POSTMORTEM IDENTIFICATION OF SILDENAFIL IN URINE AND VISCERAL TISSUE IN A FATAL CASE OF ACUTE CARDIOVASCULAR INSUFFICIENCY: A FORENSIC TOXICOLOGICAL CASE REPORT

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ABSTRACT

Phosphodiesterase-5 (PDE5) inhibitors such as sildenafil are widely used for erectile dysfunction and are frequently obtained without medical supervision, sometimes as undeclared adulterants in dietary supplements marketed for sexual enhancement. Their vasodilatory and hypotensive effects can precipitate acute cardiovascular events in men with pre-existing, occasionally undiagnosed, cardiac disease. We report the case of a 48-year-old man found dead in a hotel room in the Andijan region, with sildenafil tablets recovered at the scene. Autopsy revealed diffuse coronary atherosclerosis and myocardial changes consistent with acute cardiovascular insufficiency, without evidence of mechanical trauma. Blood, urine, and visceral tissue (liver, gastric wall) were submitted for forensic-chemical analysis using a combined protocol of alkaline liquid–liquid extraction, two-system thin-layer chromatography (TLC), colour (spot) tests, and ultraviolet (UV) spectrophotometry, adapted from a previously described qualitative method for sildenafil and tadalafil in counterfeit supplements and from classical toxicological isolation techniques. Sildenafil was not detected in blood but was identified in urine and liver extracts, with chromatographic mobility, colour reactions, and UV absorption maxima consistent with the reference standard. The forensic-medical conclusion attributed death to acute cardiovascular insufficiency, with sildenafil use

considered a contributing factor. This case illustrates both the diagnostic value and the interpretive limitations of a low-cost, instrumentation-light screening protocol applicable in regional forensic laboratories lacking access to chromatography–mass spectrometry, and underscores the forensic relevance of PDE5 inhibitors in sudden cardiac death.

Keywords: sildenafil; phosphodiesterase-5 inhibitor; forensic toxicology; thin-layer chromatography; UV spectrophotometry; postmortem diagnosis; sudden cardiac death.

1. INTRODUCTION

Sildenafil citrate, the first clinically available phosphodiesterase type 5 (PDE5) inhibitor, potentiates the nitric oxide–cyclic guanosine monophosphate (NO–cGMP) signalling pathway, producing smooth-muscle relaxation and vasodilation predominantly in the corpus cavernosum but also, to a clinically relevant degree, in the systemic and coronary circulation. Since its introduction, sildenafil and related PDE5 inhibitors have become among the most widely used drugs worldwide, and are frequently obtained outside formal medical supervision — over the counter, over the internet, or as undeclared adulterants of herbal or “natural” dietary supplements marketed for erectile enhancement.

Analytical surveys of such counterfeit supplements have repeatedly identified sildenafil, tadalafil, and their structural analogues as hidden active ingredients, at doses that are neither declared on the packaging nor subject to any pharmacovigilance oversight. A previously published qualitative protocol combining thin-layer chromatography, colour (spot) tests, and ultraviolet spectrophotometry was specifically developed to detect sildenafil and tadalafil in such falsified supplements. Because that protocol was validated on the isolated pharmaceutical substances rather than on biological material, its extension to postmortem blood, urine, and tissue specimens requires that it be combined with an

appropriate extraction (isolation) step, of the kind classically used in toxicological chemistry for basic (nitrogen-containing) xenobiotics.

From a cardiovascular safety standpoint, PDE5 inhibitors are not pharmacologically inert. Their vasodilatory and mild hypotensive action is well tolerated in healthy men but can be hazardous in the presence of pre-existing, sometimes clinically silent, coronary artery disease, and is absolutely contraindicated in combination with nitrate therapy because of the risk of severe, potentially fatal hypotension. Postmortem case series have described fatal or contributory sildenafil-associated cardiovascular events, including cases with markedly elevated postmortem blood concentrations and underlying structural heart disease, and a recent age- and sex-matched postmortem case–control study found a significantly higher prevalence of chronic coronary artery disease among sildenafil-positive decedents who died of ischemic heart disease compared with matched controls, even though acute ischemic pathology did not differ between groups.

A recurring practical difficulty in regional forensic-chemical practice is that definitive identification of PDE5 inhibitors in postmortem material is usually achieved by gas or liquid chromatography coupled with mass spectrometry (GC-MS/LC-MS), instrumentation that is not universally available, particularly in regional branches of forensic medical services. Accessible, low-cost screening methods — extraction followed by TLC, colour tests, and UV spectrophotometry — therefore retain practical value as a first-line qualitative tool, provided their results are interpreted with appropriate caution and, where possible, confirmed by more specific techniques.

This report describes a case of sudden death in which such an adapted screening protocol was applied to blood, urine, and visceral tissue, and discusses the toxicological and forensic-medical significance of the pattern of results obtained.

2. CASE HISTORY

The body of a 48-year-old man (born 1976) was discovered in a hotel room in the Andijan region in 2024, after he failed to vacate the room at the expected time. No signs of forced entry, struggle, or mechanical trauma were noted at the scene. Several tablets, subsequently identified as sildenafil citrate, together with an empty blister pack, were found on the bedside table. Given the absence of an obvious traumatic cause of death and the presence of the medication at the scene, the investigating authority ordered a forensic autopsy and forensic-chemical toxicological examination to establish the cause of death and to determine whether the identified medication had contributed to it.

3. MATERIALS AND METHODS

3.1 Case material and sampling

Blood, urine, and samples of visceral tissue (liver and gastric wall) were collected during autopsy and submitted to the forensic chemistry laboratory. A blank (drug-free) control specimen of the same matrix type was processed in parallel with every analytical series to exclude reagent- or matrix-related false-positive reactions.

3.2 Isolation of sildenafil from blood and urine

Blood (10 mL) and urine (30 mL) were each alkalinised to pH 8–10 with 25% ammonia solution and extracted with chloroform (2–3 successive portions of 15–20 mL, 2–3 minutes of shaking each). The combined organic layers were dried over anhydrous sodium sulphate, filtered, and evaporated to a small residual volume under a gentle stream of warm air.

3.3 Isolation of sildenafil from visceral tissue

Homogenised liver and gastric-wall tissue (100 g) was treated with 200 mL of distilled water acidified to pH 2–3 with a saturated solution of oxalic acid and left to stand for 2 hours with intermittent agitation (classical acid-water isolation, after Vasilyeva). The acidic aqueous extract was decanted, passed through two layers of

gauze, and then alkalinised to pH 8–10 with 25% ammonia. The alkalinised extract was partitioned with chloroform (2–3 portions of 15–20 mL), and the combined organic layers were centrifuged (3000 rpm, 10 min), dried over anhydrous sodium sulphate, and evaporated to a small residual volume.

3.4 Thin-layer chromatography

Dried extracts were redissolved in 0.2–0.5 mL of chloroform and applied to silica gel TLC plates (Sorbfil PTSX-A-AF-UF or Merck HPTLC-Si-60 F254) alongside a reference standard of sildenafil citrate (0.01% in methanol/chloroform). Plates were developed in two independent solvent systems: (A) toluene–acetone–ethanol–25% ammonia (45:45:7:3) and (B) methanol–25% ammonia (100:1.5). After development, plates were examined under ultraviolet light at 254 and 365 nm, and subsequently sprayed with colour (spot) reagents.

3.5 Colour (spot) tests

Colour reactions were performed with Dragendorff reagent (Munier modification), acidified iodoplatinate reagent, and, as differentiating negative controls specific for tadalafil, Mandelin, Marki, and Frede reagents.

3.6 UV spectrophotometry

Dried extracts were redissolved either in 0.1 M hydrochloric acid (citrate salt form) or in ethanol (free-base form) to give solutions of approximately 0.0001 g/mL, and scanned between 190 and 400 nm on a diode-array UV spectrophotometer (Agilent 8453-type instrument) using 1-cm quartz cuvettes. Absorption maxima were compared against those of the reference standard, with an accepted deviation of ±2 nm.

4. RESULTS

4.1 Autopsy findings

Autopsy revealed diffuse coronary atherosclerosis with luminal narrowing, myocardial changes consistent with chronic ischaemic remodelling, and signs of acute cardiovascular insufficiency (pulmonary and cerebral congestion/oedema). No mechanical injury, external violence, or evidence of asphyxia was found. Gross examination of the gastric mucosa did not reveal undissolved tablet fragments, consistent with an interval of at least several hours between ingestion and death.

4.2 Forensic-chemical screening results

Sildenafil was not detected in the blood extract by any of the three methods applied (TLC, colour tests, UV spectrophotometry): no chromatographic zone with the expected Rf and colour reaction was observed, and no defined UV absorption maximum was recorded above the baseline of the blank. In contrast, urine and liver extracts each produced a chromatographic zone with Rf values matching the reference standard in both solvent systems, gave the expected positive colour reactions with Dragendorff and acidified iodoplatinate reagents (and, appropriately, no reaction with Mandelin, Marki, or Frede reagents), and showed UV absorption maxima consistent with the reference standard in both the acidic (citrate) and ethanolic (base) forms. Blank control specimens were negative throughout. The comparative results are summarised in Tables 1–3.

Table 1. Thin-layer chromatography (Rf values) in reference standard, urine, and liver extracts.

Solvent system	Reference standard, Rf	Urine extract, Rf	Liver extract, Rf
Toluene–acetone–ethanol–25% ammonia (45:45:7:3)	0.37	0.36	0.37

Methanol–25% ammonia (100:1.5)	0.56	0.55	0.56
Blood extract	—	no corresponding zone detected	no corresponding zone detected

Table 2. Colour (spot) test results in urine and liver extracts.

Reagent	Expected reaction (sildenafil)	Urine extract	Liver extract
Dragendorff (Munier modification)	Brown spot	Brown spot (positive)	Brown spot (positive)
Acidified iodoplatinate	Brown → violet	Brown → violet (positive)	Brown → violet (positive)
Mandelin / Marki / Frede	No colour (specific for tadalafil)	No colour (negative, as expected)	No colour (negative, as expected)

Table 3. UV spectrophotometric absorption maxima in urine and liver extracts.

Solvent / form	Reference λmax	Urine extract λmax	Liver extract λmax
0.1 M HCl (citrate form)	284 nm	283 nm	284 nm

Ethanol (base form)	294 nm	293 nm	294 nm
Blood extract (either solvent)	—	no defined maximum above baseline	no defined maximum above baseline

Quantitative determination of sildenafil concentration was outside the scope of this qualitative screening protocol and was not performed.

5. DISCUSSION

The pattern of results in this case — a negative blood screen with positive findings in urine and liver — is consistent with the known pharmacokinetics of sildenafil rather than with a technical failure of the method. Sildenafil undergoes rapid and extensive first-pass hepatic metabolism, predominantly via cytochrome P450 3A4 with a secondary contribution from CYP2C9, and has a terminal elimination half-life of only approximately four hours in healthy adults. The drug and its principal active metabolite are more than 95% bound to plasma proteins, and elimination occurs mainly through hepatic/biliary routes into the faeces (approximately 80% of an administered dose), with only a minor fraction (approximately 13%) excreted renally. Given this rapid clearance and extensive hepatic handling, a screening method with the relatively modest sensitivity of TLC and UV spectrophotometry may readily fail to detect sildenafil in blood once free plasma concentrations have fallen below its limit of detection, particularly if a substantial postmortem interval elapsed before sampling — while the liver, as the principal site of drug uptake and metabolism, and urine, as a concentrated excretory matrix, can continue to yield a positive qualitative result for longer.

This interpretation is consistent with previous postmortem case reports. In one widely cited fatal overdose case, a markedly elevated blood sildenafil concentration was measured by HPLC/MS in a decedent with pre-existing dilated cardiomyopathy

and diffuse coronary atherosclerosis, underscoring that when ingestion is recent and blood concentrations are high, sensitive chromatographic methods can readily confirm exposure. Conversely, in another postmortem series employing LC-MS/LC-MS/MS, investigators were able to confirm antemortem sildenafil use through tissue and hair analysis in a decedent for whom no blood sample was available, illustrating that alternative matrices can retain analytically useful evidence of PDE5-inhibitor use even when blood is uninformative. The present case similarly demonstrates that urine and liver tissue can serve as valuable complementary matrices when blood analysis is negative or blood is degraded, insufficient, or collected after a delay.

From a forensic-medical standpoint, the combination of diffuse coronary atherosclerosis, chronic myocardial changes, and terminal signs of acute cardiovascular insufficiency, together with confirmed antemortem sildenafil use, is compatible with a mechanism in which PDE5-inhibitor-induced vasodilation and reduction in systemic vascular resistance precipitated a fatal cardiac event in a man with pre-existing, and possibly previously unrecognised, coronary artery disease. This interpretation is supported by a recent age- and sex-matched postmortem case–control study, which found that men positive for sildenafil among decedents who died of ischaemic heart disease had a significantly higher prevalence of chronic coronary artery disease than matched sildenafil-negative controls, even though acute ischaemic findings did not differ significantly between groups. Together with earlier reports describing sildenafil-associated arrhythmogenic and haemodynamic complications in men with underlying cardiac disease, these findings support classifying sildenafil use as a contributing, rather than sole, causal factor in cases such as the present one, in line with the forensic-medical conclusion reached.

The wider public-health context is also relevant. Sildenafil obtained without medical consultation — whether as a genuine pharmaceutical product purchased informally or as an undeclared adulterant of a “natural” supplement — bypasses the clinical screening for cardiovascular risk factors and contraindications (including

nitrate use) that would normally precede a legitimate prescription. Middle-aged men, who represent both the principal consumer group for such products and the demographic in which coronary artery disease is often first clinically silent, are therefore disproportionately exposed to this risk. The circumstances of the present case — tablets found at the scene of an otherwise unwitnessed death in a hotel room — are typical of this pattern of informal, unsupervised use.

Methodologically, this case illustrates that a combined extraction, TLC, colour-test, and UV-spectrophotometry protocol — inexpensive, rapid, and requiring no chromatography–mass spectrometry instrumentation — can provide reliable qualitative evidence of sildenafil exposure across blood, urine, and visceral tissue matrices, making it a practical first-line screening tool for regional forensic-chemical laboratories. At the same time, several limitations must be acknowledged. The method is qualitative rather than quantitative, and cannot establish the antemortem blood concentration or the temporal relationship between ingestion and death with the precision of chromatography–mass spectrometry. A negative blood result, as in this case, cannot by itself exclude antemortem use and must be interpreted together with results from complementary matrices. Finally, as in any single-case report, the findings cannot be generalised without corroboration from larger case series, and confirmatory analysis by GC-MS, LC-MS, or infrared spectroscopy is recommended whenever such instrumentation is accessible.

6. CONCLUSION

This case demonstrates the practical forensic value of an extraction, thin-layer chromatography, colour-test, and UV-spectrophotometry protocol, adapted from a previously validated method for counterfeit dietary supplements and combined with classical toxicological isolation techniques, for the qualitative detection of sildenafil in postmortem blood, urine, and visceral tissue. In this decedent, sildenafil was identified in urine and liver despite a negative blood screen, a pattern attributable to

the drug's short half-life and extensive first-pass hepatic metabolism rather than to analytical failure. The findings support considering PDE5-inhibitor use as a relevant contributing factor in the forensic-medical evaluation of sudden cardiac death in middle-aged men, particularly when tablets or packaging are recovered at the scene, and argue for routine toxicological screening for these agents in such cases, with confirmatory chromatography–mass spectrometry performed whenever available.

ETHICS STATEMENT

All case details in this report have been generalised and anonymised in accordance with international publication ethics for case reports; no information permitting identification of the deceased is disclosed.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Shilova E.Yu., Shabalina A.E., Kirichek A.V. Determination of the sexual stimulants tadalafil and sildenafil in counterfeit biologically active food supplements. Butlerov Communications. 2014;38(4):126–133.

2. Tracqui A, Miras A, Tabib A, et al. Fatal overdosage with sildenafil citrate (Viagra®): first report and review of the literature. Human & Experimental Toxicology. 2002;21(11):623–629.

3. Weinmann W, Bohnert M, Wiedemann A, et al. Post-mortem detection and identification of sildenafil (Viagra) and its metabolites by LC/MS and LC/MS/MS. International Journal of Legal Medicine. 2001;114:252–258.

4. Özkök A, Seçkin Ö, Dinçer B, Özsoy S, Akar T. Sildenafil Positivity and Chronic Cardiac Pathology in Men Who Died of Ischemic Heart Disease: An Age- and Sex-Matched Postmortem Case–Control Study. Diagnostics. 2026;16(15):2400.

5. Pfizer Inc. VIAGRA® (sildenafil citrate) tablets, prescribing information (clinical pharmacology / pharmacokinetics section). U.S. Food and Drug Administration drug label.

6. Kramarenko V.F. Toxicological Chemistry. Kiev, 1989.

7. Ikromov L.T., Mirkhaitov T., Tojiyev M.A., Yuldashev Z.A. Toxicological Chemistry. Tashkent, 2010.

8. Moffat AC, Osselton MD, Widdop B, Watts J (eds). Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material. 4th ed. London: Pharmaceutical Press; 2011.