A non-fatal self-poisoning attempt with Sildenafil

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Abstract

The phosphodiesterase type 5 inhibitor, sildenafil, is not generally known for its use as a self-poisoning drug. However, intoxication cases with lethal outcome have been described. The case presented here is of a 56-year-old man who claimed to have undertaken an unsuccessful suicide attempt by mono-ingestion of 65 tablets of 100 mg sildenafil. He arrived at the emergency department 24 hours after intake with severe vomiting and symptoms of blurred vision. Clinical examination revealed no priapism. Of note was a sinus tachycardia of 100bpm without signs of hypotension. In order to quantify the sildenafil level in serum, an HPLC-PDA method was developed and validated according to European Medicines Agency (EMA) guidelines. The intoxicated patient had a serum concentration of 22.2 µg/mL sildenafil, at the time of presentation, which is far above the therapeutic peak concentration. The serum concentration further declined to 9.2 and 2.3 µg/mL, respectively 5 and 14 hours later, revealing a biological half-life of 4.2 hours.

To the best of our knowledge, this patient took the highest sildenafil dose, which resulted in the highest serum level, ever reported. In this subject, sildenafil showed good tolerability since few symptoms occurred and only moderate supportive therapy was needed for full recovery without sequelae.

Introduction

Sildenafil or 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine was originally investigated as an antihypertensive agent and is used for the treatment of pulmonary hypertension under the trade name Revatio® (Pfizer) (1), although its use as Viagra® in the setting of erectile dysfunction is generally better known (2). Its mechanism of action is the inhibition of phosphodiesterase type 5 (PDE_5), which results in an increase in NO-stimulated release of cGMP and subsequent smooth muscle relaxation and vasodilation. Inhibition of PDE_5 in the corpus cavernosum smooth muscle facilitates erectile response to sexual stimulation (3).

Sildenafil has a good safety profile in the registered once daily doses of 25-100 mg (4). Adverse effects associated with sildenafil therapy are transient, moderate and dose-dependent effects linked to vasodilation, including headache, facial flushing and nasal congestion (4–7). Occasional visual disturbances like bluish haze or blurred vision are explained by its action on PDE_6 which is present in the retina (3, 6). An overdose of sildenafil may theoretically result in hypotension, tachycardia and cardiac arrest (5). However, a post-marketing safety analysis of overdose cases revealed that exceeding the maximum recommended daily dose or dose frequency only results in an increased occurrence and severity of the known adverse drug reactions, including a dose-related increase in the frequency of visual adverse events, but no clear relationship between dose and maximum decreases in blood pressure and no clinically significant changes in electrocardiogram. Due to the small number of cases, a relationship between sildenafil overdose and increased cardiac risk could not be excluded. The highest reported single dose in their post-marketing survey was of a 33-year old man who took 24 tablets of 100 mg sildenafil. He was diagnosed with annular scotoma, defective color vision, vascular retinal dilatation, visual field defect and papilledema. He recovered from all except for visual field defect and annular scotoma (4). Although experience with sildenafil overdose is limited, a few cases (6, 8–13) are reported in literature including two with a fatal outcome (Table 1). One due to an omental varix rupture in 41-year old male with advanced alcoholic cirrhosis (9) and

another due to sudden cardiac death in a 56-year old male, with pre-existing risk factors for coronary artery disease like hypertension, diabetes mellitus and excessive alcohol consumption. Furthermore, he received a multidrug treatment for essential hypertension and an anxio-depressive disorder (6). The symptoms in the other non-fatal overdose cases included optic neuropathy and cilioretinal artery obstruction resulting in complete blindness (8), prolonged priapism (10, 12), mild tachycardia (13) and rhabdomyolysis (11). Aortic and vertebral artery dissection have been associated with chronic sildenafil abuse (14, 15).

Case history

A 56-year-old man contacted the emergency services after an attempted suicide with 65 tablets of 100 mg sildenafil citrate (Viagra or Kamagra, the latter being available for on-line purchase without prescription) approximately 24 hours earlier. He experienced severe vomiting and reported symptoms of blurred vision and difficulties in distinguishing facial expressions. The subjective visual perception included a dark view with occasional light flashes. Clinical examination revealed a pulse of 100 beats/min, a blood pressure of 125/70 mm Hg and normal body temperature. Sinus tachycardia of 118/min was found on ECG and head CT showed dilatation of the ventricular system and subarachnoid spaces. Lab results were only minimally abnormal with a slightly diminished kidney function (GFR: 53.8 mL/min/1.73m^2) and increase in inflammatory parameters (elevated CRP: 10.6 mg/L (normal: < 5 mg/L) and leucocytosis (13.9 10^9/L, reference range: 3.45-9.76 10^9/L)). A transient increase in troponin I was also noted, with no clinical signs or echocardiographic abnormalities. Supportive care was given and an HPLC-DAD method was optimized and validated in order to quantify the sildenafil serum level.

Experimental

Reagents and chemicals

Sildenafil citrate was purchased from Sigma-Aldrich (Bornem, Belgium) and dissolved in methanol at a stock concentration of 2 mg/mL. Prazepam (LGC Standards, Molsheim, France) was used as internal

standard (IS) in a 0.75 µg/mL methanol working solution. All other reagents were purchased from Merck (VWR, Haasrode, Belgium). Methanol and acetonitrile were HPLC grade.

Sample preparation

Venous blood samples were collected in EDTA serum geltubes, centrifuged and stored at 4°C until analysis. To 350 µL serum, 35 µL methanol, 35 µL IS working solution, 100 µL 1M carbonate buffer pH 9.5, 400 µL ethyl acetate and 950 µL n-hexane were added. After mixing vigorously on a vortex shaker for 30 s, the samples were rotated for 5 min at a speed of 20 rpm, again vortexed during 5 s and centrifuged at 10,000 g for 5 min. The upper layer was transferred to another vial and dried under a nitrogen stream. The residue was reconstituted in 200 µL methanol and again dried after a 30 s vortex step and centrifugation at 5,000 g during 2 min. The residue was resolved in 70 µL of a water:methanol mixture (75:25, v/v). Twenty µl of this solution was injected into the HPLC system.

Chromatography

HPLC was carried out on an Agilent 1100/1200 series HPLC equipped with an autosampler, quaternary pump, column oven and photodiode array (PDA) detector. System management and data acquisition were performed with the Agilent Chemstation software. The analytical HPLC column was a C8 ZORBAX Eclipse Plus (Agilent), 150 mm x 3.0 mm internal diameter with 3.5 µm particle size. The column temperature was held at 40°C. The run was performed with a gradient shown in Table 2, and the mobile phase was a mixture of solution A (10mM phosphate buffer, pH 2.3) and solution B (10 mM phosphate buffer pH 2.3: acetonitrile (20:80)). Detection was performed at 225 nm.

Method validation

The quantification of sildenafil in serum was validated according to European Medicines Agency (EMA) guidelines (16). Ten sources of blank serum were individually analyzed and evaluated for interference. Carry-over effects were studied by measuring blank samples after analysis of samples with high sildenafil concentration. Verification of the linearity was performed by analysing 7

calibration standards (0.025, 0.05, 0.1, 0.25, 0.5, 1 and 2.5 µg/mL) on 6 different days. A calibration curve was constructed by plotting the peak area ratio (Y-axis) of the target compound to the IS versus the nominal concentrations (X-axis) with a least-squares linear regression. The method was considered linear if the coefficient of determination (r^2) was equal to or better than 0.99. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration plot with an imprecision and bias of < 20%. In order to evaluate the accuracy and precision of the method, quality control (QC) samples at 4 different concentrations are analysed in sixfold on one day (intra-day accuracy and precision) or on 6 consecutive days (inter-day accuracy and precision). The following QC concentrations were used: 0.025 µg/mL (LLOQ), 0.075 µg/mL (low QC), 0.25 µg/mL (medium QC) and 2 µg/mL (high QC). In order to meet the EMA accuracy criterion, the mean value of the calculated concentrations should be within 15% of the actual spiked value. The precision is reported as the variation coefficient (%) and should not exceed 15% at every concentration. The recovery was assessed by comparing the peak area ratios (sildenafil/IS) between samples in which sildenafil was added before and after extraction. This was repeated 6 times for both the low and high QC concentration. A tenfold dilution of serum was also tested (n=6) due to the high expected sildenafil concentration in the patient serum samples.

Data analysis was generated using GraphPad Prism software version 6.05 for Windows.

Results

Method validation

The analytical peaks of sildenafil and IS are well resolved with retention times of 10.3 and 16.6 minutes respectively. Chromatograms of blank and calibrator are presented in Figure 1A and 1B. No interfering peaks were observed in the extracts of 10 different blank plasma samples and no carry-over from high concentrated samples was found. The limit of detection (LOD) was 0.008 µg/mL. The sildenafil calibration curve ranging from 0.025 to 2.5 µg/mL, exhibited a good linearity with a coefficient of determination (r^2) of 0.999. Both intra- and inter-day variation coefficients were below

15 % and error percentages were between -15 % and 15 % (Table 3). The recovery rates were 79 ± 8 % for the low QC level and 80 ± 6 % for the high level. A tenfold dilution of high sildenafil-concentrated serum resulted in concentrations of 97 ± 10 % of the expected values.

Patient results

The diagnosis of sildenafil intoxication was confirmed by the analysis of multiple serum specimens of the patient during 3 days in which the clearance of the drug could also be studied. The first sample was drawn at the time of hospital admission, which was approximately 24 hours after the sildenafil intake, and showed the highest concentration (22.2 µg/mL). The chromatogram of this sample (tenfold diluted) is shown in Figure 1C. Since the patient presented himself hours after the intoxication, the initial concentration had probably been higher (about 900 µg/mL). Moreover, as adsorption of sildenafil to the separator gel in the serum collection tubes cannot be excluded, *in vivo* concentrations might have been even higher. The subsequent measurements revealed a rapid decline in sildenafil concentration to 9.2 and 2.3 µg/mL, respectively 29 and 38 hours after intake. Sildenafil clearance showed first order kinetics in which the plasma concentration-time profile during the elimination phase decreases exponentially in the plot with linear axes (Figure 2) and is linear if plotted on a semi-logarithmic scale (Figure 3). In a fourth serum sample, taken 64 hours after ingestion, sildenafil could be detected but the concentration was below the LOQ and the sample was thus discarded from further analysis. Linear regression analysis of the data in Figure 3 resulted in the following equation $y = -0.16x + 7.0$ with an r^2 value of 0.9995. Extrapolation revealed a sildenafil half-life in this patient of 4.2 hours which corresponds to the previously reported half-lives between 1.4 and 4.5 hours (5).

Discussion

The pharmacokinetics of sildenafil, when used in therapeutic doses, has been studied extensively in the past. For example in a study of Nichols *et al.*, oral administration of sildenafil in adults resulted in peak plasma concentrations of 0.13, 0.27 and 0.56 µg/mL after 25, 50 and 100 mg sildenafil

respectively (7) which is comparable to the 0.08, 0.16 and 0.33 µg/mL reported by Milligan *et al.* after the same doses of sildenafil (17). Peak concentrations were reached approximately 1h postdose. The results of both pharmacokinetic studies imply that plasma concentrations are proportional to the administered sildenafil dose (7, 17). However, according to the latter study of Milligan *et al*, there is evidence for a non-proportionality at higher doses since ingestion of 200 mg sildenafil resulted in a C_{max} of 0.90 µg/mL which was a 30% increase in bioavailability relative to the other doses (17).

Little is known about the relation between non-therapeutic sildenafil doses, sildenafil serum concentrations and clinical toxicology. The highest sildenafil concentration found in our patient was 22.2 µg/mL, which is about 3 times higher than the 6.3 µg/mL that was detected in one of the fatal case reports (6). After studying the few reported overdose cases (Table 1), it becomes clear that there is also not a good correlation between the administered dose and the clinical outcome, which might be explained by pre-existing risk factors or pharmacokinetic differences. Especially these last differences are hard to unravel since sildenafil concentration measurements were not performed in most case reports.

In the case described by Hung *et al.* (13), only mild symptoms (facial flushing, headache and mild tachycardia) occurred, while the case reported by Tracqui *et al.* (6) was fatal after ingestion of a threefold lower sildenafil dose (12x50 mg versus 20x100 mg). However, one might assume that the pre-existing risk factors for coronary artery disease in the deceased patient will have contributed significantly to the evocation of sudden cardiac death with this lower sildenafil dose (6). Our patient had no noteworthy medical history.

The sildenafil dose of the second fatal case (9) is unknown and the serum concentrations were not measured, but again the existence of a predisposing risk factor, advanced alcoholic cirrhosis, might have contributed to the fatal outcome as the liver is the predominant route of elimination of sildenafil (7). Serum aspartate transaminase (AST) activity is one of the covariates that significantly

influence the sildenafil clearance, together with age and co-administration of potential CYP3A4 inhibitors. For every 10 units increase in AST the clearance of sildenafil decreases by 6% (17). The AST levels in the cirrhotic patient were not reported (9) but it is clear that the diminished metabolisation capacity will have had an effect on the clearance of sildenafil. Our patient had a well-preserved liver function, shown by normal levels of AST, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase.

Sildenafil is metabolized by CYP3A4 and is therefore potentially sensitive to multiple drug interactions. Our patient reported the regular use of both lormetazepam and haloperidol. Although the latter drug is a potent CYP2D6 inhibitor, both drugs will not interfere with sildenafil metabolisation. Oral bioavailability of sildenafil normally averages 41%, but food causes small reductions in rate (t_{max} prolonged by 1 hour) and extend (29% reduction of C_{max}) of systemic exposure (7). Food intake is not reported in any of the overdose cases.

To the best of our knowledge, the sildenafil intoxication case that we presented ingested the highest sildenafil dose which resulted in the highest sildenafil serum concentration ever reported. The serum concentration exceeded those of both fatal (6) and non-fatal cases (10) and is far above the peak concentration after therapeutic doses. The ingested dose was well tolerated, with the expected increase in severity of the normal adverse effects, especially for the visual disturbances, but none of the other reported toxicity symptoms, like rhabdomyolysis (11) or blindness (8), occurred. Although our patient, who did receive psychological follow-up, recovered well from his sildenafil intoxication, the unofficial sale of sildenafil and variants and the exponential increase in online pharmacies remains a major concern, especially for patient with pre-existing comorbidities or multiple drug intake.

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287 **Tables**

288 Table 1: Sildenafil intoxication reports ranked by increasing dose

dose	age (years)	gender (M/F)	symptoms	lethal	serum concentration	ref
unknown	41	M	variceal rupture	yes	not reported	(9)
75mg (5mg/kg)	2	M	facial flushing, transient penile engorgement (bilateral rhonchi, diarrhea)	no	not reported	(12)
200mg	54	M	combined nonarteritic anterior ischemic optic neuropathy and cilioretinal artery occlusion	no	not reported	(8)
250mg	45	M	rhabdomyolysis subjective changes in visual perception	no	not reported	(11)
6x50mg (30mg/kg)	1.5	M	mild facial flushing mild, asymptomatic tachycardia prolonged priapism	no	3.9 µg/mL after 7h	(10)
600mg	56	M	cardiomegaly with dilated cardiomyopathy, diffuse coronary atherosclerosis and extensive myocardial fibrosis	yes	6.3 µg/mL	(6)
2000mg	42	F	flushing, headache, mild tachycardia	no	not reported	(13)
2400mg	33	M	defective color vision, vascular retinal dilatation, visual field defect, papilledema, annular scotoma	no	not reported	(4)

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299 Table 2: Chromatographic conditions

Time (min)	% solution A	% solution B	Flow (mL/min)
0	95	5	0.625
19	0	100	0.595
23	0	100	0.625
24	95	5	0.625
30	95	5	0.625

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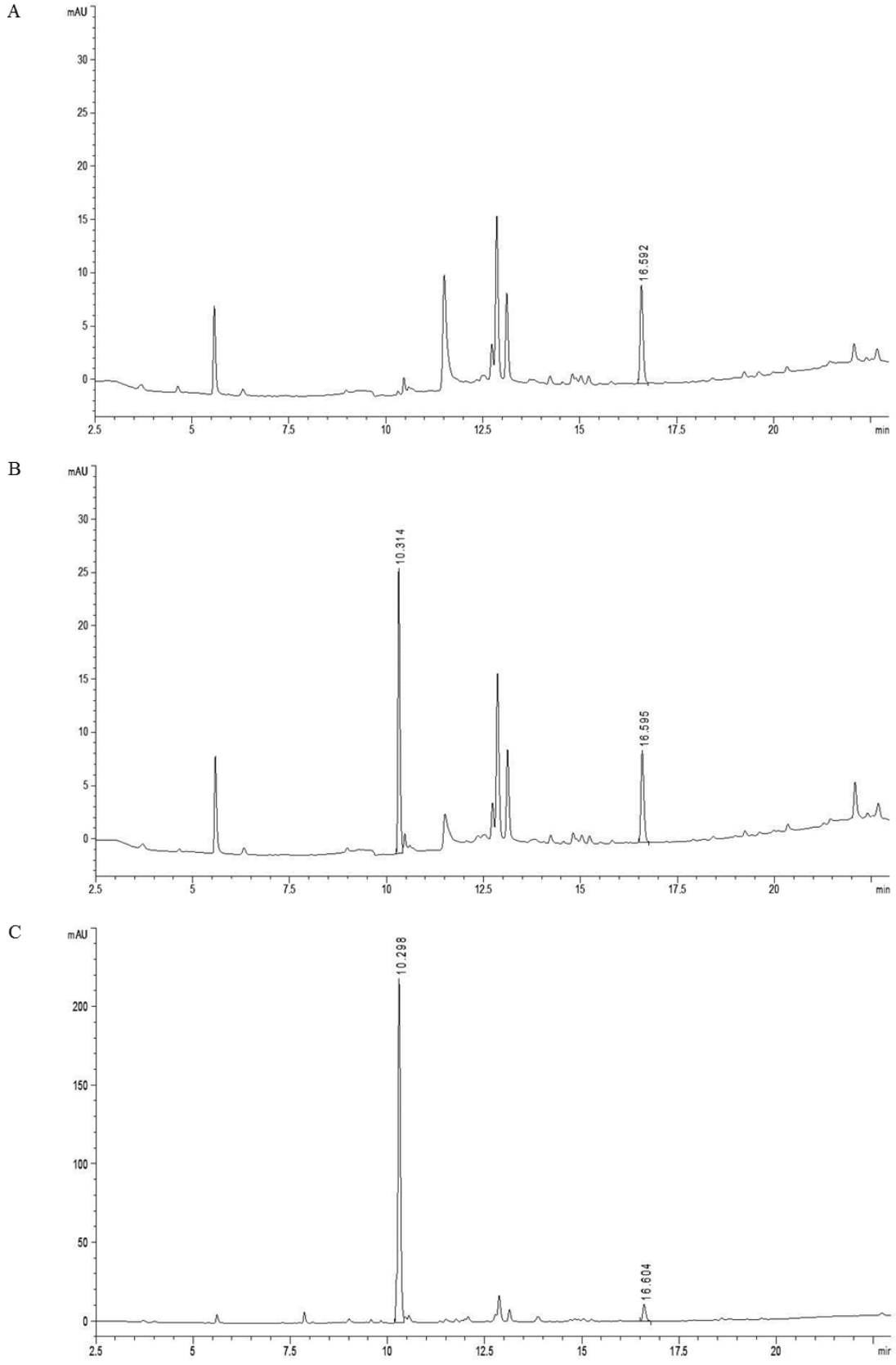
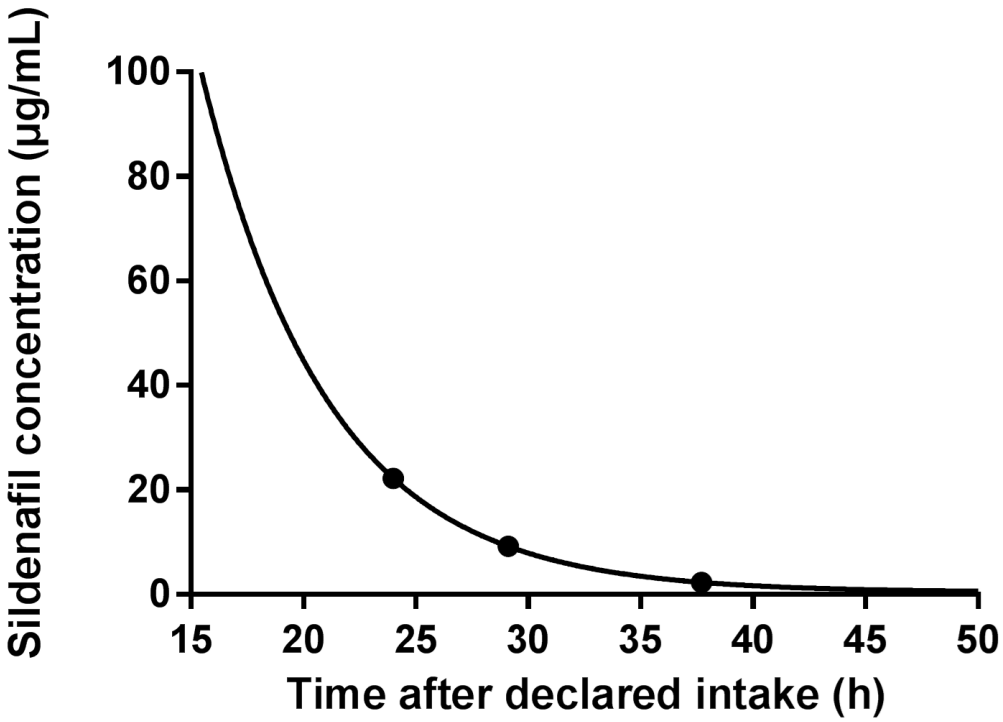
Table 3: Analytical performance (precision and accuracy)

targetconc. (µg/mL)	intra-day (n = 6)			inter-day (n = 6)		
	conc. (µg/mL)	CV%	% error	conc. (µg/mL)	CV%	% error
0.025	0.029	8.3	16.5	0.025	12.9	-1.3
0.075	0.068	7.8	-9.7	0.071	9.2	-5.8
0.250	0.226	7.6	-9.8	0.240	7.7	-3.9
2.000	1.831	10.2	-8.5	2.008	6.9	0.4

Figure 1: Chromatograms of blank serum (A), 0.25 µg/mL sildenafil calibrator (B) and tenfold diluted patient sample (24 hours after intake) (C). The retention times of sildenafil and IS are 10.3 and 16.6 minutes respectively.



345 **Figure 2**: Elimination of sildenafil on a linear plot



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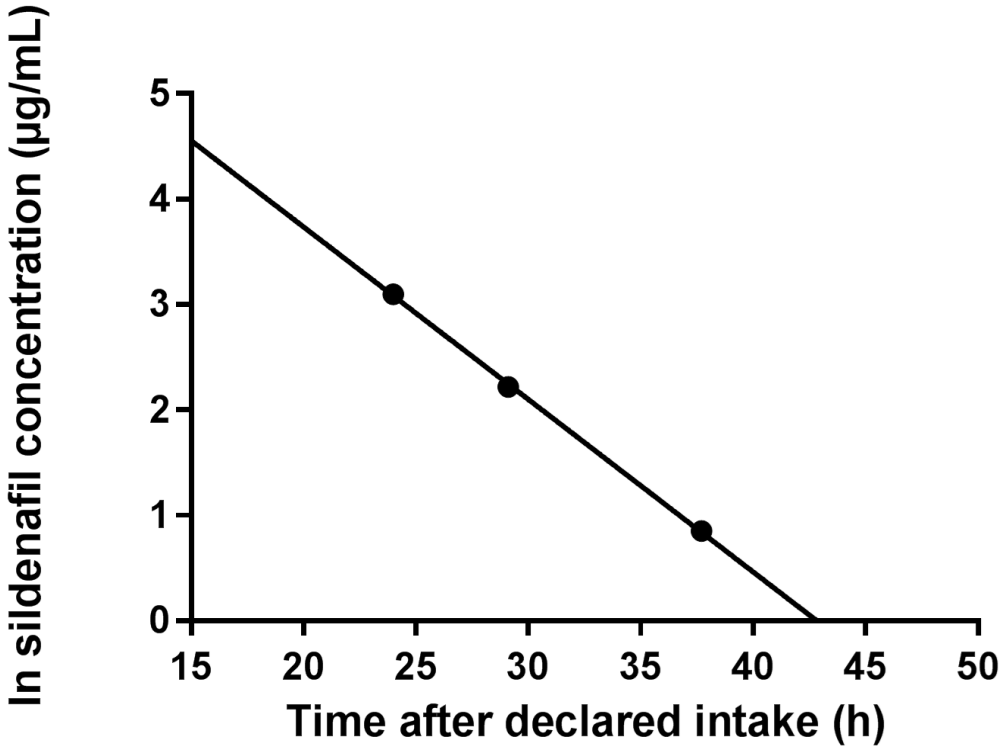
355 **Figure 3**: Elimination of sildenafil on a semi-logaritmic plot



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