

# Hair Analysis to Demonstrate Administration of Sildenafil to a Woman in a Case of Drug-Facilitated Sexual Assault

Pascal Kintz<sup>1,\*</sup>, Julie Evans<sup>2</sup>, Marion Villain<sup>1</sup>, Craig Chatterton<sup>3</sup>, and Vincent Cirimele<sup>1</sup>

<sup>1</sup>Laboratoire ChemTox, 3 rue Gruninger, 67400 Illkirch, France; <sup>2</sup>Eurofins Genetic Services, Chorley, United Kingdom; and <sup>3</sup>The Forensic Science Service, Chorley, United Kingdom

## Abstract

The drug sildenafil (Viagra<sup>®</sup>, Pfizer) and, more recently, tadalafil (Cialis<sup>®</sup>, Lilly-Icos) and vardenafil (Levitra<sup>®</sup>, Bayer), has drawn public attention to aphrodisiacs. The search for such substances dates back millennia. Adverse effects associated with these drugs include hypotension, tachycardia, headache, flushing, blurred vision, dyspepsia, and musculoskeletal pain. Although sildenafil has been marketed for erection of the penis, recent attention has been paid to its application for women, including enhancement of success of *in vitro* fertilization but also better sexual responses (increased desire, satisfaction, and orgasm) in cases of sexual disorders. Today, there is a debate on internet forums about the potential properties of sildenafil to enhance women's sexual pleasure. This laboratory was asked to analyze a 12-cm length of light brown hair submitted by a British police force following an allegation that a young female had been subjected to sexual assaults over a two-year period. The female was 15–17 years of age at the time. The alleged perpetrator was her stepfather, and there was some suspicion that drugs may have been administered to facilitate the attacks. After decontamination and segmentation (6 × 2-cm section), the specimen was analyzed by liquid chromatography coupled with tandem mass spectrometry after alkaline (pH 9.5) extraction using dichloromethane/isopropanol/*n*-heptane (25:10:65, v/v/v). The limit of quantitation was 5 pg/mg. The proximal segment tested positive for sildenafil at 38 pg/mg, and all others proved negative. This was in accord with the victim's claim. In the absence of any controlled studies, it was impossible to put any quantitative interpretation on the measured concentration.

## Introduction

Sildenafil (Viagra) was the first effective oral therapy for the treatment of erectile dysfunction. It is known to allow men with erectile dysfunction to engage in sexual activity, specifi-

cally to achieve and maintain an erection. Penile erection is caused by the local release of nitric oxide (NO) into the corpus cavernosum by sexual stimulation. This results in increased levels of cyclic guanosine monophosphate (cGMP), a vasodilator. Sildenafil inhibits cGMP (of type 5)-specific phosphodiesterase (PDE-5), which is responsible for degradation of cGMP in the corpus cavernosum. This causes smooth muscle relaxation and allows blood to flow into the corpus cavernosum, which enables penile erection (1).

In forensic toxicology, sildenafil is associated with serious health problems, including death (2,3), and there have been issues with counterfeit pills entering the market (4,5).

The social stigma attached to the problem of erectile dysfunction and the effectiveness of the drug have led to cases of uncontrolled use of sildenafil. This was observed in subjects abusing the drug for recreational purposes and also in patients with heart conditions to whom the drug would not normally be deemed suitable for treatment. This misappropriation has resulted in cardiovascular complications requiring the intervention of both physicians and pharmacists (6).

More recently, new therapeutic applications of the use of sildenafil, including the treatment of pulmonary arterial hypertension, have been proposed (7,8). As a consequence, it was suggested that sildenafil may enhance performance at high altitude (9) and could be referred to as a doping substance.

Some indications have also been aimed at the female market: treatment of cellulite (10), treatment of orgasmic dysfunction (11), and treatment of antidepressant-associated sexual dysfunction (12). The latter two may offer an explanation for its use in sexual assaults.

The use of a drug to modify a person's behavior for criminal gain is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault or DFSA, robbery, sedation, etc.) has caused an increase in literature production. After ethanol and cannabis, benzodiazepines and hypnotics are the most frequently observed compounds in cases of alleged drug-facilitated crime (13). Until now, sildenafil had never been featured in literature

\* Author to whom correspondence should be addressed. E-mail: pascal.kintz@wanadoo.fr.

relating to the drugs that have been employed in these types of crimes. A literature search for papers on "impairment or crime by sildenafil" cited in the "Analytical Abstracts" and "Medline" databases was unable to produce any citation as searched during March 2009. The precise reason for the use of sildenafil in this type of crime is unknown, but speculation has centered on its reported ability to improve sexual arousal in women.

We present here an original method to test for sildenafil in hair by liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) and its application to a sexual assault case.

## Case History

Hair was submitted by a British police force following an allegation that a young female had been subjected to sexual assaults over a 2-year period when she was 15–17 years of age. The alleged perpetrator was her stepfather, and there was some suspicion that drugs may have been administered to facilitate the attacks. At the time, the stepfather admitted to consensual sexual intercourse with the girl but denied administering drugs. Lorazepam tablets were recovered from the home of the suspect, and intelligence suggested that he might also have had access to sildenafil and ketamine.

The assault was not reported to the police until some days after the last event, and around five days had passed between that last incident and a medical examination. Thus, it was believed that examination of blood or urine would offer little assistance in determining if drugs had been employed. Consequently, it was decided that a hair sample should be obtained from the victim for analysis. This was taken around two weeks after the last incident.

The laboratory received a lock of hair that was 12 cm in length and light brown in color with the request that it be analyzed for sedatives (including lorazepam and ketamine) and sildenafil by segmentation.

## Materials and Methods

### Specimen

Sildenafil-free hair samples were obtained from laboratory personnel. They were screened for sildenafil before being used for both calibrators and negative control.

### Chemicals and reagents

Acetonitrile, isopropanol, *n*-heptane, and methylene chloride were HPLC-grade (Merck, Darmstadt, Germany). Chemicals for the ammonium formate used in the mobile phase, ammonium chloride buffer used for the extraction [(NH<sub>4</sub>)<sub>2</sub>Cl, adjusted to pH 9.5], and formic acid were purchased from Fluka (Saint-Quentin Fallavier, France). Diazepam-d<sub>5</sub> was purchased from Promochem (Molsheim, France). Sildenafil citrate was obtained from Pfizer (Paris, France).

### Extraction

Hair strands were twice decontaminated using methylene chloride (5 mL, 2 min) and then segmented (segments of 2 cm). Each segment was cut into small pieces (< 1 mm). About 50 mg were incubated overnight in 1 mL of ammonium buffer at pH 9.5 in the presence of 10 ng of diazepam-d<sub>5</sub>, which was used as an internal standard (IS), at 40°C without agitation. After a liquid-liquid extraction with 5 mL of a mixture of dichloromethane/isopropanol/*n*-heptane (25:10:65, v/v/v) and evaporation to dryness, the residue was reconstituted in 50 µL of acetonitrile/2 mM formate buffer (20:80, v/v).

### LC-MS-MS procedure

LC was performed using a Waters Alliance 2695 system (North Brunswick, NJ). Chromatography was achieved using an XTerra MS C<sub>18</sub> column (100 × 2.1 mm, 3.5 µm) eluted at a flow rate of 0.2 mL/min with a gradient (20–80% to 80–20% at 9 min) of acetonitrile and formate buffer adjusted to pH 3.0 with 0.1% formic acid. An injection volume of 10 µL was used in all cases. A Quattro Micro triple-quadrupole MS (Micro-mass-Waters, Milford, MA) fitted with a Z-Spray ion interface was used for analyses. Ionization was achieved using electrospray in the positive ionization mode.

The following conditions were found to be optimal for the analysis of sildenafil and the IS: capillary voltage, 1.0 kV; source block temperature, 120°C; and desolvation gas (nitrogen) heated to 350°C and delivered at a flow rate of 550 L/h. In order to establish appropriate multiple reaction monitoring conditions, the cone voltage was adjusted to maximize the intensity of the protonated molecular ion, and collision-induced dissociation of both species was performed. Collision gas (argon) pressure was maintained at 3.0 × 10<sup>-3</sup> bar, and the collision energy (eV) was adjusted to optimize the signal for the two most abundant product ions and a third specific transition for sildenafil, even with lower abundance (Table I). MassLynx 4.0 software was used for quantitation.

### Method validation

A standard calibration curve was prepared in hair fortified with the drug and obtained by preparing spiked standards containing 5, 10, 50, 100, 200, 500, and 1000 pg/mg of sildenafil. Within-batch precision (*n* = 8) was determined using blank hair spiked with sildenafil at 100 pg/mg. The limit of detection (LOD) was evaluated by decreasing concentrations of

**Table I. MRM Transitions and Conditions for the Measurement of Sildenafil and the Internal Standard**

Compound	Parent Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
Sildenafil	475.0	57.8	40	45
		99.6	40	30
		283.0	40	42
Diazepam-d <sub>5</sub>	289.9	154.0	40	32
		198.1	40	32

sildenafil until a response equivalent to three times the background noise was observed. Relative extraction efficiency of the liquid–liquid procedure was determined by comparing the representative peak area of sildenafil extracted from negative control hair spiked at the final concentration of 100 pg/mg with the peak area of a methanolic standard at the same concentration.

The specificity of the method was evaluated by analyzing hair extracts from 12 non-drug-consuming subjects. The matrix effect on the ESI response was evaluated by comparing the instrumental response for a calibrator at 100 pg/mg directly injected in methanol (*a*) and the same amount of compound added to pre-extracted samples (*b*). The data for the calibrator in methanol provide a relative 100% value, and the matrix effect was defined as  $100 \times (a - b)/a$ . Ion suppression was established by infusing sildenafil at 1 mg/L and evaluating the deviation in signal response at its retention time when blank hair ( $n = 12$ ) are injected at the same time.

## Results and Discussion

### Validation

To be considered positive, the specimens required to produce transition ratios within 20% compared to that of the calibration standards.

Linearity was observed for sildenafil concentrations ranging from 5 to 1000 pg/mg with a correlation coefficient of 0.9988 ( $y = 2091.1x + 9.213$ ). Within-batch precision at 100 pg/mg was 12.8%, and the relative extraction efficiency was 72%. The LOD was 2 pg/mg with a limit of quantitation (LOQ) of 5 pg/mg. Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any extractable endogenous materials present in hair ( $n = 12$ ). Ion

suppression nor matrix effects higher than 10% were observed. Vardenafil (retention time = 7.01 min,  $m/z$  481 > 151 and 481 > 312) and tadalafil (retention time 9.38 min,  $m/z$  390 > 135 and 390 > 268) did not interfere with the assay.

### Detection in hair

Screening for sedatives (including lorazepam and ketamine) was negative in the hair using an LC–MS–MS procedure (14).

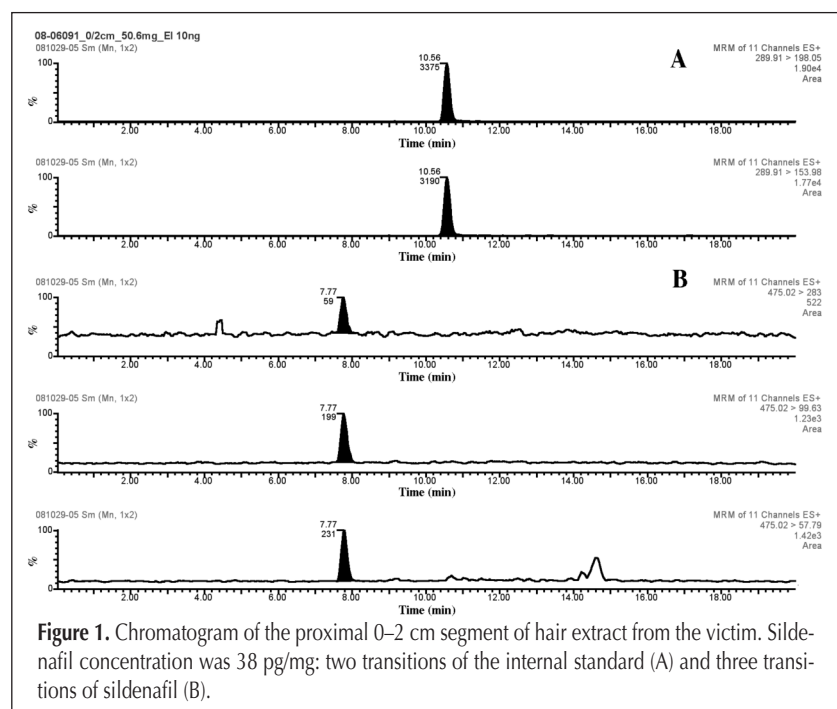
Figure 1 is the chromatogram obtained after extraction of the proximal segment of hair. The sildenafil concentration in this segment was determined to be 38 pg/mg. Segmental analyses demonstrated that only this proximal segment tested positive for sildenafil with all of the five consecutive segments moving away from the proximal segment having a concentration lower than the LOQ. No interpretation of the measured concentration in terms of dosage or frequency of exposure could be made because of the lack of controlled studies involving sildenafil. Nevertheless, its presence in a single segment suggested that the drug had not been administered over a prolonged period. At this stage, no information has been obtained from the perpetrator as to his reasons for administering sildenafil to the victim.

Very few papers dealing with sildenafil in hair are available in the literature. Dumestre-Toulet et al. (3) identified the drug at 177 pg/mg in the hair of a subject found dead in a hotel room after a sexual encounter with a colleague. A blood concentration of 105 ng/mL was also determined in this case. Saisho et al. (15) detected sildenafil in the hair from two patients who were reported to take the drug at regular intervals at 19,800 and 55,900 pg/mg. These latter two concentrations appear to be very elevated in comparison with the case of Dumestre-Toulet et al. (3).

The major practical advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2–4 days). For practical purposes, the two tests complement each other. Urine and blood analyses provide short-term information of an individual's drug exposure, whereas long-term histories are accessible through hair analysis. In addition, hair analysis may be especially useful when a history of drug use is difficult or impossible to obtain. The difference between a single exposure and long-term use can be documented by multisectional analysis (16).

## Conclusions

Hair testing should be used to complement conventional blood and urine analysis because it increases the window of detection and permits differentiation, by segmentation, of long-term therapeutic use from a single exposure. Selectivity and sensitivity of MS–MS are a prerequisite, given the low concentra-



**Figure 1.** Chromatogram of the proximal 0–2 cm segment of hair extract from the victim. Sildenafil concentration was 38 pg/mg; two transitions of the internal standard (A) and three transitions of sildenafil (B).

tions to be measured. The administration of sildenafil to obtain some gain during sexual assault was considered as a drug-facilitated crime.

## References

1. E.G. Boyce and E.M. Umland. Sildenafil citrate: a therapeutic update. *Clin. Ther.* **23**: 2–23 (2001).
2. W. Weinmann, M. Bohnert, A. Wiedenmann, M. Renz, N. Lehmann, and S. Pollak. Post-mortem detection and identification of sildenafil (Viagra) and its metabolites by LC/MS and LC/MS/MS. *Int. J. Leg. Med.* **114**: 252–258 (2001).
3. V. Dumestre-Toulet, V. Cirimele, S. Gromb, T. Belousoff, D. Lavault, B. Ludes, and P. Kintz. Last performance with Viagra: post-mortem identification of sildenafil and its metabolites in biological specimens including hair sample. *Forensic Sci. Int.* **126**: 71–76 (2002).
4. M. Thevis, Y. Schrader, A. Thomas, G. Sigmund, H. Geyer, and W. Schänzer. Analysis of confiscated black market drugs using chromatographic and mass spectrometric approaches. *J. Anal. Toxicol.* **32**: 232–240 (2008).
5. P. Kintz, M. Villain, J. Simonin, and V. Cirimele. Aspects médico-légaux de la prolifération des médicaments destinés au traitement des troubles de l'érection. *Ann. Toxicol. Anal.* **18**: 17–23 (2006).
6. K.M. Smith and F. Romanelli. Recreational use and misuse of phosphodiesterase 5 inhibitors. *J. Am. Pharm. Assoc.* **45**: 63–75 (2005).
7. J. Stehlik and M.A. Movsesian. Combined use of PDE5 inhibitors and nitrates in the treatment of pulmonary arterial hypertension in patients with heart failure. *J. Card. Fail.* **15**: 31–34 (2009).
8. S. Alp, M. Skrygan, W.E. Schmidt, and A. Bastian. Sildenafil improves hemodynamic parameters in COPD—an investigation of six patients. *Pulm. Pharmacol. Ther.* **19**: 386–390 (2006).
9. L.J. Rubin and R. Naeije. Sildenafil for enhanced performance at high altitude. *Ann. Int. Med.* **141**: 233–235 (2004).
10. K. Altabas, V. Altabas, M.C. Berkovic, and V.Z. Rotkvic. From cellulite to smooth skin: is Viagra the new dream cream? *Med. Hypotheses.* **73(1)**: 118–119 (2009).
11. A.L. Cavalcanti, V.R. Bagnoli, A.M. Fonseca, R.A. Pastore, E.B. Cardoso, J.S. Paixão, J.M. Soares, Jr., F. Saad, and E.C. Baracat. Effect of sildenafil on clitoral blood flow and sexual response in postmenopausal women with orgasmic dysfunction. *Int. J. Gynaecol. Obstet.* **102**: 115–119 (2008).
12. H.G. Nurnberg, P.L. Hensley, J.R. Heiman, H.A. Croft, C. Debatista, and S. Paine. Sildenafil treatment of women with antidepressant-associated sexual dysfunction: a randomized controlled trial. *J. Am. Med. Assoc.* **300**: 395–404 (2008).
13. M.A. LeBeau. Guidance for improved detection of drugs used to facilitate crime. *Ther. Drug Monit.* **30**: 229–233 (2008).
14. M. Villain, M. Concheiro, V. Cirimele, and P. Kintz. Screening method for benzodiazepines and hypnotics in hair at pg/mg level by liquid chromatography–mass spectrometry/mass spectrometry. *J. Chromatogr. B* **825**: 72–78 (2005).
15. K. Saisho, K.S. Scott, S. Morimoto, and Y. Nakahara. Hair analysis for pharmaceutical drugs. II. Effective extraction and determination of sildenafil (Viagra) and its N-desmethyl metabolite in rat and human hair by GC–MS. *Biol. Pharm. Bull.* **24**: 1384–1388 (2001).
16. P. Kintz. Bioanalytical procedures for detection of chemical agents in hair in the case of drug-facilitated crime. *Anal. Bioanal. Chem.* **388**: 1467–1474 (2007).