center for Drugs and Drug Addiction (EMCDDA) (2008)
241 Sexual assaults facilitated by drugs or alcohol.
242 http://www.emcdda.europa.eu/attachements.cfm/att_50544_EN_TDS_sexual_assa
243 ult.pdf (Accessed January 2012)

244 6. Cruz-Landeira A, Quintela-Jorge O, Lopez-Rivadulla M (2008) Chemical
245 submission, epidemiology and some clues for the diagnosis. Med Clin (Barc.) 131:783-
246 789. doi: 10.1016/S0025-7753(08)75505-2

247 7. Scott KS (2009) The use of hair as a toxicological tool in DFC casework. Sci
248 Justice 49:250-253. doi: 10.1016/j.scijus.2009.09.013

249 8. Madea B, Musshoff F (2009) Knock-out drugs: their prevalence, modes of action,
250 and means of detection. Dtsch Arztebl Int 106:341-347. doi:
251 10.3238/arztebl.2009.0341

252 9. Gaulier JM, Fonteau F, Jouanel E, Lachatre G (2004) Rape drugs: pharmacological
253 and analytical aspects. Ann Biol Clin 62:529-538.

254 10. Negrusz A, Juhascik M, Gaensslen RE (2005) Estimate of the incidence of drug-
255 facilitated sexual assault in the U.S.
256 http://www.ncjrs.gov/pdffiles1/nij/grants/212000.pdf (Accessed January 2012).

257 11. Negrusz A, Gaensslen RE (2003) Analytical developments in toxicological
258 investigation of drug-facilitated sexual assault. Anal Bioanal Chem 376:1192-1197.
259 doi: 10.1007/s00216-003-1896-z

260 12. Kintz P (2011) Segmental hair analysis can demonstrate external
261 contamination in postmortem cases. Forensic Sci Int Epub ahead of print. doi:
262 10.1016/j.forsciint.2011.01.041

263 13. Goulle JP, Cheze M, Pepin G (2003) Determination of endogenous levels of GHB
264 in human hair. Are there possibilities for the identification of GHB administration
265 through hair analysis in cases of drug-facilitated sexual assault? J Anal Toxicol
266 27:574-580.

267 14. Frison G, Favretto D, Tedeschi L, Ferrara SD (2003) Detection of thiopental and
268 pentobarbital in head and pubic hair in a case of drug-facilitated sexual assault.
269 Forensic Sci Int 133:171-174. doi: 10.1016/S0379-0738(03)00064-1

15. Villain M, Cheze M, Tracqui A, Ludes B, Kintz P (2004) Testing for zopiclone in hair application to drug-facilitated crimes. Forensic Sci Int 145:117-121. doi: 10.1016/j.forsciint.2004.04.026

16. Kintz P, Villain M, Ludes B (2004) Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence. Ther Drug Monit 26:211-214.

17. Kintz P, Villain M, Cheze M, Pepin G (2005) Identification of alprazolam in hair in two cases of drug-facilitated incidents. Forensic Sci Int 153:222-226. doi: 10.1016/j.forsciint.2004.10.025

18. Rossi R, Lancia M, Gambelunghe C, Oliva A, Fucci N (2009) Identification of GHB and morphine in hair in a case of drug-facilitated sexual assault. Forensic Sci Int 186:e9-e11. doi: 10.1016/j.forsciint.2009.01.017

19. Kintz P, Evans J, Villain M, Chatterton C, Cirimele V (2009) Hair analysis to demonstrate administration of sildenafil to a woman in a case of drug-facilitated sexual assault. J Anal Toxicol 33:553-556.

20. Kintz P, Villain M, Barguil Y, Charlot JY, Cirimele V (2006) Testing for atropine and scopolamine in hair by LC-MS-MS after Datura inoxia abuse. J Anal Toxicol 30:454-457.

21. Kintz P, Evans J, Villain M, Salquebre G, Cirimele V (2007) Hair analysis for diphenhydramine after surreptitious administration to a child. Forensic Sci Int 173:171-174. doi: 10.1016/j.forsciint.2006.12.006

22. Pujol ML, Villain M, Salquèbre G, Vallet E, Cirimele V, Kintz P (2006) Scopolamine : un nouveau cas de soumission médicamenteuse sur des enfants. Ann Toxicol Anal 18:207-212. doi: 10.1051/ata:2006012

23. Kintz P, Villain M, Cirimele V (2008) Chemical abuse in the elderly: evidence from hair analysis. Ther Drug Monit 30:207-211.

24. Society of Hair Testing (1997) Statements of the Society of Hair Testing concerning the examination of drugs in human hair. Forensic Sci Int 84:3-6.

25. Lendoiro E, Quintela O, de Castro A, Cruz A, López-Rivadulla M, Concheiro M (2011) Target screening and confirmation of 35 licit and illicit drugs and metabolites in hair by LC-MSMS. Forensic Sci Int. Epub ahead of print.

26. Tsanaclis L, Wicks JF (2008) Differentiation between drug use and environmental contamination when testing for drugs in hair. Forensic Sci Int 176:19-22. doi: 10.1016/j.forsciint.2007.08.009

311 Table 1. Retention time (Rt), MRM transitions and optimal cone voltage and

312 collision energy conditions for scopolamine and benzoylecgonine-d3 (BE-d3).

Compound	Rt (min)	MRM transition	Cone voltage (V)	Collision Energy (eV)
Scopolamine	4.6	[a]304.3 > 138.2 304.3 > 156.2 304.3 > 103.0	34	24 16 41
BE-d3	5.6	293.2 > 171.2	30	20
[a]MRM transition was used for quantification				

313

314 Table 2. Peak area of scopolamine (transition 304.3>138.2) in the incubated hair

315 and in the wash solvents of segments S1, S2 and S3 (S1, proximal segment; S3,

316 distal segment).

Segment	Scopolamine Peak Area		
	Wash 1	Wash 2	Incubated hair
S1	682294	387479	9216
S2	628211	26911	885
S3	106345	2859	625

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Figure 1. Scopolamine chromatograms in the first and second wash solvents, compared to chromatograms in hair (S1, proximal segment; S3, distal segment).