Int J Legal Med (2001) 114 : 252–258

CASE REPORT

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Post-mortem detection and identification of sildenafil (Viagra) and its metabolites by LC/MS and LC/MS/MS

Received: 8 February 2000 / Accepted: 10 June 2000

Abstract The highly putrefied corpse of an 80-year-old man was found in the apartment which he had rented to a prostitute. A package of Viagra 25 was found beside the corpse and three tablets were missing. Autopsy revealed severe coronary artery sclerosis as well as signs of previous myocardial infarctions. For the detection and identification of sildenafil and three metabolites in urine and tissue samples, solid-phase extraction, LC/MS and MS/MS methods were developed. Blood was not available for toxicological analysis due to the putrefaction. For method development, urine from a volunteer who had ingested 25 mg sildenafil was collected over 8 h, and three metabolites were identified by MS/MS. These metabolites were also found in the victim's urine. These findings prove that sildenafil was taken some time prior to death, but the causality of sildenafil intake and fatal cardiac failure could not be proven, since no blood was available for analysis. However, the administration of sildenafil was contraindicated due to several previous myocardial infarctions.

Keywords Sildenafil · Viagra · Tandem mass spectrometry · Electrospray ionisation · Cardiac failure

Introduction

Erectile dysfunction is widespread in males with cardiovascular disease, probably as a result of a combination of factors that impair haemodynamic mechanisms in the penis and general arteriosclerotic vascular disease [1, 2]. Sildenafil (Viagra) is known to allow men with erectile dysfunction to engage in sexual activity, specifically, to achieve and maintain an erection. Penile erection is caused by local release of NO into the corpus cavernosum by sexual stimulation, which results in increased levels of cyclic guanosine monophosphate (cGMP), a vasodilator. Sildenafil inhibits cGMP (type V)-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 [3].

Sildenafil is readily absorbed and eliminated, partly as an active N-demethyl metabolite with an in vitro potency for PDE-5 of approximately 50% by hepatic metabolism. Sildenafil is reported to have systemic vasodilator properties that result in lowered blood pressure in healthy patients, but patients with underlying cardiovascular disease could be affected adversely by such vasodilator effects, especially in combination with sexual activity. Serious cardiovascular events, including myocardial infarction, sudden cardiac arrest, ventricular arrhythmia, cerebrovascular haemorrhage, transient ischaemic attack and hypertension, have been reported post-marketing in temporal association with the use of sildenafil. Most, but not all of the patients involved had pre-existing cardiovascular risk factors. Many of these events were reported to have occurred during or shortly after sexual activity, and a few were reported shortly after the use of sildenafil without sexual activity. Others were reported to have occurred hours to days after the use of sildenafil and sexual activity.

However, the quantitation of sildenafil has only been performed in very few cases, and the metabolites only have been tested for in controlled pharmacokinetic studies. The following pharmacokinetic data and case reports have been published: after a single oral dose of 50 mg sildenafil during an open, randomised, three-way crossover study, a maximum plasma concentration of 260 ng/ml was found [4]. After oral intake of 100 mg of sildenafil, plasma concentration was found to reach a peak level of 450 ng/ml within 30–120 min and fell to about 80 ng/ml after 6 h. After a single oral dose of 0.68 mg/kg to three male volunteers the following mean peak plasma concentrations of sildenafil and metabolites were found after approximately 1 h: sildenafil 212 ± 59 ng/ml, demethylsildenafil 100 ng/ml, deethylsildenafil 49 ng/ml [5]. Mean plasma concentra-

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tions of sildenafil and demethylsildenafil fell to concentrations below 50 ng/ml in approximately 6 h. The plasma elimination half-life after oral administration of sildenafil was 3.7 ± 1.4 h. Using radiolabelled sildenafil, 76% of the dose was found to be excreted in the faeces and 13% in the urine in 120 h after oral administration. However, only a total amount of 34% of the dose was excreted in the first 24 h. Excretion could be determined over 5 days in faeces. A urine concentration of 400 ng/ml sildenafil was reported in one fatal case, where no sildenafil was detectable in blood above a detection limit of 100 ng/ml [6]. Four metabolites of sildenafil have been detected in human urine and faeces, however, the activity has not yet been investigated [5]. The safety and efficacy aspects of sildenafil have been discussed in several articles [7, 8, 9, 10, 11, 12, 13, 14]. In the following report a fatality with a combination of cardiovascular disease and self-medication of erectile dysfunction is presented.

Materials and methods

For general unknown screening, urine, stomach contents, and liver were analysed. Urine and stomach contents were analysed by GC/MS (HP 5970 MSD, Hewlett-Packard, Waldbronn, Germany) using standard procedures: liquid-liquid extraction with diethylether/ethyl acetate (1:1, v/v) at pH 7 and pH 10, full-scan GC/MS analysis with library searching [15]. Furthermore, one aliquot of the urine sample was hydrolysed by addition of concentrated hydrochloric acid and refluxed for 30 min, liquid-liquid extracted at pH 10 and acetylated prior to full-scan GC/MS analysis. Mass spectra library searching was employed using the drug libraries of Pfleger/Maurer/Weber [15], NIST and Wiley. Liver was homogenised using a homogeniser stick (Ultra-Turrax, IKA, Staufen, Germany) after dilution (1:1) with phosphate-buffer (pH 6). The liver homogenate was then extracted by solid-phase extraction after ultrasonication and centrifugation. Analysis of all extracts was also performed by high-performance liquid chromatography with photodiode array detection (HPLC/PDA) coupled on-line to an electrospray-mass spectrometer (ESI-MS). UV spectrum libraries with approximately 1700 compounds [16] and an ESI/CID-mass spectrum library developed in-house [17] with 430 compounds (ESI/CID: electrospray-ionisation/collision induced dissociation) were used for compound identification. Urine was further analysed by immunoassay for common drugs of abuse. Furthermore, urine and homogenised muscle (1 g muscle, 1 g water added and homogenised with homogeniser stick) were analysed for ethanol with headspace-GC and alcohol dehydrogenase.

For HPLC/PDA/MS the following instrumentation was used: HPLC system (Shimadzu, Duisburg, Germany): pump LC10AD, low pressure gradient mixer, photo-diode array detector (PDA) SPDM10A, interface module CBM10A and 486/100 DOS PC; column: RP-C8-select B, 2 mm i.D.x125 mm, 5 µm particle size (Merck, Darmstadt, Germany). This HPLC/PDA system was coupled without splitting to a PE/SCIEX API 365 triple-quadrupole mass spectrometer with a turbo-ion spray source (PE-SCIEX, PE Biosystems, Langen, Germany) equipped with an Apple Macintosh G3 Power PC, MassChrom and Multiview 1.4 software, an ion spray-CID mass spectra library of drugs and an ion spray-MS/MS library of drugs (ChemicalSoft, Freiburg, Germany). Deionized water (<0.1 µS from a cartridge deioniser, Memtech, Moorenweis, Germany), gradient grade acetonitrile, 25% aqueous ammonia and formic acid (analytical grade, Merck) were used as HPLC solvents and for redissolving drug standards and extracts. The HPLC solvents were solvent A (1 mM ammonium formate/0.1% formic acid, pH 3) and solvent B (acetonitrile/0.1% formic acid). The gradient was 10–30% B (0–6.6 min), 30–70% B (6.6–26.6 min), 70–90% B (26.6–33.2 min), 90% B (33.3–35.2 min). Sildenafil was obtained from a tablet of Viagra 25, since no reference compound was available at the time of analysis. The internal standard D_3-doxepine was obtained from Promochem/Radian (Wesel, Germany). This standard has been successfully used with the following solid-phase extraction (SPE) method for general unknown screening by LC/MS in our laboratory. Extraction of urine and homogenised tissue was performed by SPE using mixed-mode SPE-cartridges (Chromabond Drug, 3 ml/200 mg, Macherey-Nagel, Düren, Germany) and an automated SPE-device (RapidTrace, Zymark, Idstein, Germany [18]). The extraction method has been described for the extraction of drugs of abuse from serum samples [19]. The evaporated eluate was redissolved in 100 µl HPLC solvent (A:B, 1:1, v/v) and 20 µl was injected into the LC/MS/MS system in positive ionisation mode. Full-scan spectra of a reference compound and extracts were acquired in the single-quadrupole mode (Q1-scan) with a scan range of 50–550 amu using a looped experiment with orifice voltage switching (20, 50 and 80 V) between each scan [17], a dwell-time of 2 ms and a step-size of 0.5 amu. Increase of orifice-voltage induces fragmentation of molecular ions of most substances for structural identification – a method which had been developed for setting-up the LC/ESI-CID/MS library in our laboratory [17]. For the identification of sildenafil metabolites a volunteer's urine sample was analysed by MS/MS in product-ion scan mode.

Quantitation of sildenafil and its metabolites in urine and tissue samples

For quantitation of sildenafil and its metabolites in urine, control urine samples were spiked with 50, 100, 250, 500 and 1000 ng/ml sildenafil, respectively, and 100 ng/ml D_3-doxepine as internal standard. Using peak areas of the extracted MH^+-ion chromatogram (Q1-scan, 20 V orifice) linearity was found in this range (r = 0.98). The limit of detection (LOD) and limit of quantitation (LOQ) were determined using linear regression with an α-error of 1% and a relative confidence interval of 33% (k = 3) [20]. The LOD was 14 ng/ml, the LOQ was 25 ng/ml for the MH^+ (m/z 475) at 20 V orifice-voltage. The day-to-day precision and accuracy was tested on five consecutive days: urine samples spiked with sildenafil at two concentration levels (250 and 1000 ng/ml) were analysed three times on each day yielding mean values of 241 ng/ml and 978 ng/ml,

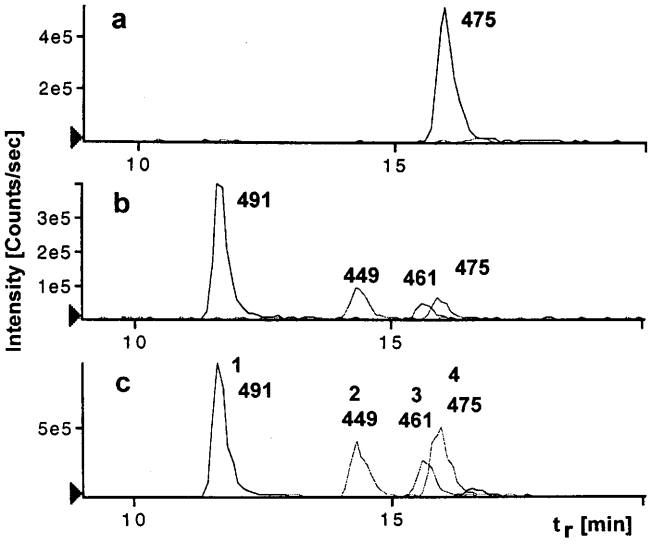


Fig. 1 Reconstructed ion chromatograms of **a** spiked urine, **b** victim's urine, **c** volunteer's urine. MH^+ ions of sildenafil (peak *4*, m/z 475) and three metabolites (peaks *1–3*, m/z 491, 449 and 461) were extracted from a full-scan spectrum using Q1 scan mode. Peak numbers (1–4) are also used in the following figures

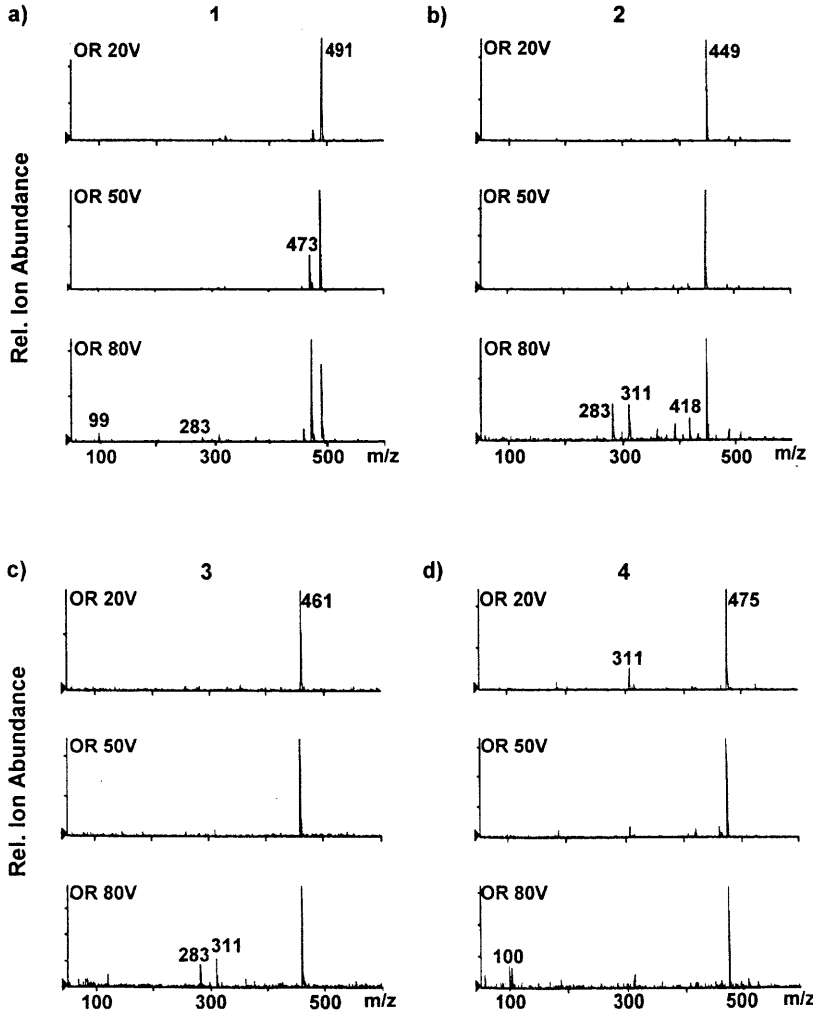


Fig. 2 ESI/CID-mass spectra of sildenafil and sildenafil metabolites using orifice-voltage switching (20, 50 and 80 V) with Q1 scan mode (*1–4* refer to the chromatographic peaks as specified in Fig. 1)

with coefficients of variation (CV) of 8% and 7%, respectively. Sildenafil was quantified by LC/MS in the urine samples obtained from the volunteer and the victim (Table 1). For quantitation of sildenafil metabolites in the volunteer's urine, UV peak areas of metabolites and sildenafil were compared. Assuming a similar extinction coefficient for sildenafil and all metabolites (see Fig. 4), peak areas at 225 nm were used for quantitation of compounds in the volunteer's urine (see Fig. 3). For quantitation of sildenafil and its metabolites different extraction efficiencies were taken into account (Table 2). The extraction efficiency for sildenafil by solid phase extraction from urine was determined by repeated analysis (n = 3) of urine spiked with 250 ng/ml sildenafil prior to extraction, and of blank urine with 250 ng/ml sildenafil added to the eluate after extraction. Internal standard D_3-doxepine (100 ng/ml) was added to sildenafil-spiked and to blank urine prior to extraction. From the concentration difference, calculated after normalisation by the internal standard, the extraction efficiency was calculated for sildenafil (79 ± 6%) (Table 2). Since no reference compounds for the metabolites were available the extraction efficiency was determined by extracting two 1 ml-aliquots of the volunteer's urine sample, one eluate was directly analysed by LC/MS, the other eluate was redissolved with 1 ml drug-free control urine, extracted a second time with SPE and then analysed by LC/MS. From the differences in ion abundances, the extraction efficiencies were determined for sildenafil and its metabolites (Table 2).

Table 1 Concentrations of sildenafil and its metabolites ([a] determined by PDA at 225 nm, [b] determined by MRM using response factors, [c] not detected. Concentrations of metabolites are only putative, assuming similar extinction coefficients for the different analytes at 225 nm)

Substance	Volunteer's urine	Victim's urine	Liver	Muscle	Brain
Propyl-oxidated Sildenafil (1)	1876 ng/ml[a]	843 ng/ml[b]	126 ng/g[b]	2.2 ng/g	–[c]
Deethylsildenafil (2)	586 ng/ml[a]	138 ng/ml[b]	56 ng/g[b]	1.3 ng/g	–[c]
Demethylsildenafil (3)	233 ng/ml[a]	44 ng/ml[b]	11 ng/g[b]	–[c]	–[c]
Sildenafil (4)	526 ng/ml	63 ng/ml	74 ng/g	0.5 ng/g	1.2 ng/g

Table 2 Analytical data of sildenafil and metabolites

[a] Extraction efficiency determined by extraction once versus twice
[b] Extraction efficiency determined by repeated extractions of urine samples 250 ng/ml sildenafil added prior to extraction ($n = 3$) and to the eluate after extraction ($n = 3$)

Substance	Retention time (min)	MH^+	MS response factor	Transitions for MRM (m/z)	MRM response factors	Extraction efficiency of SPE
Propyl-oxidated sildenafil	11.8	491	0.46	491 → 310	0.27	72%[a]
Deethylsildenafil	14.6	449	0.64	449 → 283	2.8	72%[a]
Demethylsildenafil	15.8	461	1.3	461 → 283	4.7	68%[a]
Sildenafil	16.4	475	1	475 → 283	1	81%[a] (79 ± 6%[b])
D_3-Doxepine	18.7	283	–	283 → 107	–	69%[a]

Mass spectrometric peak areas of the quasi-molecular ions were used for the quantitation of compounds in the victim's urine since UV signals at 225 nm were not sufficiently abundant for quantitation. For this purpose, mass spectrometric response factors (Table 2) for the molecular ions of the metabolites were calculated in the following way: the ratio of ion-intensities of metabolite and sildenafil were divided by the concentration ratios as determined by UV at 225 nm:

$$\text{MS response factor}_{MET} = \frac{(\text{int. } MH^+_{MET}) / (\text{int. } MH^+_{SIL})}{[Met]_{UV} / [Sil]_{UV}} \quad (1)$$

where *int. MH*$^+$ is the intensity of the molecular ion of metabolite (*Met*) and sildenafil (*Sil*).

For the analysis of tissue samples a multiple-reaction-monitoring (MRM) experiment was set up for higher selectivity and sensitivity. Product ion spectra (MS/MS) of sildenafil and D_3-doxepine (m/z 283 → 107) were recorded. Optimisation of the MS/MS transition of sildenafil (m/z 475 → 283) was performed by infusion of a sildenafil solution (1 µg/ml) using a syringe pump. The same MS/MS conditions were used for the analysis of demethylsildenafil (m/z 461 → 283), deethylsildenafil (m/z 449 → 283) and propyl-oxidated sildenafil (m/z 491 → 310) (see Fig. 5 and Table 2). The concentration of sildenafil in the tissue samples was determined using a calibration curve obtained from SPE-extracted standard solutions (sildenafil: 0.5–50 ng/ml with D_3-doxepine as internal standard) by LC/MS/MS in MRM mode. The extract of the volunteer's urine was analysed after dilution and the response factors for the transitions of the metabolites (Table 2) were calculated using a formula similar to equation (1), but instead of the intensities of MH^+ ions, the intensities of the MRM transitions were used.

For the extraction of tissue samples 8 ml of phosphate buffer was added to 0.5–1.5 g of sample. The tissue samples were homogenised (Ultra-Turrax, IKA, Staufen, Germany) ultrasonicated, centrifuged and filtered, internal standard (D_3-doxepine) was added and the supernatant was extracted by SPE. The eluates were evaporated, redissolved in mobile phase (A:B, 1:1, v/v), and analysed by LC/MS/MS in the MRM mode.

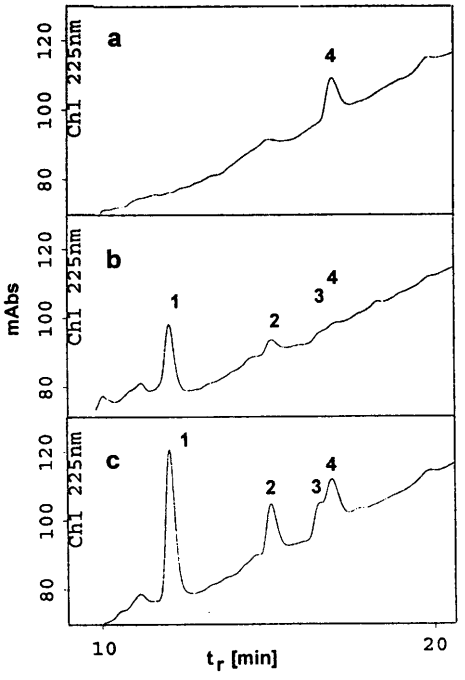


Fig. 3 UV chromatograms at 225 nm of **a** spiked urine; **b** victim's urine; **c** volunteer's urine (*1–4* refer to the chromatographic peaks as specified in Fig. 1)

Case report

An 80-year-old man was found dead on the floor in a single-room apartment. The fully dressed body was already in a state of putrefaction, since it had been lying there for approximately 3 days in midsummer at temperatures up to 35 °C. The apartment was rented to a prostitute whom he frequently met there. His personal valuables were missing. Beside the corpse, a package of Viagra 25 was found from which three tablets were missing.

According to the report of his family, the man had suffered from chronic heart insufficiency, hypertension, and had taken beta-receptor antagonists and diuretics regularly. Some of the prescribed drugs were found in his car. An autopsy was ordered by the public prosecutor because of the suspicious circumstances of the case.

Results

Autopsy results

The autopsy was performed the same day the corpse was found. No injuries could be detected by external and internal examination. The major internal findings were severe arteriosclerosis, pulmonary artery sclerosis, coronary artery sclerosis, enlargement of the heart, and several scars on the myocardium after myocardial infarction at the apical region and at the interventricular septum. Histological examination of the cardiac tissue revealed fibrotic areas and hypertrophic myocardial fibres, but no signs of acute ischaemic damage. Due to the suspicious nature of the scene of discovery (i.e., a box of Viagra had been placed beside the corpse and the victim's valuables and credit card had been stolen), a toxicological analysis was ordered in addition to the autopsy.

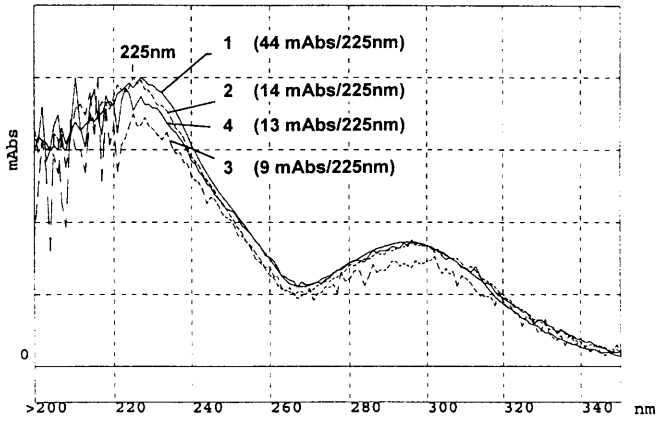
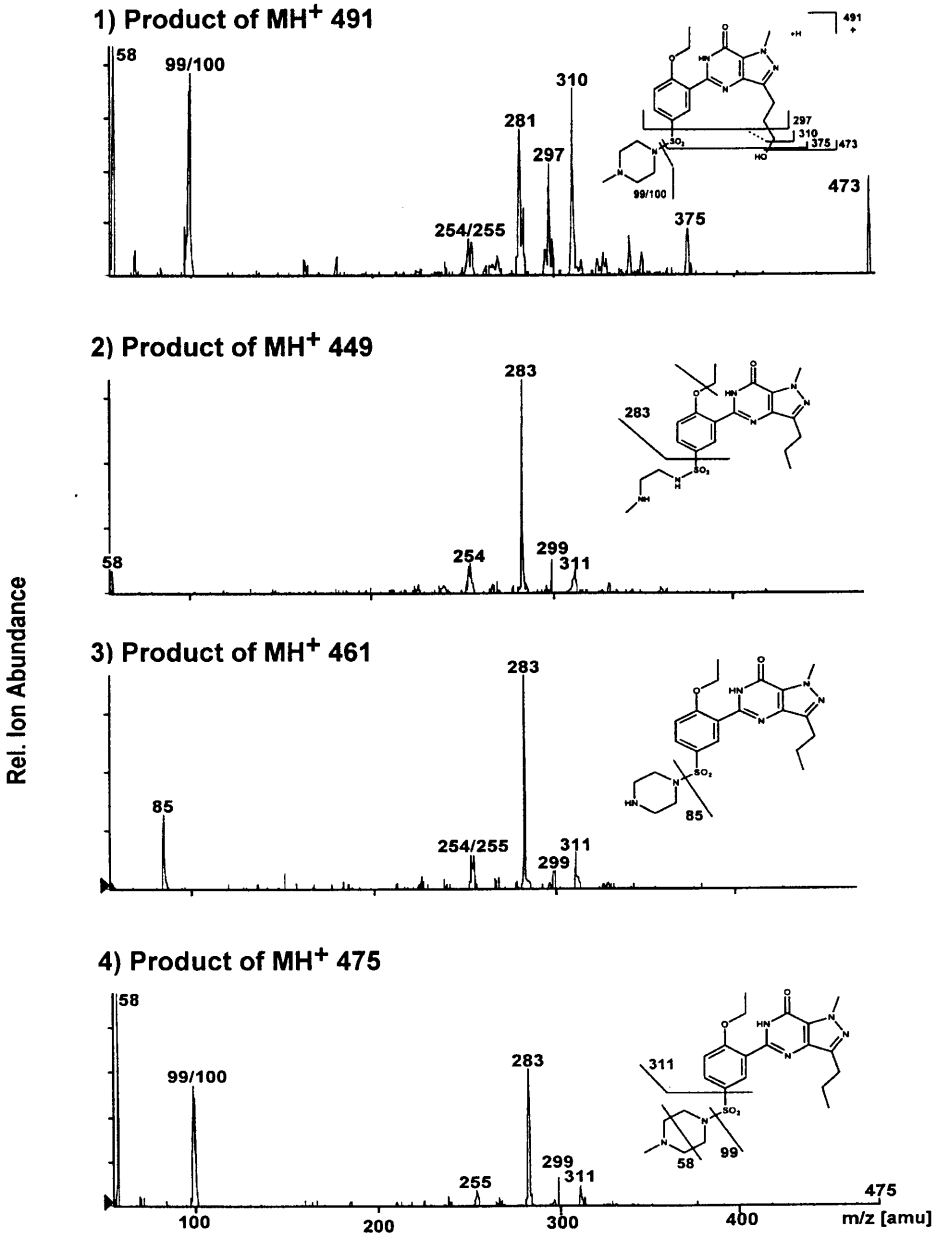


Fig. 4 UV spectra of sildenafil and sildenafil metabolites (*1–4* refer to the chromatographic peaks as specified in Fig. 1)

Results of the toxicological analysis

Apart from sildenafil and its metabolites in urine and tissue samples, xipamide (a diuretic) was also found in the stomach contents and urine. No other drugs or metabolites could be detected in urine, stomach content and liver. Alcohol was not present in the urine and muscle samples above 50 µg/g. For the identification of sildenafil metabolites, the extract of a volunteer's urine sample which was collected 8 h after intake of one Viagra 25 tablet was first analysed by LC/MS in Q1-scan mode and four compounds were found with protonated molecular ions of m/z 491 (1), 449 (2), 461 (3) and 475 (4) (see Fig. 1c). The extracted ion chromatograms of the spiked urine sample (500 ng/ml sildenafil) and of the victim's urine sample are shown in Fig. 1a,b. The same metabolites were found in the victim's urine as were detected in the volunteer's

Fig. 5 MS/MS product ion spectra of sildenafil and sildenafil metabolites (*1–4* refer to the chromatographic peaks as specified in Fig. 1)



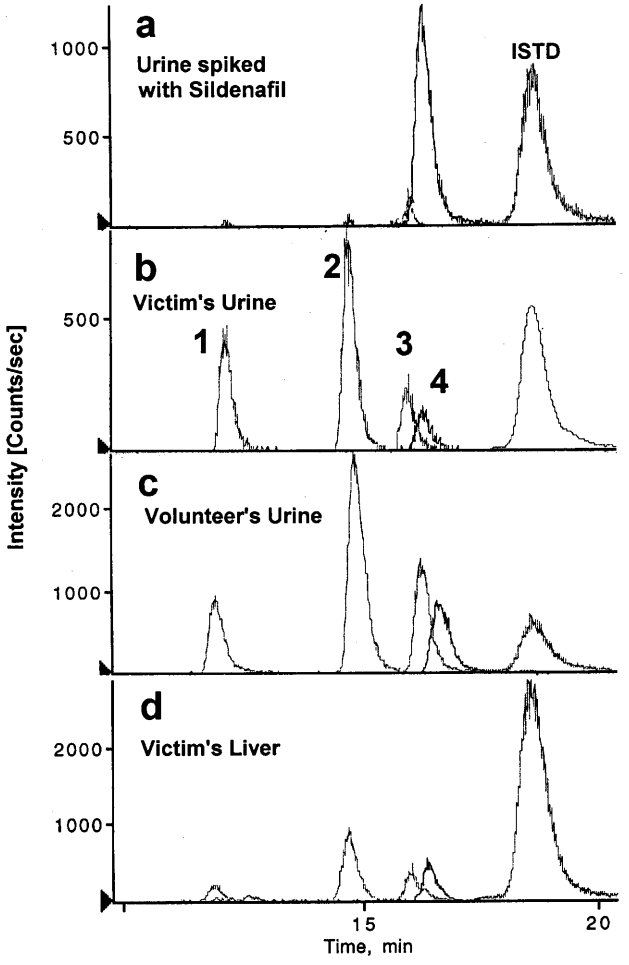


Fig. 6 LC/MS/MS analyses using MRM for the determination of sildenafil and sildenafil metabolites in urine and tissue samples. Concentrations determined by these experiments are listed in Table 1 (*ISTD* internal standard D_3-doxepine)

urine. The Q1-mass spectra at three orifice-voltages (20, 50 and 80 V, respectively) are shown in Fig. 2.

HPLC-chromatograms at 225 nm of the same urine samples are shown in Fig. 3. UV spectra of sildenafil and three metabolites from the volunteer's urine are displayed in Fig. 4. The sildenafil concentration in the volunteer's urine sample was quantified by LC/MS using a calibration curve of spiked urine samples. From the similarity of the UV spectra it was assumed that the metabolites and sildenafil have approximately the same extinction coefficients at 225 nm, thus for semiquantitative determination, the absorption at 225 nm of the three metabolites was compared with that of sildenafil. Quantitation of sildenafil and metabolites was performed by multiple reaction monitoring in tissue samples and urine (see Fig. 6), using mass spectrometric response factors. The concentrations of sildenafil and its metabolites are listed in Table 1.

For the mass spectrometric identification of the metabolites 1, 2 and 3, MS/MS product-ion scanning of the molecular ions was used with the volunteer's urine sample. Product-ion mass spectra of the MH^+ ions and proposed structures of the three metabolites and characteristic fragmentation sites are shown in Fig. 5. All three metabolites have recently been identified by Walker et. al. in a pharmacokinetic study of sildenafil in human urine or faeces; however, complete structural identification by mass spectrometry was not shown [6].

Discussion

From March until September 1998, 69 deaths of males taking sildenafil were reported in the USA. No cause and effect for these fatalities has yet been proven, but it is suspected that some of these patients would not have been taking Viagra 25 if proper medical precautions had been observed [21]. Most of the deaths reported since Viagra went on the market in late March 1998 in the US have been cardiac-related [2]. Although the reported cardiovascular effects of sildenafil in randomised controlled clinical trials were minor, there is a risk since patients suffering from heart disease represented only a small fraction of the patients studied. Moreover, patients with cardiac failure, patients with myocardial infarction or stroke within the 6 previous months, or patients with uncontrolled hypertension were not included in these studies [1].

In the case presented here, concentrations of sildenafil and metabolites in urine were low compared to data from the literature and compared to the concentrations found in a volunteer's urine after intake of a low dose of sildenafil. Also concentrations in liver and muscle were low and no sildenafil was detectable in stomach contents. Since blood was not available for analysis, the contribution of sildenafil to the cause of death could not be estimated. The metabolite spectrum found in the victim's urine was comparable to that found in the volunteer's urine sample collected for 8 h after intake of 25 mg sildenafil. It should be emphasized that the major detectable metabolite in urine from the victim and the volunteer as well as in the victim's liver and muscle was propyl-oxidated sildenafil, in addition to lower concentrations of piperazine-N,N′-deethylated sildenafil, piperazine-N-demethylated sildenafil and sildenafil. Therefore, for toxicological analysis of urine samples it is important to search not only for sildenafil itself, but also for the oxidated and dealkylated metabolites. The propyl-oxidated sildenafil was found with high peaks by PDA and mass spectrometric detection at a significantly shorter retention time than sildenafil. However, no pharmacokinetic data of the propyl-oxidated sildenafil in blood or plasma are yet available.

In the case presented here, there was no final proof that the intake of sildenafil was the cause of death, but after toxicological investigation it was clear that the deceased had taken sildenafil before he died. Sildenafil was contraindicated due to previous myocardial infarctions, severe arteriosclerosis and reported hypertension.

References

1. Conti CR, Pepine CJ, Sweeney M (1999) Efficacy and safety of sildenafil citrate in the treatment of erectile dysfunction in patients with ischemic heart disease. Am J Cardiol 83: 29C–34C
2. Cheitlin MD, Hutter AM, Brindis RG, Ganz P, Douglas PS, Faxon DP, Fisher JD, Gibbons RJ, Halperin JL, Hochman JS, Weintraub WS, Winters WL, Wolk MJ (1999) Use of sildenafil (Viagra) in patients with cardiovascular disease. Circulation 99: 168–177
3. Goldenberg MM (1998) Safety and efficacy of sildenafil citrate in the treatment of male erectile dysfunction. Clin Ther 20: 1033–1048
4. Cooper JDH, Muirhead DC, Taylor JE, Baker PR (1999) Development of an assay for the simultaneous determination of sildenafil (Viagra) and its metabolite (UK-103,320) using automated sequential trace enrichment of dialysates and high-performance liquid chromatography. J Chromatogr B 701: 87–95
5. Walker DK, Ackland MJ, James GC, Muirhead GJ, Rance DJ, Wastall P, Wright PA (1999) Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. Xenobiotica 29: 297–310
6. Ng TL, Chow ST, Yao YJ, Koh TH, Lo DST (1999) Sildenafil detected in urine of first Viagra-related death in Singapore. TIAFT Bulletin 14/4: 20–22
7. Morales A, Gingell C, Collins M, Wicker PA, Osterloh ICH (1998) Clinical safety of oral sildenafil citrate (Viagra) in the treatment of erectile dysfunction. Int J Impot Res 10: 69–73
8. Derry FA, Dinsmore WW, Fraser M, Gardner BP, Glass CA, Maytom MC, Smith MD (1998) Efficacy and safety of oral sildenafil (Viagra) in men with erectile dysfunction caused by spinal cord injury. Neurology 51: 1629–1633
9. Licht MR (1999) Sildenafil (Viagra) for treating male erectile dysfunction, Cleve Clin J Med 65: 301–304
10. Sperling H, Michel MC, Rubben H (1999) Viagra: tolerability, contraindications and drug interactions. Urologe A28: 124–127
11. Jarow JP, Carson CC, Basskin LE, Parham W, Chang CF, Rajfer J, Kloner RA, Rowland DL, Leider H, Sadovsky R, Sharlip ID (1999) An overview of erectile dysfunction. Am J Manage Care 4: S7–S14
12. Jarow JP, Carson CC, Basskin LE, Parham W, Chang CF, Rajfer J, Kloner RA, Rowland DL, Leider H, Sadovsky R, Sharlip ID (1999) Cardiovascular risk factors and erectile dysfunction. Am J Manage Care 5: S31–S39
13. Rolf C, Nieschlag E (1998) Sildenafil (Viagra) in erectile dysfunction. Effective treatment with noteworthy side effects. Dtsch Med Wochenschr 123: 1356–1361
14. Meinhardt W, Kropman RF, Vermeij P (1999) Comparative tolerability and efficacy of treatments for impotence. Drug Saf 20: 133–146
15. Pfleger K, Maurer HH, Weber A (eds) (1992) Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites. VCH Verlagsgesellschaft mbH, Weinheim
16. Pragst F, Erxleben B-T, Herre S (1997) UV-Spektren toxischer Verbindungen. In: Pragst F (ed) Photodiodenarray-UV-Spektrenbibliothek von Medikamentenwirkstoffen, illegalen Drogen, Pestiziden, Umweltnoxen und anderen Giften, 2nd edn. Institut für gerichtliche Medizin der Humboldt-Universität, Berlin
17. Weinmann W, Wiedemann A, Eppinger B, Renz M, Svoboda M (1999) Screening for drugs in serum by electrospray ionization/collision-induced dissociation and library searching. J Am Soc Mass Spec 10: 1028–1037
18. Diamond FX, Vickery WE, DeKanel J (1996) Extraction of benzoylecgonine (cocaine metabolite) and opiates (codeine and morphine) from urine samples using the Zymark RapidTrace. J Anal Toxicol 20: 587–591
19. Weinmann W, Svoboda M (1998) Fast screening for drugs of abuse by solid-phase extraction combined with flow-injection ionspray-tandem mass spectrometry. J Anal Toxicol 22: 319–328
20. Funk W, Damman V, Donnevert G (eds) (1995) Quality assurance in analytical chemistry. VCH Verlagsgesellschaft, Weinheim/Germany, pp 32–40
21. Viagra's licence and the internet (editorial) (1998) Lancet 352: 751