Drug-facilitated sexual crime by use of ketamine and diazepam by a gynaecologist

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Introduction

Drug facilitated crimes (DFCs) can be defined as criminal acts carried out by means of administering a substance to a person with the intention of impairing behaviour, perceptions, or decision-making capacity. It also extends to taking advantage of an impaired person after voluntary intake of an incapacitating substance. Crimes include robbery, money extortion, and maltreatment of the elderly, children, or mentally ill patients.[1] Rape or other types of sexual assault are referred to as drug facilitated sexual assault (DFSA). In DFSA cases, the victim is subjected to a sexual act without legal consent as a result of the pharmacological effects of alcohol and/or drug(s).

A large number of psychoactive substances have the potential to alter the victim's state of mind, alcohol being the number one candidate because of its widespread use.[2] Illicit drugs, psychoactive prescription drugs and even over-the-counter medicines are also likely candidates, either consumed alone or in combination with alcohol. The resulting pharmacological effects may include relaxation, euphoria, and lack of inhibition on the one hand and drowsiness, loss of motor function, unconsciousness, and amnesia on the other hand.

Information on the frequency of DFC cases is scarce but there has been a significant increase in reports worldwide. However, several factors complicate the registration of the actual number of DFSA cases. Governmental statistics show a general underreporting of sexual assault crimes. The impact that central nervous system depressant drugs have on memory and consciousness might lead to a situation where the victim is not able to remember what has actually happened and chooses not to report the incident. If the incident is reported, police officers often assume that the victim was simply drunk rather than drugged. If an investigation is initiated, the delay between the collection of biological evidence and the alleged assault can be between several hours and more than a day.

This case report describes a DFSA case in a medical setting: a gynaecologist was suspected of assaulting a young patient after drug administration. In the presented case, the time delay between the alleged assault and the sampling was 30 h. Evidence collection via a standardized sampling kit and the choice of sample in such cases is discussed. The analytical strategy is described with regard to the choice of methods, uncertainty measurement, and limitations of the methods which are important during interpretation of the analytical results. Finally, interpretation of this specific case, but also interpretation issues of DFSA cases in general, is discussed.

Case history

A young woman of 29 was searching for a gynaecologist via the Internet. After about five consultations at that gynaecologist's practice, she asked the physician to place an intra-uterine device as contraception. On the day of the alleged incident, she was alone in his office. During the procedure she felt enormous pain due to an injection in the fold of her elbow. She lost consciousness for a while and when she regained it, she felt a second injection from which she collapsed again. When she awoke she noticed that the gynaecologist was touching her intimately. The doctor was very nervous when he noticed that she was awake. She started vomiting and only after he had cleaned up the office did he let her contact her boyfriend. While the doctor insisted that she stayed for observation, the couple left the medical centre about an hour after the young woman had entered. The next morning, the victim went to the police to press charges for sexual assault. Physical examination of the victim by a medical doctor revealed the presence of two injection marks in her arm fold. Blood and urine samples for genetic and toxicological analysis were collected between 9:30 PM and 11:15 PM, while the alleged assault was estimated to have occurred between 4:00 PM and 5:00 PM the day before. The evidence for genetic analysis was stored without further analysis since the declarations of the victim did not indicate that sexual contact had occurred. No medication or drug use occurred in the week before the sampling according to the victim.

Materials and methods

Sexual aggression set (SAS)

The SAS used in this case has been developed by our institute to harmonize the approach in cases of sexual assault, to provide a rapid and standardized evidence collection, and to provide tools for professional support for the victim. Sets are distributed to pathologists or medical doctors working in a hospital that have signed a protocol of collaboration with the local judicial authority. They contain material for the medical doctor for sampling (24 'steps'), manuals for the police officer and the health professional, a medical report and questionnaire and material (e.g. disposable clothing), and information for the victim. Each SAS has a unique ID number that is used by the police in their report; all 24 'steps' are sub-numbered and no name is mentioned on these items. Immediately after use, the set is sealed by the police officer and sent to the laboratory as soon as possible, preferably within 24 h.

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In addition to evidence seals for genetic analysis, a urine sample and two 5-ml blood tubes with sodium fluoride and potassium oxalate as anticoagulant are provided for toxicological analysis. The questionnaire contains information on the date and time of collection, the date and time of the alleged assault, the date and time of the last consented sexual relations, and the use of alcohol, drugs and medication in the week before the assault.

Screening techniques

Two different chromatographic screening techniques for urine or blood were applied: a gas chromatography–mass spectrometric (GC-MS) method and a high performance liquid chromatography (HPLC) method combined with a diode-array detector (DAD) (Agilent Technologies, Santa Clara, CA, USA). The sample preparation step of the GC-MS screening comprised an enzymatic hydrolysis of 2 ml of urine using β-glucuronidase obtained from *Helix pomatia* (Sigma Aldrich, St Louis, MO, USA) or 500 μl of whole blood and a liquid-liquid extraction (LLE) using Toxitube A (Varian, Palo Alto, CA, USA). An internal standard (IS) prazepam (400 ng/ml, LGC Standards, Teddington, UK) and an external standard SKF-525 (proadifen, Sigma Aldrich, St Louis, MO, USA) were used. The organic phase was evaporated and reconstituted in 50 μl of anhydric ethylacetate. One μl was injected in splitless mode onto the 6890 GC- 5973N MS from Agilent Technologies (Santa Clara, CA, USA). After injection, the organic phase was acetylated using acetic anhydride and pyridine and injected again. Chromatographic separation occurred on a DB-5MS (0.2 μm, 30 m x 0.250 mm) column (Agilent Technologies, Santa Clara, CA, USA) and the temperature program was as follows: 100 °C (1 min) to 325 °C with an increase of 10 °C

per min. Total run time was 28.5 min for one run. The Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) permitted the search in different published mass spectral libraries (MPW, Wiley-VCH, 2011; NIST 05, Agilent Technologies, 2008; RTL 01.00, Agilent Technologies; DD2011, Wiley-VCH, 2011) together with a 'home made' library. One ml of blood sample was also screened using an HPLC-DAD method as published by Herzler *et al.*[3] using a UV library of 2682 compounds. The chromatographic system consisted of a reversed-phase Lichrospher-RP8ec column (5 μM, 250 x 4.0 mm) in a HP 1100 Series (Agilent Technologies, Santa Clara, CA, USA) HPLC-DAD.

Target techniques

The methods used for target quantification in urine and blood are described in the literature.[4–12] The applied method to detect benzodiazepines and their metabolites as well as several hypnotics in blood and urine is described by our research group.[7] However, the HPLC method was transferred to a UPLC method. Twenty tree compounds were detected using an Acquity UPLC coupled to a Quattro premier XE Mass spectrometer (Waters, Milford, MA, USA). An LLE using chlorobutane after alkalization of the sample was applied for 250 μl of blood and 200 μl of hydrolyzed urine using β-glucuronidase (*Helix pomatia*, Sigma Aldrich, St Louis, MO, USA). The limit of quantification (LLOQ) of diazepam, nordiazepam and temazepam were 10 ng/ml and 1 ng/ml in urine and blood, respectively. The uncertainty measurement (U) was determined to be 10%.

Ketamine and its metabolites were quantified in urine and blood according to the method published by Ramirez-Fernandez *et al.*[11] An SPE using Oasis MCX cadriges (Waters, Milford, MA, USA) was applied to extract 500 μl of sample after acidification. A Sunfire
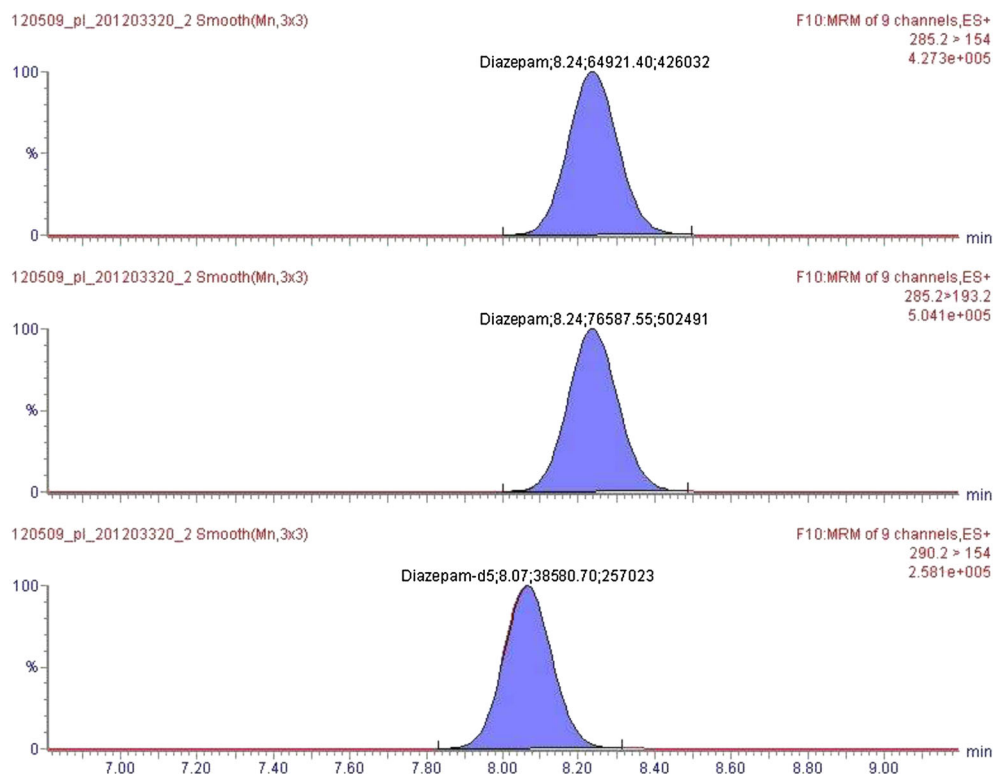

Figure 1. Multiple Reaction Monitoring chromatograms of diazepam (28 ng/ml), nordiazepam (8 ng/ml) and temazepam (2 ng/ml) in the blood sample. Deuterated analogues (d-5) were used as IS at a concentration of 80 ng/ml.
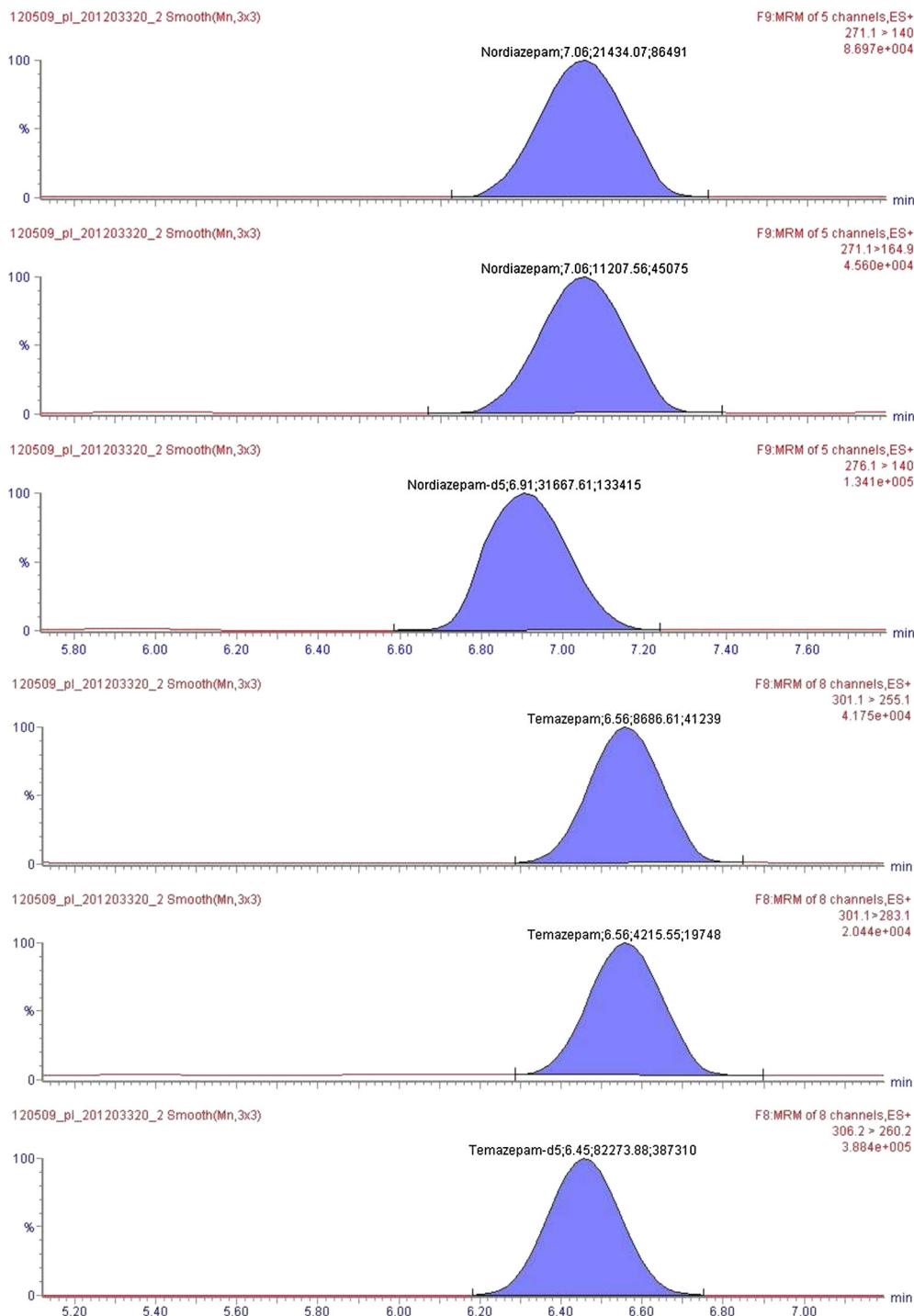
Figure 1. (Continued)

C8 3.5 μm, 2.1 mm x 100 mm (Waters) on an Alliance 2695 HPLC (Waters, Milford, MA, USA) was used to separate bufotenine, cathinone, psilocine, mescaline, scopolamine, 2-oxo-3-hydroxy-LSD, norketamine, ketamine, ritalinic acid, ibogaine, LSD and chlorpheniramine in 22 min. A Quattro Ultima Tandem MS (Waters, Milford, MA, USA) used in ESI+ mode was coupled to the HPLC system. The LLOQ of ketamine was 0.5 ng/ml in urine and blood with U = 13%. The LLOQ for norketamine was 2 ng/ml in urine and 0.5 ng/ml in blood with U = 12%.

Results and discussion

The time elapsed between the alleged facts and the sampling was about 30 h. This is not unusual in a DFSA case since several psychotropic substances have an impact on memory and consciousness, which makes it very difficult for the victim to act decisively and initiate a complaint. Consequently, this important delay has an impact on the data interpretation as compounds with short half-lives will no longer be detected in blood. In such cases, collection

of a urine sample is always recommended to allow for the detection of a wide panel of potent fast-acting central nervous system depressants that will incapacitate the victim rapidly, even after administration of a relatively low (single) dose.

For DFSA cases, the use of screening methods with AMDIS facilitates the detection of a large number of compounds. In this case, in the blood sample, norketamine was detected via GC-MS screening and diazepam and nordiazepam via HPLC-DAD. GC-MS analysis of the urine sample revealed ketamine, norketamine, nordiazepam, and temazepam. However, to detect compounds at their concentration level 24 h to several days after a single administration, very sensitive and specific techniques such as UPLC-MS/MS or GC-MS/MS are usually required.[13] Our analytical strategy consists of GC-MS and HPLC-DAD analysis for a general and broad screening of drugs of abuse (e.g. designer drugs), while in addition all samples are systematically analyzed using the previously described LC-MS/MS target techniques to screen as well as quantify drugs in very low concentrations. These methods are validated according to international guidelines.[14] Moreover, Quality Assurance (QA) according to ISO 17025 principles using within-run certified quality controls and proficiency tests to evaluate intra laboratory results and uncertainty measurement were applied.[14]

Urine analysis using LC-MS/MS revealed concentrations of temazepam at 25 ng/ml, nordiazepam at 10 ng/ml, ketamine at 26 ng/ml and norketamine at 31 ng/ml. The creatinine concentration in urine was lower that the proposed limit of 20 mg/dl,[15] which could lead to falsely negative analytical results, especially if only traditional screening methods are used.

The blood target analysis revealed a concentration of 28 ng/ml of diazepam, 8 ng/ml of nordiazepam and 2 ng/ml of temazepam (Figure 1). Diazepam is a benzodiazepine utilized for its anxiolytic myorelaxant, antiepileptic, sedative and anaesthetic properties, commercialized under the form of tablets but also as injectable solutions. In Belgium, the only legal injectable solution sold has a concentration of 10 mg/2 ml. The prosecutor requested an estimate of the dosage and the associated effects at the time of the alleged assault. Therefore, the blood diazepam concentration at the time of the alleged assault and the administered dose were estimated, based on half-life and the assumption that linear pharmacokinetic data can be applied at low concentrations for diazepam. The reported half-life of diazepam is about 20–40 h[16] and is age and sex dependent. In addition, metabolization rates can differ considerably between subjects. As indicated in Table 1, due to uncertainty measurement and half-life differences, back-

calculations result in a broad range (79.5–138.5) of possible plasma concentrations. According to The International Association of Forensic Toxicologists (TIAFT)[17] diazepam has anxiolytic effects at plasma concentrations from 125 to 250 ng/ml. The concentration range observed in this case thus ranges from sub therapeutic to low therapeutic range. The estimated dose administered ranges from 2 to 20 mg (Table 1).

In addition to diazepam and its metabolites, ketamine and norketamine were detected in the blood sample at concentrations of 2 and 6 ng/ml. respectively (Figure 2). Ketamine is used as a general anesthetic. In Belgium it is delivered only in a hospital environment as an injectable solution (Ketalar®) with a concentration of 50 mg/ml (10 ml solution). The injection is performed intramuscularly or intravenously. Within 30 s of the intravenous injection analgesia starts with a loss of consciousness, catalepsy, amnesia, hypnosis and a final sedation and analgesia; it seems that the patient is disconnected from his environment and not really asleep. Side effects of ketamine can be cardiovascular effects such as augmentation of the cardial rhythm and arterial pressure, respiratory effects such as severe respiratory depression and apnoea during a fast injection of the drug, gastro-intestinal effects such as nausea, vomiting and hyper salivation, but also psychological such as confusion, excitation, an irrational behaviour and hallucinations. The plasma levels of ketamine have a complex logarithmic decline with an initial half-life of minutes and a second half-life of 2–3 h,[18] which in addition is sex-dependent.[19] The half-life of norketamine is 4 h.[16] During a short-term high-dose ketamine infusion, norketamine concentrations will be lower than ketamine concentrations. However, after termination of the infusion, norketamine concentrations will exceed those of ketamine.[19] The requested back-calculation of the blood concentration and association to administered dose is difficult for ketamine due to the three-compartment pharmacokinetic model and as the elapsed time window between sampling and facts represents approximately 10 half-lives. Ketamine and its metabolite can persist in trace amounts in plasma at least for 24 h following a single intravenous dose.[18] Therefore, sensitive methods are still able to detect these compounds after a time delay of 30 h. In addition, diazepam has shown to alter ketamine metabolism in animals.[18]

Diazepam is combined with ketamine during induction of anaesthesia to have a better control of the vital functions of the patient and the general anaesthesia but also to diminish the psychological effects of ketamine. The Food and Drug Administration (FDA)[20] suggests an injection of diazepam with a dose of 2 to 5 mg after

Table 1. Analytical results and back-calculation of the estimated range of injected dose.
Conc: Concentration, U: uncertainty measurement, T=30: Time delay between alleged assault and sampling, T=0: Time point of alleged assault

|	Diazepam	Nordiazepam	Temazepam	Ketamine	Norketamine
ANALYTICAL RESULT					
Measured Blood Conc T=30h (ng/ml)	28	8	2	2	6
U (%)	10	10	10	13	12
Blood concentration range (ng/ml)	[25.2-30.8]	[7.2-8.8]	[1.8-2.2]	[1.7-2.3]	[5.3-6.7]
INTERPRETATION					
Plasma/Blood ratio[22]	1.8			0.6	
Plasma concentration range (ng/ml)	[45.4-55.4]			[1.0-1.4]	
Plasma Half-life[22] (h)	20-40			minutes / 2-3	
Plasma concentration range at T=0 (ng/ml)	[79.5-138.5]			?	
Distribution Volume[22] (l/kg)	[0.5-2.5]			4	
Estimated Dose (mg) / 60 kg	[2.4-20.8]			?	
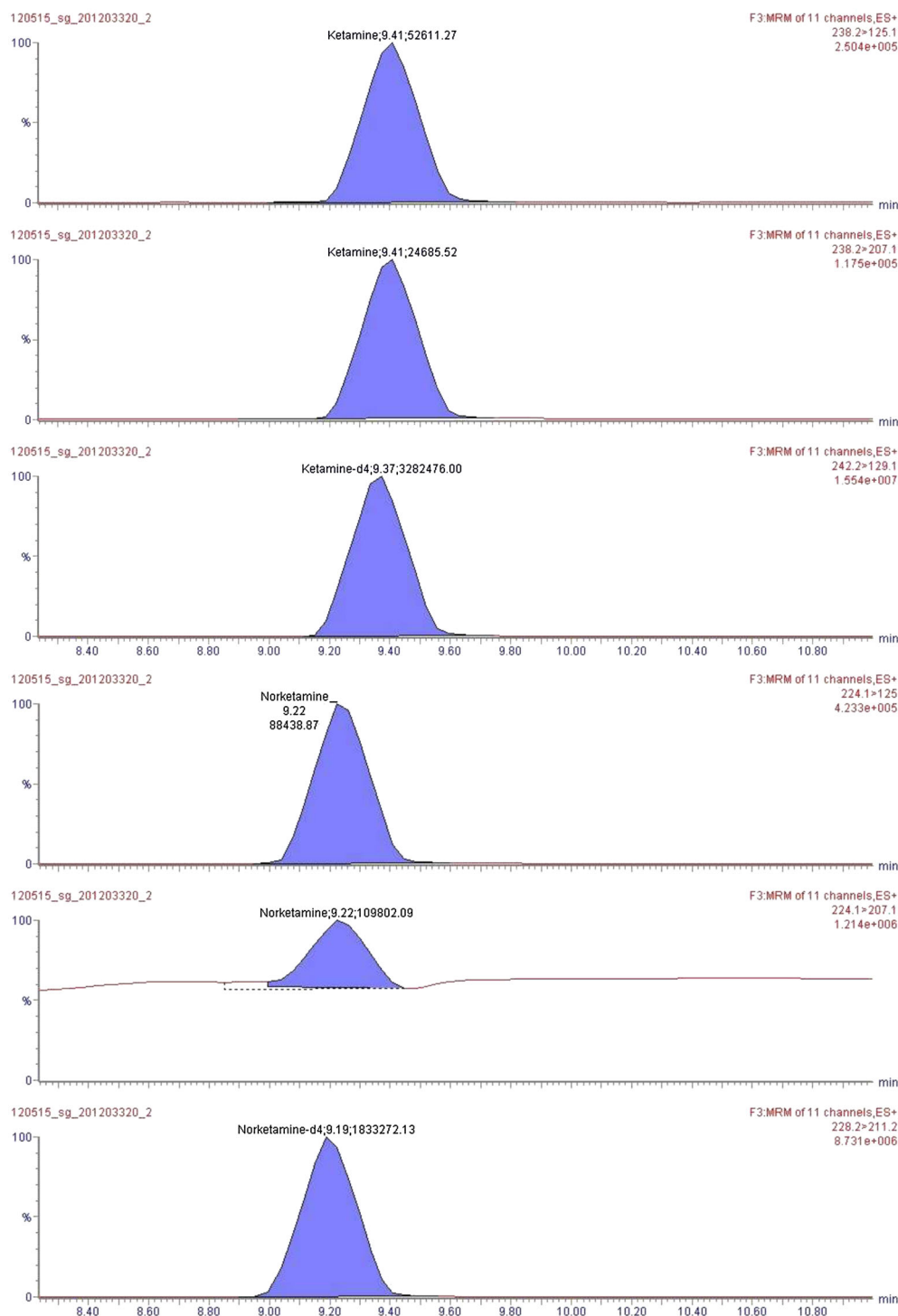
Figure 2. MRM chromatograms of ketamine (2 ng/ml) and norketamine (6 ng/ml) in the blood sample. Deuterated analogues (d4) are used as IS at a concentration of 100 ng/ml.

an intravenous injection of ketamine of 1 to 2 mg/kg to induce and maintain anaesthesia. Administration of both compounds intravenously will result in two injection marks as ketamine and diazepam have to be injected separately to avoid chemical reactions.

The final interpretation of the results is not straightforward. It is clear that the side effect of irrational behaviour and hallucinations due to ketamine can make it difficult for the investigators to evaluate the testimony of the victim. Moreover, ketamine is an illicit substance that is also abused. Analysis of a hair sample would have been of interest to distinguish between a naïve usage and a chronic presumably illicit drug use. Unfortunately, collection of a hair sample was refused by the victim. On the other hand, according to the Belgian law ketamine should only be used in a hospital environment and the question arises why a gynaecologist uses ketamine in his private practice to place an intra-uterine device. An additional police

investigation of the doctors' practice revealed the presence of other regulated substances for the hospital environment in expired ampoules containing fentanyl and midazolam. In this case, the toxicological data have contributed to the investigation, by supporting the declarations of the victim and the observations during the physical examination. However, toxicologists should always be aware that a positive finding does not mean that the drug was actually administered and a negative result does not always imply that there was no drug consumption at the time of the facts.

Conclusion

This case report demonstrates the difficulties in DFSA cases. First of all, biological evidence should be properly collected and stored. Toxicologists should have sensitive and specific methods to be able to detect traces of compounds in blood and urine, and in hair. A list of targeted compounds and their detection limits should be available. Analytical interpretation with regard to uncertainty measurement should be general knowledge if the obtained data is to be linked to data from interpretation lists such as the ones provided by TIAFT. While back-calculations and dose estimations can be done, it still remains ambiguous due to uncontrolled parameters such as metabolization rates. However, prosecutors often demand the effects of drug at the time of the alleged facts. Finally, the toxicologists should interpret the analytical data with caution and inform the judicial authorities of all possible angles.

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