

Review

Phosphodiesterase-5 (PDE-5) Inhibitors as Emergent Environmental Contaminants: Advanced Remediation and Analytical Methods

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Abstract: Pharmaceuticals, fundamental in therapy and the prevention of known pathologies, are responsible for environmental pollution. These substances, called “emerging contaminants,” are harmful to human health because they enter the environment in quantities exceeding the natural self-capacity purification of the ecosystems. Furthermore, wastewater treatment plants (WWTPs) cannot remove these substances, which can undergo chemical/biological transformations in the environment, thus forming by-products, sometimes more toxic than the parent molecules; successively, they move into rivers and could reach the drinking water supplies. All these phenomena represent a severe public health problem. Therefore, the Water Framework Directive by European Union imposed the monitoring of drugs' levels in aqueous matrices. Every two years, the EU carefully updates the list of potential water pollutants, called the Watch List, including pharmaceuticals, to evaluate their risk on the aquatic environment. The last Commission Implementing Decision (EU 2018/840) comprises several substances of primary concern. In addition, the scientific community is giving particular attention to other pharmaceuticals not yet on the Watch list, whose markets are in growth; particularly, the Phosphodiesterase 5 (PDE-5) inhibitors used for the pharmaceutical treatment of erectile dysfunction (ED) in men. This review discusses the presence of PDE-5 inhibitors in environmental systems, their toxic effects, the different kinds of removal, and the analytical methods normally adopted for their detection. In addition, the study helps figure out the best possible strategy to tackle pharmaceutical pollution by using analytical and advanced diagnostic methods.

Keywords: phosphodiesterase-5 (PDE-5) inhibitors; environment contamination; wastewater plants (WWTPs); advanced oxidation process (AOP); eco-toxicity; analytical methods



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1. Introduction

Emerging pollutants (EPs) are synthetic or naturally occurring compounds not commonly examined in the environment [1]. Still, they can enter ecosystems and cause recognised or supposed adverse effects on ecology and human health [1]. Moreover, they undergo chemical/biological transformations, forming by-products sometimes more toxic than the parent molecules. The final issue is the accumulation of original and transformed substances in water bodies [2]. In particular, Zuccato et al. [3] evidenced the presence in ground and surface waters of drugs and pharmaceuticals having polar structures, probably coming from WWTP effluents.

When absorbed by the body, a pharmaceutical substance enters the circulation and distributes to reach the target site to perform its function [2,4]. When the metabolism

process is activated, some molecules reach the target site and others transform into inactive metabolites, which are substances no longer producing effects into the body. Many medicines, however, are excreted without being metabolised or at least without being completely inactivated [2,5]. These, together with sewage, reach the WWTP, where organic loads degrade and water is purified. Unfortunately, these structures are often not designed to degrade active substances of pharmaceutical origin, which, therefore, once again manage to resist, unharmed, and maintain their effectiveness. As a result, the purified water (still rich in active ingredients) flows into the receiving channels, carrying a load of pollutants to rivers and lakes. Thus, tons of active substances such as antibiotics, anti-neoplastic, estrogens and others are poured into surface waters [6,7]. Once in the environment, the drug eventually degrades or can persist very long, resulting in noticeable build-ups [8]. In the sediments of some Italian rivers such as the Po, Lambro and Adda rivers, as well as in the aqueducts of the towns of Varese and Lodi, traces of various drugs were present in different amounts, including antibiotics (lincomycin and erythromycin), anticancer (cyclophosphamide), anti-inflammatory (ibuprofen), diuretics (furosemide) and antihypertensive (atenolol) drugs [9]. The existence of these compounds in environmental systems is of concern since they constitute a complex assortment, which could induce the occurrence of undesirable synergistic effects and could be responsible for many health adverse effects such as allergies, development of antibiotic-resistance phenomena, disorders of the endocrine system, cytolytic or cytostatic effects and others [10–12].

The European Parliament regulated with the Directive 2008/105/EC environmental quality standards in the field of water policy and the range of inorganic and organic contaminants to be monitored in water [13]. The last Commission Implementing Decision (EU 2018/840) included several new substances of primary concern [14]. The scientific community is also paying attention to other pharmaceuticals having a growing market in the last years: phosphodiesterase-5 (PDE-5) inhibitors (sildenafil citrate, tadalafil, avanafil and vardenafil marketed as Viagra[®], Cialis[®], Spedra[®] and Levitra[®]) (Figure 1) titled for the cure of erectile dysfunction (ED) [15].

ED, an increasing disorder [16], affects 25 to 35 million men over 18 years in Europe [17]. Following many statistic studies, it seems caused by (i) neurologic (deficit in nerve signalling to the “*corpora cavernosa*”), (ii) psychological (depression, stress, anxiety) or (iii) endocrinologic (low testosterone levels or other hormone imbalances) problems, but most often, it is the result of cardiovascular disease associated with other health problems such as diabetes, smoking, hypertension, hyperlipidaemia, metabolic syndrome and other [18–20]. Pharmaceuticals used to reduce this disorder act as phosphodiesterase-5 (PDE-5) inhibitors, a family of enzymes typically active in cyclic guanosine monophosphate (cGMP) degradation. The inhibition of PDE-5 results in the intracellular accumulation of cGMP, which plays a central role in signal transduction and regulates several physiological responses. In the penis, cGMP induces corpora cavernosa smooth muscle relaxation and thus increases the blood supply, leading to the erection of the penis. PDE-5 inhibitors prevent the degradation of cGMP, thus enhancing and prolonging erections and increasing sexual satisfaction [21]. ED products are medicines approved by the US Food and Drug Administration. The European Medicines Agency (EMA) approved Viagra, Cialis and Levitra in 1998, 2002 and 2003, respectively. The last one was Spedra in 2013. Doctors also prescribe these compounds for other syndromes such as arterial hypertension, coronary artery disease and benign prostatic hyperplasia (BPH) [22,23].

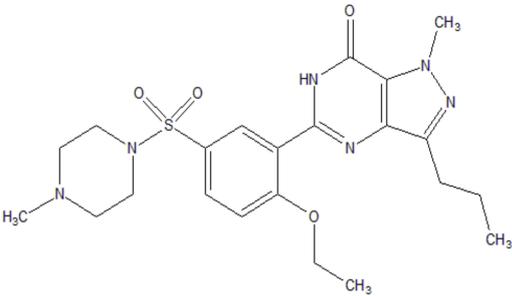
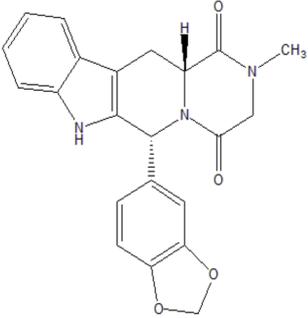
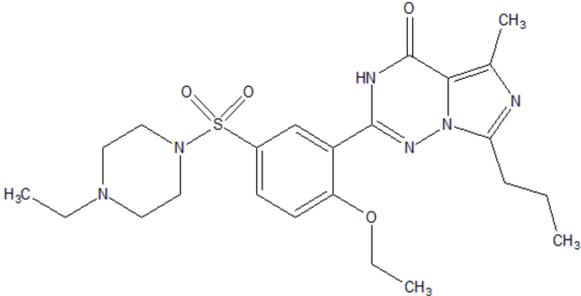
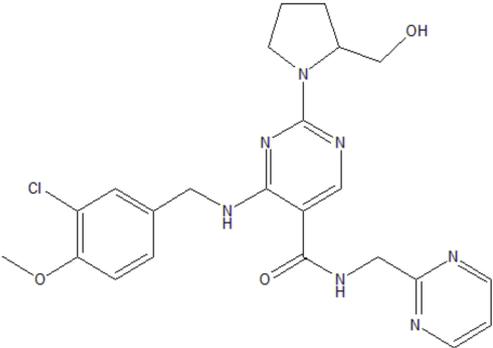
<p style="text-align: center;">SILDENAFIL</p>  <p>Iupac name: 5-[2-ethoxy-5-(4-methylpiperazin-1-yl) sulfonylphenyl]-1-methyl-3-propyl-6H-pyrazolo [4,3-d]pyrimidin-7-one</p> <p>Commercial name: Viagra®</p> <p>Chemical formula: C₂₂H₃₀N₆O₄S</p> <p>Molecular weight: 474.6 g mol⁻¹</p> <p>Water solubility (pH dependent): 0.46–3.5 mg mL⁻¹</p> <p>LogP: 1.9 Amphoteric: pKa 6–11</p>	<p style="text-align: center;">TADALAFIL</p>  <p>Iupac name: (2R,8R)-2-(1,3-benzodioxol-5-yl)-6-methyl-1,3,6,17-triazatetracyclo[8.7.0.0.3,8.0.11,16]heptadeca-1(10),11,13,15-tetraene-4,7-dione</p> <p>Commercial name: Cialis®</p> <p>Chemical formula: C₂₂H₁₉N₃O₄</p> <p>Molecular weight: 389.4 g mol⁻¹</p> <p>Water solubility: practically insoluble</p> <p>LogP: 1.7</p>
<p style="text-align: center;">WARDENAFIL</p>  <p>Iupac name: 2-[2-ethoxy-5-(4-ethylpiperazin-1-yl) sulfonylphenyl]-5-methyl-7-propyl-3H-imidazo [5,1-f][1,2,4]triazin-4-one</p> <p>Commercial name: Levitra®</p> <p>Chemical formula: C₂₃H₃₂N₆O₄S</p> <p>Molecular weight: 488.6 g mol⁻¹</p> <p>Water solubility: 0.11 mg mL⁻¹</p> <p>LogP: 1.4</p>	<p style="text-align: center;">AVANAFIL</p>  <p>Iupac name: 4-[(3-chloro-4-methoxyphenyl) methylamino]-2-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]-N-(pyrimidin-2-ylmethyl) pyrimidine-5-carboxamide</p> <p>Commercial name: Spedra®</p> <p>Chemical formula: C₂₃H₂₆ClN₇O₃</p> <p>Molecular weight: 483.9 g mol⁻¹</p> <p>Water solubility: practically insoluble</p>

Figure 1. Structures, chemical formula and available solubility and partition properties of the primarily used PDE-5 inhibitors.

According to the 2018 annual report prepared by the Italian Medicines Agency (AIFA), these compounds' consumption had increased over time from 2.9 DDD (Defined Daily Dose assumed per 1000 inhabitants in the referred year) in 2014 to 3.6 DDD in 2018 (for example, tadalafil has risen by 3.7% since 2017, becoming the fourth most-used drug, preceded only by paracetamol, lorazepam and alprazolam). Patients usually tend to hide

their problems and the related pharmaceutical cure and choose the Internet as a way of purchasing to avoid medical prescriptions. However, Chiang et al. [24] report that illicit trading with products from the Internet and with counterfeit medicines is increasing [25], and the individuals purchasing medicines via the Internet are not wholly conscious of the risks for health concerning the quality of these products such as the possible presence of toxic impurities [26]. The high consumption of these substances, globally accomplished by legal and illegal ways, legitimates thinking that they can pose a severe threat to ecosystems and human health [27].

2. Aim and Review Methodology

This review deals with the presence of pharmaceuticals used as PDE-5 inhibitors in the environment and their toxic effects, including a standpoint of the different removal technologies and analytical methods normally adopted for their detection in environmental samples. The rationale behind the literature analysis is the prompter for the reviewing methodology, as follows: (i) PDE-5 inhibitors are widely used for different therapeutic applications; (ii) they are also marketed illegally due to their ease of availability without prescriptions outside the official health system; (iii) urban wastewater contains not only residues of the parent molecules but also their human metabolic products; (iv) the relative mixture of drugs can undergo biotic and abiotic degradation, causing new concerns of eco-toxicity. This review offers an overview of the highlighted issues, starting from the contamination sources, passing through the ability of water purification plant systems to remove these contaminants, including an evaluation of the parent molecules and by-products toxicity. Reviewing the analytical methods used to determine these substances and identifying derivatives is essential for the article's completeness, emphasising analytical methods that offer the highest resolution and accuracy.

We consulted a total of 110 articles using several keywords: PDE-5 inhibitors, the status of the environmental contamination, wastewater treatment plants (WWTPs), sewage treatment plants (STPs), advanced oxidation process (AOPs), eco-toxicity and analytic diagnostic methods.

3. Source of PDE-5 Inhibitors in the Environment

According to the "anatomical therapeutic and chemical" (ATC) classification system, PDE-5 inhibitors are available in the class ATC code G04BE. The four most significant inhibitors used are sildenafil (Viagra[®]), tadalafil (Cialis[®]), vardenafil (Levitra[®]) and avanafil (Spedra[®]), all approved by Food and Drug Administration (FDA). In addition, there are other pharmaceuticals that are non-FDA approved and commercially available in some countries, such as udenafil (Zydena[®]) in South Korea and Malaysia, mirodenafil (Mvix[®]) in South Korea and lodenafil carbonate (Helleva[®]) in Brazil [28].

Sildenafil (C₂₂H₃₀N₆O₄S) was discovered in 1989 by the Pfizer Cardiovascular Research and Development Group (Sandwich, Kent, UK) during research focused on identifying PDE-5 inhibitors to treat angina pectoris due to the abundant presence of the PDE-5 enzymes in platelets and vascular smooth muscle cells. It showed low effectiveness on angina pectoris tests and penile erection as the primary collateral effect [29]. The FDA, in 1998, approved sildenafil for erectile dysfunction treatments and then in 2005, the European Medicine Agency (EMA) approved it for class II and class III pulmonary hypertension treatments. Vardenafil (C₂₃H₃₂N₆O₄S) and tadalafil (C₂₂H₁₉N₃O₄) were introduced clinically in 2003, while avanafil (C₂₃H₂₆ClN₇O₃) in 2013. Although the structural differences between these compounds are minor, they have different pharmacokinetic properties (absorption, distribution, metabolism and excretion). In addition, they can exert their activity also on other PDE-types, as reported in Table 1 [30].

All four PDE-5 inhibitors are rapidly absorbed from the gastrointestinal tract and show broadly similar T_{max}, except for tadalafil which has the longest T_{max}. Peak plasma concentration (C_{max}) of sildenafil is reached in less than 1 h [31]. Vardenafil and avanafil have sim-

ilar pharmacokinetics to sildenafil, with T_{max} approximately 1 h [32] and 30–45 min [33]. Tadalafil reaches its maximum concentration in plasma after about 2 h [34].

Sildenafil, vardenafil and avanafil have a terminal half-life ($T_{1/2}$) of between 4 and 5 h, and tadalafil has a half-life of 17.5 h.

Table 1. Pharmacokinetic parameters of PDE-5 inhibitors (re-edited from [30]).

Parameters/Drugs	Sildenafil (Viagra)	Vardenafil (Levitra)	Tadalafil (Cialis)	Avanafil (Spedra)
Bioavailability	41% (mean) 25–63% (range)	15% (mean)	-	-
T_{max}	1 h (median) 0.5–2 h (range)	1 h (median) 0.5–2 h (range)	2 h (median) 0.5–6 h (range)	0.5–0.75 h (range)
Protein binding	96%	95%	94%	99%
Metabolism	Major: CYP3A4 Minor: CYP2C9	Major: CYP3A4 Minor: CYP3A5, CYP2C	CYP3A4	Major: CYP3A4 Minor: CYP2C
Active metabolite (% effect)	Yes (20%) N-demethylation	Yes (7%) Demethylation	No	Yes (4%) Methylation, glucuronidation
Half-life($T_{1/2}$)	4 h	4–5 h	17.5 h	5 h
Elimination	80% faeces 13% urine	91–95% faeces 2–6% urine	61% faeces 36% urine	62% faeces 21% urine
Ingestion with high-fat meals	↓ C_{max} 29% ↑ T_{max} by 1 h	↓ C_{max} 18–50%	Not affected	↓ C_{max} 24–39% ↑ T_{max} by 1.12–1.25 h
Additional PDE inhibition	PDE ₁ , PDE ₆	PDE ₁ , PDE ₆	PDE ₁₁	-

C_{max} = peak concentration; CYP = cytochrome P450; T_{max} = time to peak concentration.

Huang et al. [30] reported that each PDE-5 inhibitor undergoes metabolism predominantly through the hepatic isoenzyme cytochrome P450 (CYP) 3A4 pathway. Minor pathways include CYP2C9 for sildenafil, CYP3A5 and CYP2C for vardenafil and CYP2C for avanafil. Of these four pharmaceuticals, only tadalafil produces human metabolites that are not pharmacologically active. Sildenafil predominantly metabolises into an N-desmethyl metabolite that contributes to approximately 20% of the parent molecule total pharmacological activity [35]. Vardenafil and avanafil produce active metabolites that contribute 7% [36] and 4% [37] of the real pharmacological action. All the PDE-5 inhibitors mainly excrete as metabolic by-products in the faeces and to a reduced amount in the urine.

Thanks to their different features, some PDE-5 inhibitors can be used in many clinical treatments and not only for erectile dysfunction treatments. For example, the national medicines agencies approved tadalafil for: (i) the treatment of lower urinary tract symptoms (LUTS); (ii) secondary to benign prostatic hyperplasia (BPH); and (iii) for the treatment of pulmonary arterial hypertension (PAH), a condition of increased blood pressure within the arteries of the lungs [34].

Sildenafil is approved for PAH treatment because it inhibits the PDE-5 in the pulmonary blood vessels and promotes the vasodilator action of nitric oxide by maintaining high cGMP levels [35].

In addition, researchers performing mice models with Alzheimer's disease proved that PDE-5 inhibitors can effectively promote the cGMP-mediated processes involved in consolidating information in memory and countering the neurodegenerative mechanisms typical of Alzheimer's disease [38].

Schnetzler et al. [39] estimated that about 6 million men in Europe could avoid the healthcare system to get PDE-5 medicines themselves. Market globalisation and the Internet are offering new scenarios and creating unknown risks for public health due to the growing attitude to buy drugs from the illegal market and online shops, exposing in this way more and more persons to the hazards due to the intake of illicit and counterfeit

drugs [40]. Authorities developed specific legislative rules, particularly the European Directive 2011/62/UE, to avoid the trade of falsified medicinal products through the permitted supply chain. This Directive also normalises the Internet market of legal medicines by specifying that legal online pharmacies are obligated to exhibit a “common logo” on each page of the website dedicated to drugs sale [41,42]. An exciting and recent study [43] listed 80 new sexual performance enhancers detected illegally in the market that mimics the approved PDE-5 inhibitors.

4. Content of PDE-5 Inhibitors in WWTPs and STPs

Together with many other pharmaceutical products, these substances, once eliminated in faeces and urine, are transported through sewers to municipal wastewater treatment plants. Here, the parent substances and their metabolites are treated in biological reactors, which can only partially degrade them while triggering other transformation processes. They also often undergo accumulation processes before being discharged into the receiving water bodies. For this reason, investigations about the residues of these pharmaceuticals in the environment are essential to proceed with their removal. The first approach consists of determining the PDE-5 inhibitors' content in untreated wastewater samples taken at the entry into WWTPs. Despite the low or very low solubility in water and the positive values of the partition coefficients (Log P), these products can arrive at municipal wastewater treatment plants adsorbed on the solid organic materials or dispersed in the liquid mass. Their lipophilicity favours their transport.

Research performed in eight WWTPs serving the catchment inside the towns of Bristol, Brussels, Castellón, Copenhagen, Milan, Oslo, Utrecht and Zurich [44] showed the presence of sildenafil and its two human urinary metabolites, desmethyl- and desethyl-sildenafil with amounts up to 60 ng L^{-1} . They did not detect tadalafil and vardenafil in appreciable concentrations. The authors transformed the concentrations found in the collected samples to normalised loads and estimated the possible intake of sildenafil as amounts back-calculated from these loads. Moreover, they gathered the national prescription data from five countries in the form of the number of prescribed daily doses and transformed them into predicted loads for assessment. In Utrecht and Brussels, prescription data could only partially clarify the total quantity determined in wastewater.

In contrast, in Bristol, Milan and Oslo, the authors found that drug amounts in wastewater were lower than predicted from the prescription data. These studies illustrate the theoretical capacity of performed investigations to assess the use of imitating fraudulent medication and criminal online sales. Other researchers in Tarragona (Spain) and Germany determined the occurrence of these pharmaceuticals in WWTP influent and effluent water and sewage sludge [15] to evaluate the removal efficiency of the treatment systems and the possible influence of STPs on the pollution of the aquatic systems.

Sildenafil was the principal drug in all investigated water and sewage samples at a few ng L^{-1} and ng g^{-1} range, respectively. Tadalafil was not identified or below the limit of detection (LOD) in effluent water collected in Spain but was revealed in sewage sludge (12 ng g^{-1} –LOD). Vardenafil was detected only in one sludge sample and between 5 ng g^{-1} and the LOD value in effluent water. The higher elimination efficiency of the STP in Tarragona (Spain) was 68%, 69% and 80% for sildenafil, tadalafil and vardenafil, respectively.

The monitoring evidenced the maximum concentrations for all drugs during the summer, probably due to the touristic fluxes (Figure 2, from [15]).

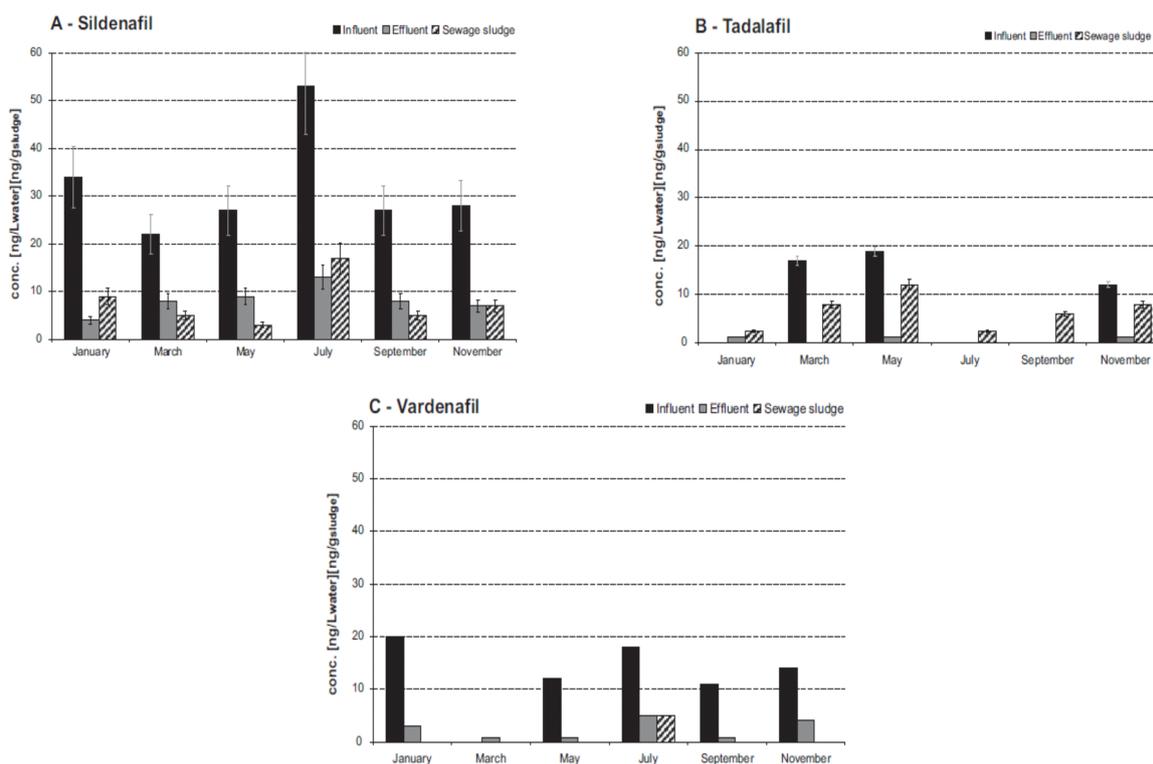


Figure 2. Amounts of sildenafil, tadalafil and vardenafil in influent and effluent wastewater and sludge samples collected from the STP Tarragona (Spain) [15]. Reprinted with permission from ref. [15]. Copyright 2021 Elsevier.

Studies conducted in several German STPs revealed that: (i) sildenafil was constantly in greater quantity (except in the case of Wiesbaden, where tadalafil was the most copious); (ii) sildenafil was detected in 100% of samples at concentrations ranging from 6 to 18 ng L⁻¹. The highest level of 18 ng L⁻¹ was determined in the STP effluent from Bad Homburg, while the lowest amount of 6 ng L⁻¹ remained in samples from Darmstadt. In general, there were no significant differences in sildenafil levels found in Spanish and German samples (Figure 3).

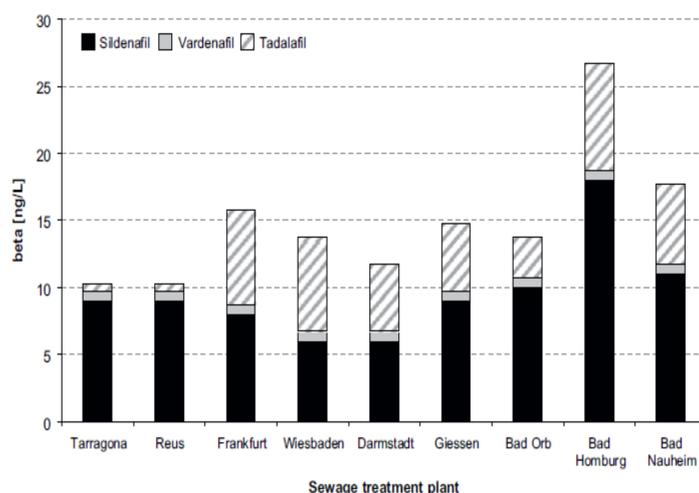


Figure 3. Amounts of drugs found in the STP effluent samples grabbed from Germany and Spain [15]. Reprinted with permission from ref. [15]. Copyright 2021 Elsevier.

Similar quantities of these drugs were also detected by Schroeder et al. in a fitness centre discharge in Germany [45] and by Papageorgiou et al. in WWTPs in Volos, Greece [46].

Beyond the levels found for these substances, it is crucial to consider that the hazard to the environment may derive from their transformation compounds, as described by Temussi et al. [47] and Eichhorn et al. [48].

5. Removal Treatments

The absence of guidelines has permitted the discharge of significant amounts of pharmaceuticals in the environment even because municipalities usually do not adopt WWTPs designed to remove these kinds of pollutants [49–52]. Unfortunately, there are no reference limits for most of these substances [53,54].

The effective removal of these compounds from wastewater before discharge into the water bodies is an emerging issue [55], and specific tertiary treatments could be indispensable.

There are different methods able to remove several emerging contaminants such as photolysis, biological degradation, filtration on carbonised materials [56,57], catalytic use of green-synthesised copper nanoparticles (Cu NPs) [58] and adsorption on activated carbon [59].

5.1. Advanced Oxidation Treatment

It is easy to think that, due to their affinity for organic material, these products remain more in sewage sludge than in wastewater. But it is also true that the release into liquid effluents could be a constant. Ordinary sewage management does not eliminate these substances from the wastewater stream and consequently releases them into the receiving body of water [60]. Once they reach the aquatic systems, a combination of different processes governs their fate. Photochemical reactions are accounted as an essential process in the natural reduction of organic micropollutants [61].

However, it is essential to remember that the contaminant must be removed entirely, including its transformation into by-products and derivatives eventually formed during the decontamination stage.

Chlorination is by far the most adopted method in the disinfection stage in WWTPs. Wastewater is frequently added with chlorine as a sodium hypochlorite solution. However, the mixture HOCl/OCl⁻, recognised as free available chlorine, is a potent non-specific oxidant that promotes transformation reactions of many micro-pollutants. As a result, chlorinated and oxidised by-products are usually obtained, which could be more toxic than the parent substances [62].

Temussi et al. [47] report remarkable results: drug chlorination experiments were performed on sildenafil and tadalafil, simulating conditions of a characteristic wastewater management procedure. The main transformation compounds were isolated and fully characterised. Sildenafil showed a high reactivity by hypochlorite addition and was completely mineralised in 1h. The reaction mixtures were treated with thiosulfate to remove the hypochlorite excess and then extracted with ethyl acetate. No appreciable variations were detected in the experiments, and three main by-products were isolated by chromatographic separation and identified by spectroscopic analyses (S1–S3) (Figure 4).

Tadalafil (T) was converted by hypochlorite addition. Indeed, the researchers observed the compounds T1–T3 (Figure 5). These substances were produced by cleaving the bond between nitrogen and benzylic carbon, followed by carbon oxidation. Contemporarily, the chlorination reaction of the aromatic ring and indolic nitrogen occurred for compounds T1 and T2 [63]. Spectroscopic features were essential to elucidate the structure of the compounds.

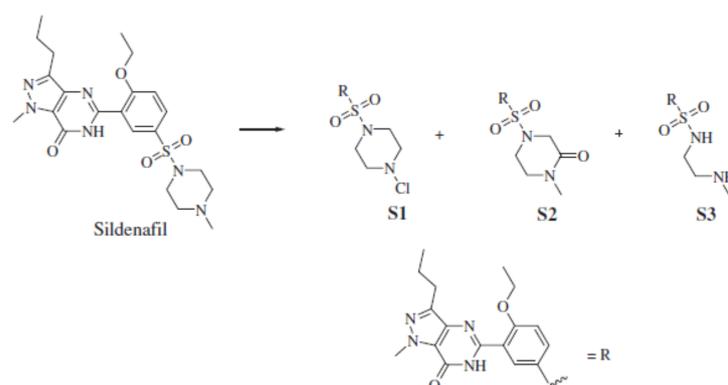


Figure 4. Sildenafil and its chlorination products [47]. It is reproduced with the permission of Elsevier. Reprinted with permission from ref. [47]. Copyright 2021 Elsevier.

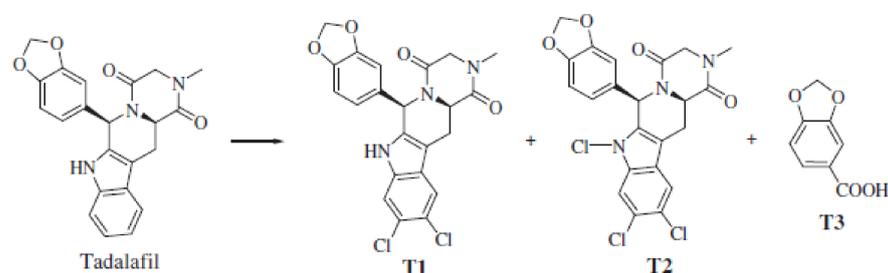


Figure 5. Tadalafil and its chlorination products [47]. It is reproduced with the permission of Elsevier. Reprinted with permission from ref. [47]. Copyright 2021 Elsevier.

5.2. Biological Degradation

De Felice et al. [64] studied the genetic outline of the microbial community emerging in a sildenafil-polluted aquatic environment. They isolated the 16S and 18S rRNA genes for bacteria and fungi, amplified by polymerase chain reaction (PCR) and separated using the denaturing gradient gel electrophoresis (DGGE) technique. Analysis of DGGE data indicated that the microbial composition changed with numerous major species in the last period of the experiment. This result suggests that great variety in microorganisms might be necessary for the effective biodegradation of pollutants. When more microbial species with different physiological capabilities are involved, many diverse organic pollutants might be degraded. In general, the use of mixed microbial consortia to remediate polluted sites is encouraged.

A few years later, Grossberger et al. [65] reported, in a study of agricultural soils irrigated with treated wastewater, that sildenafil was recalcitrant and accumulated in the surface layer. Sildenafil, not found in the soil before irrigation, was identified after irrigation. An increase of its concentration with subsequent irrigations occurs, suggesting an accumulation of the chemical in the top layer, which is noteworthy. This effect could follow the ampholytic characteristic of sildenafil, which does not ionise at environmentally relevant pH levels.

The final considerations from the literature results are that the soil composition, the structure of the contaminant molecule and an adequate microbial community influence the degradation of these kinds of pollutants.

5.3. Adsorption on Activated Carbon

Delgado et al. [59] reported the efficiency of eliminating sildenafil citrate from water using powdered activated carbon. Having obtained a removal efficiency greater than 85%, they promoted the use of activated carbon as an effective tool for removing these kinds of recalcitrant emerging pollutants.

6. Toxicity Assessment

De Felice et al. [64] tested the biological degradation and the possible toxicity of microbial transformation substances of sildenafil in several living models such as *Daphnia magna* and *Pseudokirchneriella subcapitata* microalgae and human HepG2 cells to evaluate the mitochondrial activity.

Toxicological tests showed that only the initial sildenafil concentration (400 mg L^{-1}) was toxic for *D. magna* and caused a decrease of cellular viability by 50% for HepG2 cells (Figure 6A,B, respectively). These assays revealed the absence of toxicity for the biotransformation products (Figure 6C).

Temussi et al. [47] carried out aquatic acute and chronic toxicity tests with *Brachionus calyciflorus* and *Ceriodaphnia dubia* and mutagenesis and genotoxicity analyses for some bacterial strains to evaluate the adverse environmental effects of parent compounds and transformation products obtained simulating the chlorination stage of a WWTP.

The authors selected the freshwater rotifer *B. calyciflorus* and the microcrustacean *C. dubia* as demonstrative aquatic organisms because they have an extensive geographic distribution and substantially impact water's critical ecological processes. Furthermore, the authors performed mutagenesis and genotoxicity assays using the Ames test on *Salmonella typhimurium* and the SOS Chromotest on *Escherichia coli* PQ37 to detect the induction of point mutations of the SOS DNA repair system. The results of the acute toxicity tests are in Table 2.

Table 2. Acute LC_{50} values in mg L^{-1} with confidence limits (95% probability) of sildenafil and tadalafil and their respective derivatives S1 and T1 [47]. Reprinted with permission from ref. [47]. Copyright 2021 Elsevier.

	<i>B. calyciflorus</i>	<i>C. dubia</i>
Sildenafil citrate	42.74 (34.32–53.21)	5.74 (3.08–10.71)
S ₁	19.82 (17.51–22.43)	6.60 (5.73–7.60)
Tadalafil	NE up to 20	NE up to 20
T ₁	NE up to 20	NE up to 20

NE: no effect.

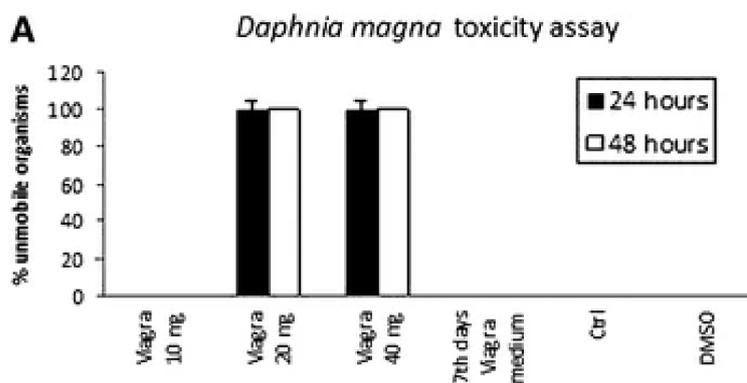


Figure 6. Cont.

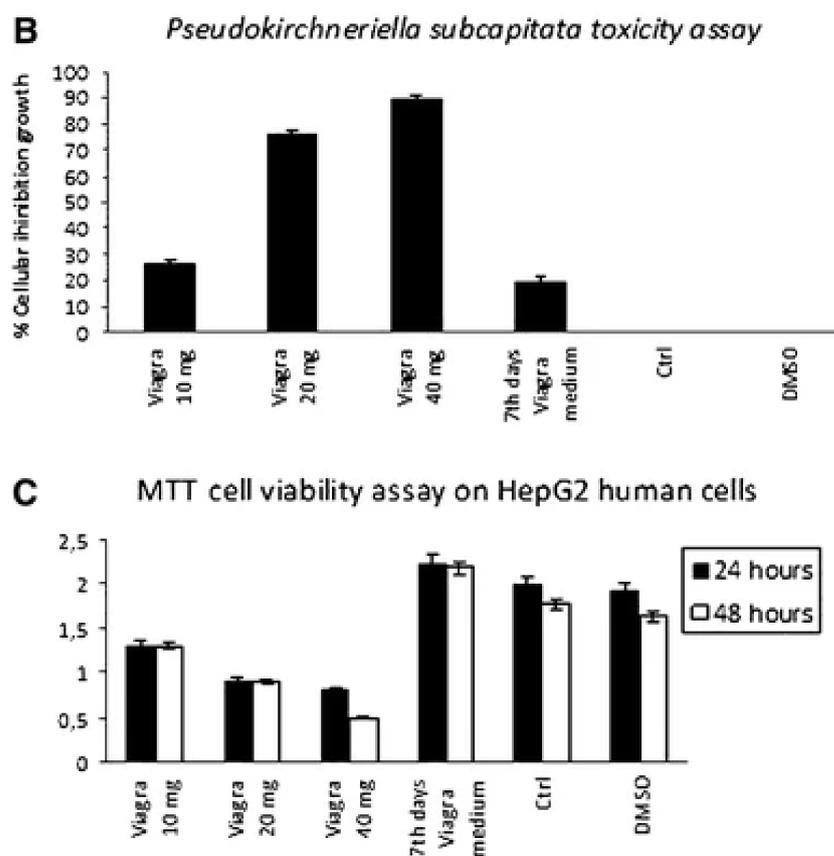


Figure 6. (A) Viability of *Daphnia magna* after 24 and 48 h incubation with sildenafil citrate (mean \pm SD). (B) Viability of *Pseudokirchneriella subcapitata* after 24 h incubation with sildenafil citrate (mean \pm SD). (C) Viability of HepG2 cells after 24 h incubation with sildenafil citrate (mean \pm SD) [64]. Reprinted with permission from ref. [64]. Copyright 2021 Springer Nature.

Chemical analysis revealed that the actual number of drugs diverged from the nominal one by less than 10%, so the researchers calculated the EC_{50} values using the nominal concentrations.

Tadalafil did not exhibit any acute consequence on both organisms up to the highest concentration tested (20 mg L^{-1}). Sildenafil did not display any effect up to the highest concentration tested (10 mg L^{-1}) for *B. calyciflorus*, while it showed an EC_{50} value of 0.64 mg L^{-1} for *C. dubia*. Chlorine derivatives of both drugs evidenced a different behaviour: the compound S1 showed long-term effects for both test organisms, while the product T1 only for crustaceans.

Literature on the additional biological effects of drugs such as mutagenesis and genotoxicity is also available [66–68]. The risk associated with small quantities of drugs is frequently due to their mutagenic and genotoxic potential already examined on mammalian cell lines or bacterial models. Sildenafil and tadalafil and the respective derivatives could not induce an SOS activation response, while the results of the Ames test were fascinating (Table 3). Tadalafil expressed a significant mutagenic activity (maximum MR = 18.4) in TA98 with a positive range of concentrations from 0.625 to $10 \text{ } \mu\text{g mL}^{-1}$, and it was positive also in TA100 (2.5 – $10 \text{ } \mu\text{g mL}^{-1}$). The derivative T1 had a mutagenic potential (2.5 – $10 \text{ } \mu\text{g mL}^{-1}$) for TA98, but it was negative for TA100. Rocco et al. [69] studied the possible genetic damage of sildenafil through a Comet assay, diffusion assay and RAPD-PCR for the erythrocytes of *Danio rerio*. They found statistically significant genotoxicity.

Table 3. Ames test results for parent compounds, chlorine derivatives and positive and negative controls. In bold are the positive MRs [47]. Reprinted with permission from ref. [47]. Copyright 2021 Elsevier.

Compounds	Ames Test					
	TA98			TA100		
	Concentration ($\mu\text{g mL}^{-1}$)	Mean Revertants/Plate ($\pm\text{SD}$)	MR ^a	Concentration ($\mu\text{g mL}^{-1}$)	Mean Revertants/Plate ($\pm\text{SD}$)	MR ^a
Negative Control2-Nitrofluoren	-	29.6 \pm 2.7	-	-	190.1 \pm 47.2	-
	2.5	61.0 \pm 5.7	2.1	-	-	-
	5	83.2 \pm 11.3	2.8	-	-	-
Sodium azide	10	178.0 \pm 15.0	6	-	-	-
	-	-	-	5	488.0 \pm 96.9	2.6
	-	-	-	10	794.7 \pm 90.9	4.2
Sildenafil citrate	-	-	-	20	1153.3 \pm 239.8	6.1
	0.625	46.7 \pm 13.1	1.6	0.625	161.0 \pm 18.7	0.8
	1.25	48.7 \pm 5.1	1.6	1.25	164.6 \pm 8.7	0.9
	2.5	72.0 \pm 11.3	2.4	2.5	190.6 \pm 12.6	1
	5	93.0 \pm 4.4	3.1	5	198.3 \pm 24.0	1
S1	10	170.7 \pm 32.0	5.8	10	220.0 \pm 46.8	1.2
	0.3125	27.8 \pm 3.2	0.9	0.3125	241.1 \pm 27.5	1.3
	0.625	37.6 \pm 4.7	1.3	0.625	291.1 \pm 21.6	1.5
	1.25	37.3 \pm 11.6	1.3	1.25	303.1 \pm 44.7	1.6
	2.5	53.6 \pm 9.0	1.8	2.5	376.6 \pm 45.3	2
	5	64.7 \pm 20.2	2.2	5	405.1 \pm 82.5	2.1
Tadalafil	10	112.4 \pm 41.5	3.8	10	444.2 \pm 83.2	2.3
	0.3125	49.5 \pm 7.7	1.7	0.3125	298.5 \pm 38.7	1.6
	0.625	67.2 \pm 12.2	2.3	0.625	318.6 \pm 65.2	1.7
	1.25	149.3 \pm 33.2	5	1.25	325.6 \pm 94.4	1.7
	2.5	223.0 \pm 54.8	7.5	2.5	425.3 \pm 80.9	2.2
	5	337.3 \pm 32.3	11.4	5	556.0 \pm 41.7	2.9
T1	10	544.0 \pm 92.1	18.4	10	880.0 \pm 237.6	4.6
	0.625	43.6 \pm 11.9	1.5	0.625	286.0 \pm 14.1	1.5
	1.25	43.5 \pm 14.0	1.5	1.25	310.7 \pm 43.9	1.6
	2.5	85.4 \pm 19.2	2.9	2.5	322.7 \pm 28.4	1.7
	5	221.8 \pm 57.2	7.5	5	288.0 \pm 40.6	1.5
	10	391.2 \pm 69.4	13.2	10	410.7 \pm 70.0	2.2

$\pm\text{SD}$ = standard deviation obtained from three independent experiments. ^a MR (mutagenic ratio): number of revertants/plate compared to the negative control.

7. Impact on the Aquatic Organisms

To better understand the mechanisms that allow marine invertebrates to survive and reproduce in contaminated and changing habitats, researchers investigated the effect of some drugs, including sildenafil, on aquatic organisms. In particular, Zanuri et al. [70] studied *Asterias rubens*, *Psammechinus miliaris* and *Arenicola marina* (Polychaeta), essential components of marine benthos. They investigated the effects of exposure time and dosage of diclofenac, ibuprofen and sildenafil citrate on sperm motility and subsequent fertilisation. Diclofenac concentrations $\geq 0.1 \mu\text{g L}^{-1}$ caused a reduction in motility for all species observed. Exposure to $\geq 1.0 \mu\text{g L}^{-1}$ ibuprofen affected only *P. miliaris* gametes and *A. marina* fertilisation. Only the spermatozoa of *A. rubens* and *P. miliaris* exposed to sildenafil citrate at concentrations ≥ 18 and $\geq 50 \text{ ng L}^{-1}$, respectively, showed higher percentage motility and a significant increase in fertilisation [70] (Figure 7; Table 4). Sildenafil citrate was non-toxic in all cases.

Su et al. [71] confirmed the influence of PDE-5 inhibitors on the sperm motility increase for aquatic species that studied this effect on the sperm of sea urchin. The data are consistent

with the hypothesis that sPDE-5 regulates cyclic guanine monophosphate (cGMP) levels in sperm, modulating sperm motility.

Rocco et al. [69] analysed the genotoxic damage caused by sildenafil citrate, a vasodilator, gemfibrozil, and atorvastatin, two regulators of the lipids' hematic level, for the teleost fish *Danio rerio* (Zebrafish). This species is a bio-indicator used for genetic toxicology studies (Table 5) [72–74]. The pharmacological agents chosen by the authors are mainly present in high concentrations in Italian-treated wastewater.

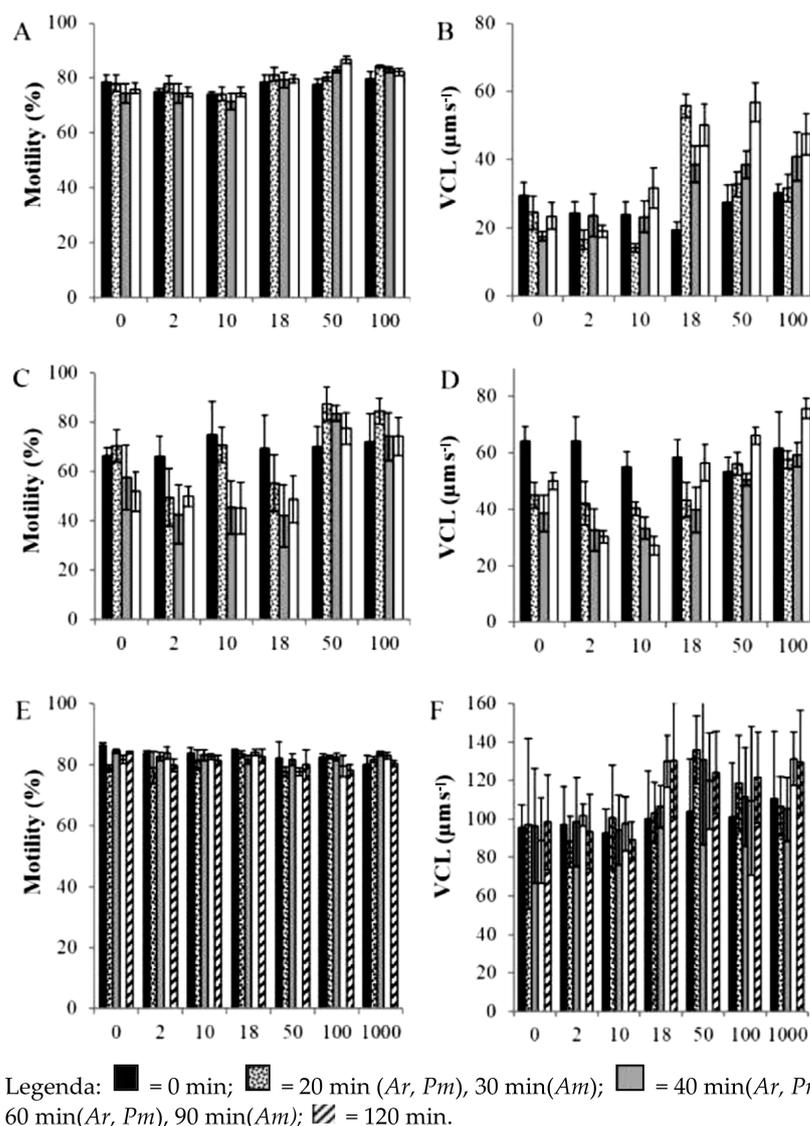


Figure 7. Percentage of sperm motility (A,C,E) and curvilinear velocity (VCL) (B,D,F) of sperm of *Asterias rubens* (A,B), *Psammechinus miliaris* (C,D), and *Arenicola marina* (E,F) after exposure to sildenafil citrate for set periods [70]. Reprinted with permission from ref. [70]. Copyright 2021 Elsevier.

Once again, Comet and diffusion assays and RAPD-PCR are the resources used to evaluate the genotoxicity of the drugs [69]. The obtained data showed a statistically significant loss of DNA integrity after 35 days of exposure to sildenafil citrate, after five days to atorvastatin and after one week of exposure to gemfibrozil. In addition, the fishes treated with sildenafil citrate at the concentration of 26.25 ng L^{-1} for 5 and 7 days exhibited a percentage of apoptotic cells similar to the control animals. However, the treatment extended to 35 days showed a significant increase of apoptotic cells (7.7%), demonstrating that this pharmacological agent induces genotoxic damage over time.

These data could suggest that there is no human health risk for exposure to sildenafil and tadalafil. However, they require great attention since this kind of contamination is present in each aquatic system investigated.

Table 4. No-observed-effects (NOEC) or lowest-observed-effects concentration (LOEC), half-minimal effective concentration (EC_{50}), and toxicity substance classification for *Asterias rubens*, *Psammechinus miliaris* and *Arenicola marina*. Toxicity classification is from EU Directive 93/67/EEC: EC_{50} ($\mu\text{g L}^{-1}$) > 100,000 = non-toxic; 10,000–100,000 = harmful; 1000–10,000 = toxic; <100–1000 = very toxic; and <100 = extremely toxic. N/A = not applicable as an EC_{50} could not be calculated and is therefore deemed non-toxic [70]. Reprinted with permission from ref. [70]. Copyright 2021 Elsevier.

Species	Test	Pharmaceutical	NOEC or LOEC ($\mu\text{g L}^{-1}$)	EC_{50} ($\mu\text{g L}^{-1}$)	Classification
<i>Asterias rubens</i>	Sperm motility	Sildenafil citrate	NOEC = 0.18	60 min = 2.25×10^{12}	Non-toxic
	Fertilisation: sperm pre-incubation	Sildenafil citrate	NOEC = 0.010	60 min = 7.15×10^{13}	Non-toxic
	Fertilisation: oocyte pre-incubation	Sildenafil citrate	NOEC = 0.10	N/A	N/A
	Fertilisation: sperm and oocyte pre-incubation	Sildenafil citrate	NOEC = 0.01	60 min = 2.37×10^{12}	Non-toxic
<i>Psammechinus miliaris</i>	Sperm motility	Diclofenac	NOEC = 0.01	60 min = 378.22	Very toxic
		Ibuprofen	NOEC = 0.1	60 min = 845.98	Very toxic
		Sildenafil citrate	NOEC = 0.018	60 min = 7.23×10^{10}	Non-toxic
	Fertilisation: sperm pre-incubation	Sildenafil citrate	NOEC = 0.10	60 min = 6.241×10^{10}	Non-toxic
Fertilisation: oocyte pre-incubation	Sildenafil citrate	NOEC = 0.01	N/A	N/A	
Fertilisation: sperm and oocyte pre-incubation	Diclofenac	LOEC = 0.01	60 min = 247.31	Very toxic	
	Ibuprofen	NOEC = 0.10	60 min = 792.96	Very toxic	
	Sildenafil citrate	NOEC = 1.0	N/A	N/A	
<i>Arenicola marina</i>	Sperm motility	Sildenafil citrate	NOEC = 1.0	N/A	N/A
	Fertilisation: sperm pre-incubation	Diclofenac	NOEC = 1.00	120 min = 565.53	Very toxic
		Ibuprofen	NOEC = 0.10	120 min = 3.24×10^9	Non toxic
		Sildenafil citrate	NOEC = 1.0	N/A	N/A
Fertilisation: oocyte pre-incubation	Sildenafil citrate	NOEC = 1.00	N/A	N/A	
Fertilisation: sperm and oocyte pre-incubation	Sildenafil citrate	NOEC = 1.0	N/A	N/A	

Table 5. Experimental conditions of treatment and percentage of DNA in the tail and apoptotic cells in *Danio rerio* erythrocytes exposed to atorvastatin, gemfibrozil and sildenafil citrate with *p*-value (* = 5 < 0.05) [69]. Reprinted with permission from ref. [69]. Copyright 2021 John Wiley and Sons.

Pharmaceutical Compound	Mean Concentration ng L ⁻¹	Number of Specimens Examined	Days of Treatment	Tail DNA Mean % ± SD		<i>p</i> -Value Tail DNA	Apoptotic Cells Mean % ± SD		<i>p</i> -Value Apoptotic Cells
				NT	T		NT	T	
Atorvastatin	13	20	5	20.41 ± 4.44	50.47 ± 9.19	0.003 *	1.0 ± 0.21	6.5 ± 1.85	0.003 *
			7	27.68 ± 13.49	40.81 ± 16.34	0.535	1.5 ± 0.31	5.7 ± 1.78	0.020 *
			14	19.96 ± 4.99	31.05 ± 13.03	0.426	1.2 ± 0.34	4.3 ± 1.86	0.101
Gemfibrozil	380	20	5	25.76 ± 12.94	55.12 ± 7.42	0.045 *	6.9 ± 0.22	8.6 ± 0.68	0.017 *
			7	27.24 ± 11.74	60.67 ± 11.78	0.044 *	5.7 ± 0.84	9.6 ± 1.34	0.013 *
			14	12.45 ± 2.32	18.47 ± 2.78	0.096	4.5 ± 0.94	5.7 ± 0.75	0.298
Sildenafil citrate	26.25	20	5	20.41 ± 4.44	25.79 ± 10.13	0.627	1.0 ± 0.28	1.8 ± 0.42	0.113
			7	27.68 ± 13.49	34.00 ± 8.19	0.688	1.5 ± 0.25	2.5 ± 1.45	0.497
			14	19.96 ± 4.99	29.93 ± 18.02	0.593	1.2 ± 0.21	2 ± 0.56	0.181
		22	21	36.56 ± 13.33	39.29 ± 14.78	0.890	4.9 ± 0.25	5.3 ± 0.21	0.220
			28	15.87 ± 5.08	12.99 ± 4.25	0.663	1.4 ± 0.25	1.1 ± 0.56	0.625
			21	35	15.36 ± 4.86	49.15 ± 16.67	0.051 *	1.3 ± 0.23	7.6 ± 2.95

SD, standard deviation; NT, untreated; T, exposed to the compound indicated.

8. Analytical Methods for PDE-5 Inhibitors Detection

Several analytical methods are suitable to estimate the concentration of PDE-5 inhibitors: spectrophotometric methods [75–80], capillary electrophoretic methods [81], atomic emission and atomic absorption spectrometry methods [82] and chromatographic techniques (GC-LC-HPLC-UHPLC) coupled with different detectors, including mass spectrometers (MS) [83–87]. Only a few reports are available in the literature for the GC-MS use to analyse PDE-5 inhibitors, maybe due to the thermal instability of these compounds and their analogues and the difficulties in derivatising them with standard silylation reagents [88–91]. Liquid chromatography techniques, instead, seem to be more suitable for the analysis of PDE-5 inhibitors.

8.1. Sample Preparation

Sample preparation is an essential task in PDE-5 inhibitors detection. Since the concentrations of the target analytes in environmental samples are expected to be in the low ng L⁻¹ range, an efficient sample preparation to extract the analytes from the respective matrix is required. For aqueous samples, solid-phase extraction (SPE) is widely used. Pressurised liquid extraction (PLE) has been successfully used for solid samples to determine pharmaceutical compounds in sewage sludge.

Solid-phase extraction (SPE) involved using a small amount of sorbent (commonly hundreds of mg) in a cartridge or syringe barrel. After activation of the sorbent, a water sample of hundreds of mL was passed through the sorbent, which retained the analytes of interest (in this case, PPCPs) whereas the water was discarded. Then, the analytes retained in the sorbent were eluted using a few mL of organic solvent. This technique has some advantages, such as the minor investment in reagent and materials and rapidity.

8.2. Spectrophotometric Methods

Spectrophotometric methods are habitually used for the chemical analyses of pharmaceuticals, thanks to their versatility and cost-effectiveness. They can be successfully applied for pharmaceutical determinations, including the quality control of commercialised substances and pharmacodynamic investigations. Since most pharmaceutical compounds show several unsaturations and aromatic rings in their structure, one of the main ad-

vantages of their analysis through spectrophotometric methods is the possibility of their determinations without preliminary derivatisation steps [92].

Ahmed et al. [80] developed a simple and accurate UV-spectrophotometric method to estimate vardenafil hydrochloride in pharmaceutical preparations and environmental wastewater samples. This compound exhibited the maximum absorption at a wavelength of 214 nm in distilled water. The proposed method was simple, straightforward and sensitive, providing 3.32 ng mL^{-1} and 10.96 ng mL^{-1} as LOD and LOQ values, respectively.

However, as sildenafil citrate is among the most widely used PDE-5 inhibitors, most research has focused on it. For example, Baokar et al. [93] validated a spectrophotometric method for detecting sildenafil in pharmaceutical formulation with good linearity in the range of 2 to 10 mg mL^{-1} , using 228 nm as the wavelength of maximum absorption. This method also showed an excellent intra-day and inter-day precision and a recovery of 99–101%.

Although the spectrophotometric analysis of these pharmaceuticals can be done quickly, conjugation with other compounds to increase sensitivity is reported in some methods, e.g., bromocresol purple (BCP) and bromophenol blue (method B) with absorption maxima at 404 and 410 nm, respectively, for determination of sildenafil [94]. Salem et al. [95] reported the use of gold nanoparticles (Au NPs) for the assay of sildenafil citrate, tadalafil and vardenafil. Gold nanoparticles showed absorption at 522 nm but, after contact with the investigated drugs, the band at 522 nm disappeared with the formation of a new red-shifted band at 673, 660 and 665 nm for sildenafil, tadalafil and vardenafil, respectively. This method showed good linearity in a concentration range of $0.1\text{--}12 \text{ }\mu\text{g mL}^{-1}$ and was then used for the determination of these pharmaceuticals in pharmaceutical dosage forms and biological fluids (human serum and urine).

Despite providing several advantages such as being easy, cheap and not time-consuming, spectrophotometric techniques alone for quantifying drugs in biological and environmental matrices do not guarantee high selectivity and suffer from the interference of many compounds, which can compromise the results of the analysis. Accordingly, a previous separation step by liquid chromatography (LC) or capillary electrophoresis (CE) is typically used before detecting pharmaceuticals.

8.3. Chromatographic Methods Coupled with UV/Vis Detectors and Mass Spectrometry (MS)

Liquid chromatography coupled with UV/Vis and diode-array detectors (DAD) is commonly used to quantify pharmaceutical compounds in biological fluids and environmental and food samples. Generally, the separation is carried out on C18 columns with different mobile phase compositions for these techniques, although the most commonly used are acidified water, acetonitrile or methanol. Fidan and Bakirdere [96] developed a sensitive analytical method for the simultaneous determination of sildenafil and tadalafil and legal drugs and illegal/counterfeit drugs in wastewater samples by using a C18 column and phosphate buffer (35%) and acetonitrile (65%) as mobile phases and 225 nm as the wavelength of absorption. They calculated LOD/LOQ values of $28/92 \text{ ng mL}^{-1}$ for sildenafil and $39/129 \text{ ng mL}^{-1}$ for tadalafil.

As said above, due to their popularity, medicinal products containing the PDE-5 inhibitors sildenafil, vardenafil and tadalafil are often subject to counterfeiting. In addition, illicit herbal dietary supplements adulterated with these substances or their analogues have appeared on the market, offering an easy and anonymous sale. Fejos et al. [97] reported an analytical method for the qualitative and quantitative screening of sildenafil, vardenafil, tadalafil and 11 of their designer analogues in illegal erectile dysfunction products by high-performance liquid chromatography with UV detection, using sildenafil as an external standard for the identification and quantification of all analytes. Also, in this case, a Kinetex C18 reverse-phased column was used. The elution was carried out with a gradient method using a mobile phase composed of a binary mixture of 200 mM ammonium acetate solution (A) and MeOH/ACN (B). The detection was performed at 290 nm.

Although HPLC-UV methods are low cost and easy to use, LC-MS and LC-MS/MS-based methods are the most popular techniques for characterising pharmaceutical products and by-products in environmental samples. Naturally, the NMR is the elected technique to obtain the absolute configuration of a compound. But, the limit of NMR is the availability of a good amount of the substance to be analysed. For the determination of pollutants in micro- or nano-grams in environmental samples, some difficulties can arise. The ability of LC-MS-based methods to provide some structural information about the detected analytes makes them indispensable in the identification of low-concentration compounds and their unknown analogues and transformation products [98]. In detail, soft ionisation techniques such as electrospray ionisation (ESI) are generally used to ionise the drugs of interest effectively. The polarity is chosen for ionisation, i.e., the positive or the negative one depends on the relative acidity (pKa) of the functional groups occurring on the molecules [99]. As regard PDE-5 inhibitors, positive polarity is typically used.

Öztürk Er et al. [100] validated a sensitive method for determining sildenafil and tadalafil using LC-QTOF-MS/MS and optimised both LC and MS parameters. They obtained very low LOD/LOQ values of 0.16/0.53 and 0.28/0.93 ng g⁻¹ for sildenafil and tadalafil, respectively. They successfully applied this method to analyse the two drugs by spiking experiments into all tested matrices into energy drinks, sewage sludge and tap water (Figure 8).

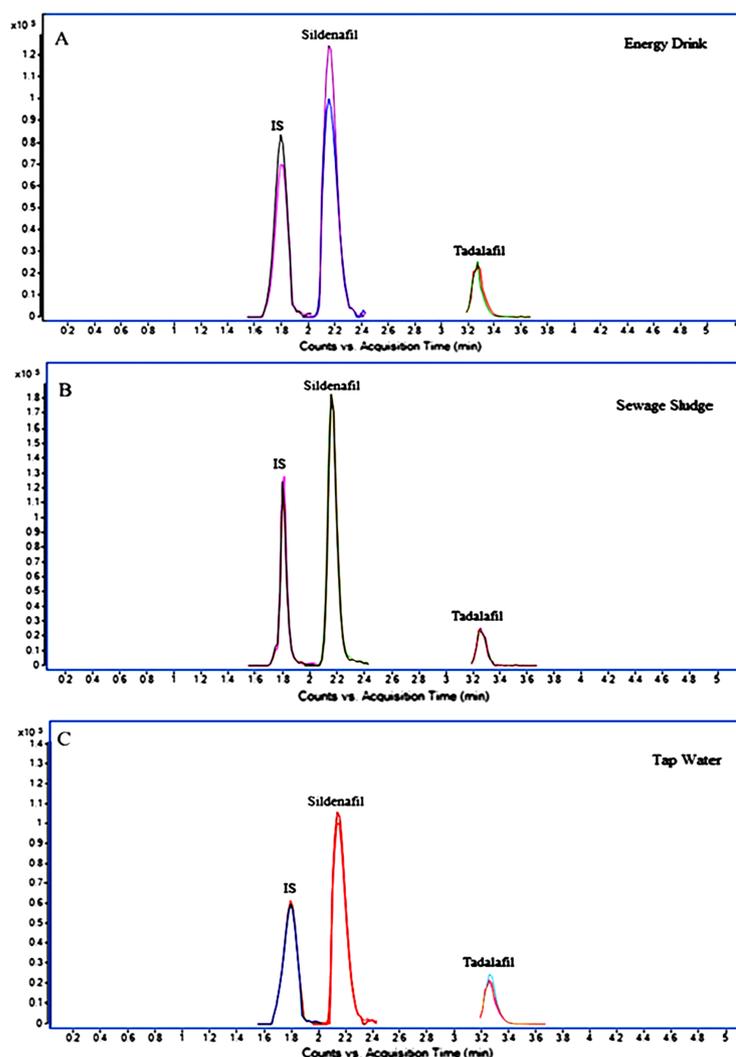


Figure 8. Spiking experiments for (A) energy drink, Brand E (B) sewage sludge and (C) tap water [100]. Reprinted with permission from ref. [100]. Copyright 2021 Elsevier.

In addition, matrix effects were low and therefore acceptable: they ranged from <9 to <19% for spiked urine, <9% to <18% for WWTP feed and <5% to <14% for WWTP effluent. As expected, matrix effects affecting ionisation increased from low-loaded effluent samples via feed samples to the heavily matrix-loaded fitness centre discharges. Therefore, LC-MS was a valid method applicable in quantification without stable isotope-labelled internal standards.

The developed method presents lower limits of detection/quantification and offers fast and direct analysis compared to other methodologies already published [101–103].

Öztürk Er et al. [104] also developed in 2019 another analytical method for the simultaneous determination of sildenafil, tadalafil, vardenafil and avanafil in human urine by the same instrumental system described above. The chromatographic separation of analytes was achieved by using a gradient elution method with a total run time of eight min and with ammonium formate/formic acid (pH = 4.60) in ultrapure water (A) and 0.10% *v/v* formic acid in acetonitrile (B) as mobile phase. This method provided a proper chromatographic separation of analytes with the retention times of 2.373, 2.845, 3.379 and 5.287 min for vardenafil, sildenafil, avanafil and tadalafil, respectively. MS/MS acquisitions were made using the electrospray interface in positive mode, and MS/MS transitions of each analyte were determined by adjusting the collision energy. The monitored transitions in the MS/MS experiments were 475.2155 → 58.0652/100.0997 *m/z* for sildenafil, 489.2247 → 151.0859/312.1574 *m/z* for vardenafil, 484.1856 → 375.1217/155.0254 *m/z* for avanafil and 390.1441 → 268.1076/135.0437 for tadalafil, as quantifier/qualifier ion, respectively (Figure 9).

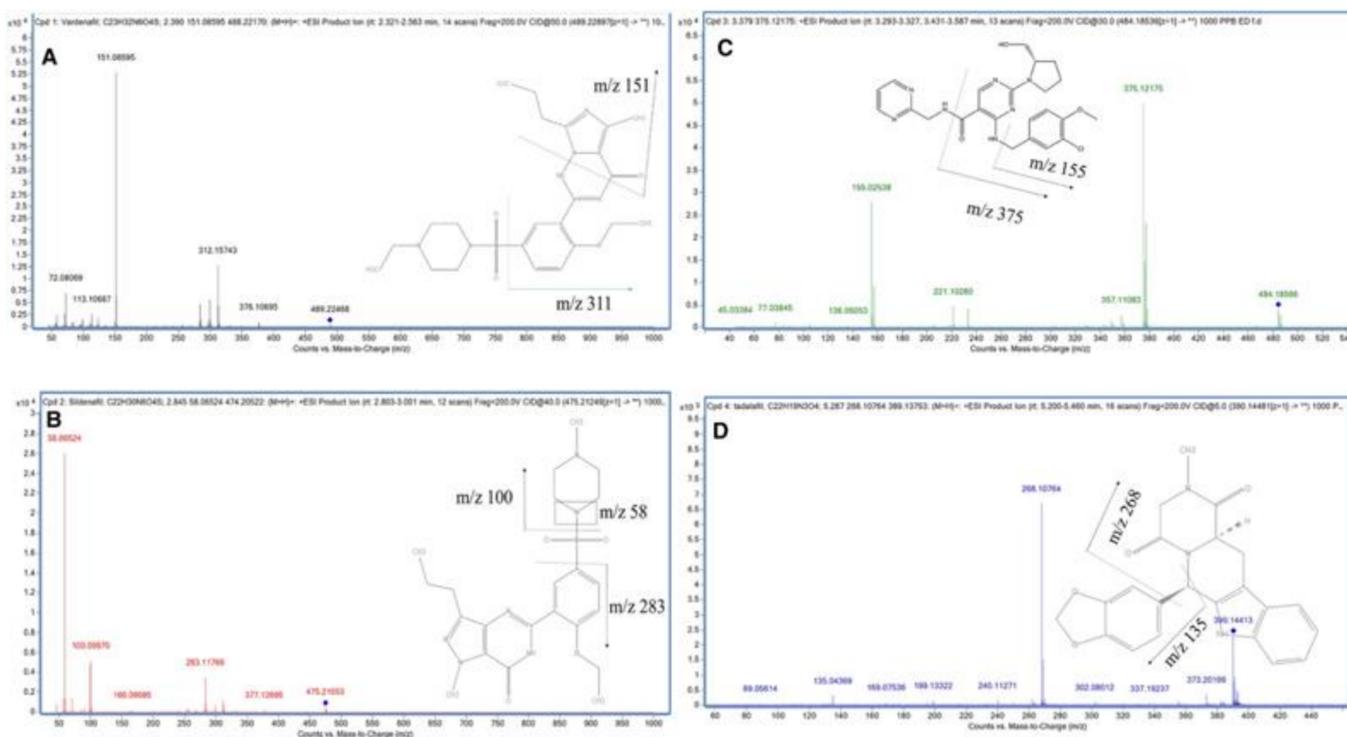


Figure 9. Full scan product ion mass spectra and proposed fragmentations of (A) vardenafil, (B) sildenafil, (C) avanafil and (D) tadalafil [104]. Reprinted with permission from ref. [104]. Copyright 2021 John Wiley and Sons.

The analytical performance of this method proved good linearity and low LOD/LOQ values, in particular, 2.28/7.59 ng g⁻¹, 2.19/7.28 ng g⁻¹, 1.63/5.43 ng g⁻¹ and 9.81/32.71 ng g⁻¹ for vardenafil, sildenafil, avanafil and tadalafil, respectively. Then, the method was successfully applied in human urine samples spiked with standard mixtures of each analyte. It was the first report for the simultaneous determination of FDA-approved active ingredients (sildenafil, tadalafil, vardenafil and avanafil) for ED drugs using MS/MS.

The evaluation of matrix effects was also performed to investigate the potential interfering compounds in the urine matrix on the ionisation source. In detail, the analyte peak areas in the spiked urine sample were compared with the analyte peak areas in ultrapure water at the concentration of 60 ng g^{-1} for each compound. The matrix effect percentage was found to be below 10%, which demonstrates a low matrix impact.

Some researchers have included sildenafil in multi-residue methods [105–107], and very few studies have analysed tadalafil and vardenafil [15] or have included metabolites and transformation products [45]. Indeed, as mentioned above, contaminants can undergo additional physical and chemical transformations in the environment due to abiotic (photolysis, hydrolysis) and biotic (biodegradation) reactions. Consequently, they can undergo structural modifications that lead to new transformation products, which may persist in the environment. LC-MS techniques play a central role in this contest because they allow elucidating structural information about TPs. Eichhorn et al. [48], Aceña et al. [108] and Herbert et al. [109] focused on some transformation products of sildenafil and vardenafil after the photodegradation process that appeared notably persistent, which might indicate a potential impact on the aquatic environment.

However, for these by-products, the literature mainly reported qualitative results. Therefore, a systematic method should be developed in the future to determine their concentration levels, LOD and LOQ values and matrix effect.

Eichhorn et al. [48] performed the photolysis of sildenafil and its human metabolite N-demethylsildenafil in ultrapure water, artificial freshwater, which resembled moderately hard water ($96 \text{ mg L}^{-1} \text{ NaHCO}_3$, $60 \text{ mg L}^{-1} \text{ CaSO}_4 \cdot 2\text{H}_2\text{O}$, $60 \text{ mg L}^{-1} \text{ MgSO}_4$ and $4 \text{ mg L}^{-1} \text{ KCl}$; pH 6.9), and natural river water (pH 7.6). After chromatographic separation of the irradiated samples, eight photoproducts for sildenafil and six photoproducts for N-demethylsildenafil were detected and characterised using a combination of ultra-performance liquid chromatography-electrospray ionisation-quadrupole time-of-flight-mass spectrometry (UPLC-ESI-QToF-MS), liquid chromatography-atmospheric pressure chemical ionisation-triple quadrupole mass spectrometry (LC-APCI-QqQ-MS) and hydrogen/deuterium exchange experiments, that allowed to propose plausible chemical structures for the photoproducts and to identify the piperazine ring as the main susceptible site of the photodegradation process (Tables 6 and 7).

Table 6. Accurate ion mass measurements of sildenafil and its phototransformation products obtained by UPLC-(+)ESI-QToF-MS in MS and MS/MS mode [48]. Reprinted with permission from ref. [48]. Copyright 2021 John Wiley and Sons.

Compound/ Photoproduct	Measured Ion Mass of [M + H] ⁺	Elemental Composition	Theoretical Ion Mass (<i>m/z</i>)	Relative Error (ppm) (DBE)
SDF	475.2128	C ₂₂ H ₃₁ N ₆ O ₄ S	475.2132	+1.9 (10.5)
	377.1285	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+1.6 (9.5)
	311.1514	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+3.5 (10.5)
	299.1153	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+4.7 (10.5)
	283.1183	C ₁₅ H ₁₅ N ₄ O ₂	283.119	−2.5 (10.5)
	100.1033	C ₅ H ₁₂ N ₂	100.0995	+38 (1.0)
	99.0938	C ₅ H ₁₁ N ₂	99.0917	+21.2 (1.5)
TP490	491.2089	C ₂₂ H ₃₁ N ₆ O ₅ S	491.2072	+3.5 (10.5)
	473.1948	C ₂₂ H ₂₉ N ₆ O ₄ S	473.1966	−3.8 (11.5)
	404.1382	C ₁₈ H ₂₂ N ₅ O ₄ S	404.1387	−1.2 (10.5)
	377.1285	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+1.6 (9.5)
	311.1518	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+4.8 (10.5)
	299.1152	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+4.3 (10.5)
	283.125	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+4.6 (10.5)
	100.1012	C ₅ H ₁₂ N ₂	100.0995	+17 (1.0)
99.0940	C ₅ H ₁₁ N ₂	99.0917	+28.3 (1.5)	

Table 6. Cont.

Compound/ Photoproduct	Measured Ion Mass of [M + H] ⁺	Elemental Composition	Theoretical Ion Mass (<i>m/z</i>)	Relative Error (ppm) (DBE)
TP488-C	489.1952	C ₂₂ H ₂₉ N ₆ O ₅ S	489.191	+4.5 (11.5)
	461.1997	C ₂₁ H ₂₉ N ₆ O ₄ S	461.1966	+6.7 (10.5)
	377.1296	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+4.5 (9.5)
	311.1511	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+2.6 (10.5)
	299.1146	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+2.3 (10.5)
	283.1183	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	−2.5 (10.5)
	85.0727	C ₄ H ₉ N ₂	85.0760	−38.8 (1.5)
TP462	463.1777	C ₂₀ H ₂₇ N ₆ O ₅ S	463.1758	+4.1 (10.5)
	435.1836	C ₁₉ H ₂₇ N ₆ O ₄ S	435.1809	+4.1 (9.5)
	418.1517	C ₁₉ H ₂₄ N ₅ O ₄ S	418.1544	−6.5 (10.5)
	311.153	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+8.7 (10.5)
	299.1155	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+5.3 (10.5)
	283.1178	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	−4.2 (10.5)
TP460	461.1986	C ₂₁ H ₂₉ N ₆ O ₄ S	461.1966	+4.3 (10.5)
	377.1285	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+1.6 (9.5)
	311.1511	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+2.6 (10.5)
	283.1185	C ₁₅ H ₁₅ N ₄ O ₂	283.119	−1.8 (10.5)
	85.0769	C ₄ H ₉ N ₂	85.0760	+9.4 (1.5)
TP448-A	449.2163	C ₂₀ H ₂₉ N ₆ O ₄ S	449.1966	+5.1 (9.5)
	418.1529	C ₁₉ H ₂₄ N ₅ O ₄ S	418.1544	−3.6 (10.5)
	392.1363	C ₁₉ H ₂₄ N ₅ O ₄ S	392.1387	−6.1 (9.5)
	377.129	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+2.9 (9.5)
	311.1489	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	−4.5 (10.5)
	299.1151	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+4.0 (10.5)
	283.1203	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+4.6 (10.5)
TP434	435.1834	C ₁₉ H ₂₇ N ₆ O ₄ S	435.1814	+5.7 (9.5)
	418.1567	C ₁₉ H ₂₄ N ₅ O ₄ S	418.1544	+5.5 (10.5)
	392.1451	C ₁₇ H ₂₂ N ₅ O ₄ S	392.1387	+5.6 (9.5)
	377.1245	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	−9.0 (9.5)
	311.1516	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+4.2 (10.5)
	299.1159	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+6.7 (10.5)
	283.1205	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+5.3 (10.5)
TP392	393.1234	C ₁₇ H ₂₁ N ₄ O ₅ S	393.1227	+1.5 (9.5)
	365.0903	C ₁₅ H ₁₇ N ₄ O ₅ S	365.0914	−3.0 (9.5)
	336.0637	C ₁₄ H ₁₄ N ₃ O ₅ S	336.0649	−3.6 (9.5)
	284.1279	C ₁₅ H ₁₆ N ₄ O ₂	284.1268	3.9 (10.0)
	256.1070	C ₁₄ H ₁₄ N ₃ O ₂	256.1081	−4.3 (9.5)
TP391	392.1365	C ₁₇ H ₂₂ N ₅ O ₄ S	392.1387	−5.6 (9.5)
	364.1056	C ₁₅ H ₁₈ N ₅ O ₄ S	364.1079	−6.3 (9.5)
	299.1157	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+6.0 (10.5)
	283.1169	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	−7.4 (10.5)

DBE = double bond equivalent.

Table 7. Accurate ion mass measurements of N-demethylsildenafil and its phototransformation products obtained by UPLC-(+)ESI-QToF-MS in MS and MS/MS mode [48]. Reprinted with permission from ref. [48]. Copyright 2021 John Wiley and Sons.

Compound/ Photoproduct	Measured Ion Mass of [M + H] ⁺	Elemental Composition	Theoretical Ion Mass (<i>m/z</i>)	Relative Error (ppm)
DM-SDF	461.1978	C ₂₁ H ₂₉ N ₆ O ₄ S C ₁₇ H ₂₁ N ₄ O ₄ S C ₁₇ H ₁₉ N ₄ O ₂	461.1966	+2.6 (10.5)
	377.1270	C ₁₅ H ₁₅ N ₄ O ₃	377.1279	−2.4 (9.5)
	311.1518	C ₁₅ H ₁₅ N ₄ O ₂	311.1503	+4.8 (10.5)
	299.1155	C ₄ H ₉ N ₂	299.1139	+5.3 (10.5)
	283.1203		283.1190	+4.6 (10.5)
	85.0803		85.0760	−11.8 (1.5)
	TP476	477.1959	C ₂₁ H ₂₉ N ₆ O ₅ S	477.1915
404.1404		C ₁₈ H ₂₂ N ₅ O ₄ S	404.1387	+7.9 (10.5)
377.1295		C ₁₇ H ₂₁ N ₄ O ₄ S	377.1278	+4.5 (9.5)
331.0836		C ₁₅ H ₁₅ N ₄ O ₃ S	331.0865	−5.7 (10.5)
312.1597		C ₁₇ H ₂₀ N ₄ O ₂	312.1581	+5.1 (10.0)
283.1205		C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+4.6 (10.5)
83.0628		C ₄ H ₇ N ₂	83.0604	+28.9 (2.5)
TP462	463.1777	C ₂₀ H ₂₇ N ₆ O ₅ S	463.1758	+4.1 (10.5)
	435.1839	C ₁₉ H ₂₇ N ₆ O ₄ S	435.1809	+6.9 (9.5)
	418.1569	C ₁₉ H ₂₄ N ₅ O ₄ S	418.1544	+6.0 (10.5)
	311.1520	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+5.5 (10.5)
	299.1122	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	−5.7 (10.5)
	283.1175	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	−5.3 (10.5)
TP448-B	449.162	C ₁₉ H ₂₅ N ₆ O ₅ S	449.1602	+4.0 (10.5)
	431.1507	C ₁₉ H ₂₃ N ₆ O ₄ S	431.1496	+2.6 (11.5)
	404.1393	C ₁₈ H ₂₂ N ₅ O ₄ S	404.1387	+0.2 (10.5)
	311.1519	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+5.1 (10.5)
	299.1122	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	−5.7 (10.5)
	283.1206	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+5.7 (10.5)
TP434	435.1801	C ₁₉ H ₂₅ N ₆ O ₅ S	435.181	−2.1 (9.5)
	418.1539	C ₁₉ H ₂₂ N ₅ O ₄ S	418.1544	−1.2 (10.5)
	392.1353	C ₁₇ H ₂₂ N ₅ O ₄ S	392.1387	−8.7 (9.5)
	377.1281	C ₁₇ H ₂₁ N ₄ O ₄ S	377.1279	+0.5 (9.5)
	311.1514	C ₁₇ H ₁₉ N ₄ O ₂	311.1503	+3.5 (10.5)
	299.1126	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	−4.3 (10.5)
	283.1197	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	+2.5 (10.5)
TP392	393.1248	C ₁₇ H ₂₁ N ₄ O ₅ S	393.1227	+5.3 (9.5)
	365.0922	C ₁₅ H ₁₇ N ₄ O ₅ S	365.0914	+2.2 (9.5)
	365.0922	C ₁₅ H ₁₇ N ₄ O ₅ S	365.0914	+2.2 (9.5)
	336.0627	C ₁₄ H ₁₄ N ₃ O ₅ S	336.0649	−6.5 (9.5)
	284.1290	C ₁₅ H ₁₆ N ₄ O ₂	284.1268	+7.7 (10.0)
	256.1060	C ₁₄ H ₁₄ N ₃ O ₂	256.1081	−8.2 (9.5)
TP391	392.1405	C ₁₇ H ₂₂ N ₅ O ₄ S	392.1387	+3.1 (9.5)
	364.1105	C ₁₅ H ₁₈ N ₅ O ₄ S	364.1079	−0.5 (9.5)
	299.1158	C ₁₅ H ₁₅ N ₄ O ₃	299.1139	+6.4 (10.5)
	283.1172	C ₁₅ H ₁₅ N ₄ O ₂	283.1190	−6.4 (10.5)

Following ESI(+), the acquisition of the product ion spectrum of the protonated sildenafil and N-demethylsildenafil gave rise to a number of characteristic fragment ions. Most of them are the same for both compounds.

The results of sildenafil and N-sildenafil photolysis evidenced that the piperazine ring in these molecules can be broken down with relative ease under the influence of artificial sunlight until reaching the stage of the sulfonic acid as a key intermediate.

It is interesting to note that the fate of sildenafil under irradiation followed a similar path that occurs in the human body. The cocktail of possible metabolites (human and not) released into the environment through wastewater discharges and the possibility of N-sildenafil undergoing photoreactions evidence the contamination issue.

Herbert et al. [109] successively compared and identified the photoproducts obtained after 48–96 h of irradiation for vardenafil and sildenafil (Table 8) by LC-ESI-MS and MS/MS. Most of these photoproducts resulted from cleavages within the alkylated piperazine ring. Several photoproducts confirmed the previous report by Eichhorn et al. [48]. The compound represented the only exception at m/z 476, a transformation product with an opening in the fused heterocyclic moieties instead of the piperazinic ring.

Sildenafil 476 and vardenafil 490 were the unique transformation substances detected with an opening of the fused heterocyclic (pyrazolopyrimidione) moiety within the two compounds; in these products, the alkylated piperazine ring remains intact. The MS/MS spectrum for sildenafil 476 included a major fragment at m/z 449. The spectrum for vardenafil 490 displayed a constituent at m/z 463, both of which point to an opening in the heterocyclic ring. These products represent a small but significant portion of the overall photoproducts observed in this study even after 96 h of photo exposure.

Table 9 reports a summary of the main LC-MS methods used to determine PDE-5 inhibitors in the last years.

Table 8. LC–MS data and proposed structures of vardenafil (VRD) and sildenafil (SLD) PT products [109]. Reprinted with permission from ref. [109]. Copyright 2021 Elsevier.

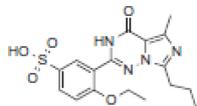
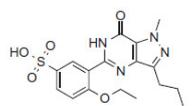
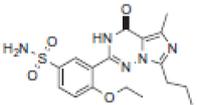
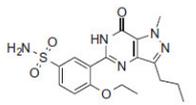
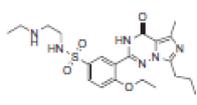
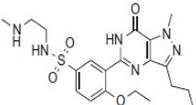
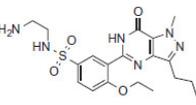
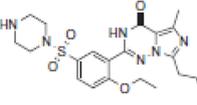
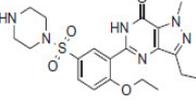
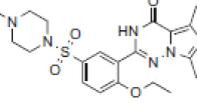
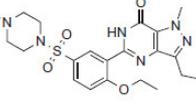
VRD Phototransformation (PT) Products				SLD Phototransformation (PT) Products			
Rt(m)	m/z (Da)	Proposed Structure	Name	Rt(m)	m/z (Da)	Proposed Structure	Name
2.8	393		VRD-392	3.8	393		SLD-392
5.3	392		VRD-391	9.6	392		SLD-391
5.6	463		VRD-462	6.9	449		SLD-448
	435	ND		6.6	435		SLD-434
6.7	461		VRD-460		461		SLD-460
6.8	489		VRD		475		SLD

Table 8. Cont.

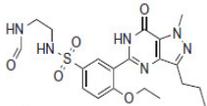
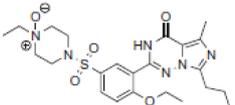
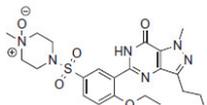
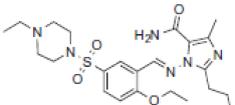
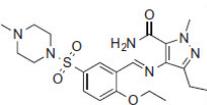
VRD Phototransformation (PT) Products				SLD Phototransformation (PT) Products			
Rt(m)	m/z (Da)	Proposed Structure	Name	Rt(m)	m/z (Da)	Proposed Structure	Name
	(463)	ND			463		SLD-462
7.1	505		VRD-504		491		SLD-490
8.4	491		VRD-490		477		SLD-476

Table 9. Main LC-MS methods reported in the literature for detecting sildenafil, tadalafil, vardenafil and avanafil.

Investigated Compound	Sample Matrix	Sample Preparation	Detection Method	LOD/LOQ	Reference
Sildenafil, Tadalafil, Vardenafil	Influent wastewater, effluent wastewater and sewage sludge samples	Wastewater samples Solid-phase extraction (SPE) Sewage sludge samples Pressurised liquid extraction (PLE)	HPLC-MS/MS	Sildenafil	[15]
				<i>Influent wastewater</i>	
				LOD: 1 ng L ⁻¹	
				LOQ: 2.5 ng L ⁻¹	
				<i>Effluent wastewater</i>	
				LOD: 1 ng L ⁻¹	
				LOQ: 1 ng L ⁻¹	
				<i>Sewage sludge</i>	
				LOD: 1 ng g ⁻¹	
				LOQ: 3 ng g ⁻¹	
				Tadalafil	
				<i>Influent wastewater</i>	
LOD: 5 ng L ⁻¹					
LOQ: 6 ng L ⁻¹					
<i>Effluent wastewater</i>					
LOD: 1.5 ng L ⁻¹					
LOQ: 2.5 ng L ⁻¹					
<i>Sewage sludge</i>					
LOD: 3 ng g ⁻¹					
LOQ: 5 ng g ⁻¹					
Vardenafil					
<i>Influent wastewater</i>					
LOD: 2 ng L ⁻¹					
LOQ: 4 ng L ⁻¹					
<i>Effluent wastewater</i>					
LOD: 1 ng L ⁻¹					
LOQ: 1.5 ng L ⁻¹					
<i>Sewage sludge</i>					
LOD: 2 ng g ⁻¹					
LOQ: 5 ng g ⁻¹					

Table 9. Cont.

Investigated Compound	Sample Matrix	Sample Preparation	Detection Method	LOD/LOQ	Reference
Sildenafil, Tadalafil, Vardenafil	Wastewater samples	Homogenisation, spiking with deuterated analogue and filtration with 0.2 µm syringe filter	UPLC-MS/MS	Sildenafil LOD: 2 ng L ⁻¹ LOQ: 6 ng L ⁻¹ Tadalafil LOD: 2 ng L ⁻¹ LOQ: 8 ng L ⁻¹ Vardenafil LOD: 7 ng L ⁻¹ LOQ: 24 ng L ⁻¹	[44]
Sildenafil, Tadalafil, Vardenafil and their metabolites	Spiked urine and wastewater samples	Solid-phase extraction (SPE)	HPLC-(HR)-MS/MS	Sildenafil <i>Spiked urine</i> LLOD: <2 ng L ⁻¹ LLOQ: 2 ng L ⁻¹ <i>Spiked wastewater</i> LLOD: <1 ng L ⁻¹ LLOQ: 2 ng L ⁻¹ Tadalafil <i>Spiked urine</i> LLOD: <2 ng L ⁻¹ LLOQ: 2 ng L ⁻¹ <i>Spiked wastewater</i> LLOD: <1 ng L ⁻¹ LLOQ: 2 ng L ⁻¹ Vardenafil <i>Spiked urine</i> LLOD: <2 ng L ⁻¹ LLOQ: 2 ng L ⁻¹ <i>Spiked wastewater</i> LLOD: <1 ng L ⁻¹ LLOQ: 2 ng L ⁻¹	[45]
Sildenafil, N-desmethyl sildenafil and their photo-transformation products	Ultrapure water, artificial freshwater, natural river water	Irradiation of tests solution in the 3 matrices, with an initial concentration of 10 mg L ⁻¹	UPLC-ESI-QToF-MS	Not reported	[48]
Sildenafil and Tadalafil	Standard solutions, tap water, energy drinks, sewage sludge	Energy drink Direct injection Tap water and sewage sludge Dilution with 50/50 (v/v) ACN/water and filtration with a 0.45 µm syringe filter	LC-QTOF-MS/MS	Sildenafil LOD: 0.16 ng g ⁻¹ LOQ: 0.53 ng g ⁻¹ Tadalafil LOD: 0.28 ng g ⁻¹ LOQ: 0.93 ng g ⁻¹	[100]

Table 9. Cont.

Investigated Compound	Sample Matrix	Sample Preparation	Detection Method	LOD/LOQ	Reference
Sildenafil, Tadalafil, Vardenafil	Standard solutions, pharmaceutical dosage forms and herbal products	Tablet samples and herbal products Mixed with 50 mL of ACN: water 50:50 (<i>v/v</i>), sonication for 15 min and filtration through a 0.45 µm syringe filter	LC-ESI-MS/MS	Sildenafil, Tadalafil, Vardenafil LLOQ: 20 ng mL ⁻¹	[101]
Sildenafil, Tadalafil, Vardenafil, Avanafil	Standard solutions, urine samples and illicit medications	Urine samples Dilution of urine samples in ultrapure water (1:10), filtration through a 0.22 µm syringe filter and spiking with standard solutions. Illicit medications Homogenisation of pills, dissolution in ultrapure water, sonication for 30 min and filtration with a 0.22 µm syringe filter.	LC-qTOF-MS/MS	Sildenafil LOD: 2.19 ng g ⁻¹ LOQ: 7.28 ng g ⁻¹ Tadalafil LOD: 9.81 ng g ⁻¹ LOQ: 32.71 ng g ⁻¹ Vardenafil LOD: 2.28 ng g ⁻¹ LOQ: 7.59 ng g ⁻¹ Avanafil LOD: 1.63 ng g ⁻¹ LOQ: 5.43 ng g ⁻¹	[104]
Sildenafil	Wastewater samples	Solid-phase extraction (SPE)	SPE-LC-MS/MS	Not reported	[106]
Sildenafil & N-desmethyl sildenafil	Surface water and drinking water	Solid-phase extraction (SPE)	UHPLC-MS/MS	Sildenafil <i>Surface water</i> LOD: 1.0 pg L ⁻¹ LOQ: 0.9 ng L ⁻¹ <i>Drinking water</i> LOD: 1.0 pg L ⁻¹ LOQ: 0.8 ng L ⁻¹ N-desmethyl sildenafil <i>Surface water</i> LOD: 1.0 pg L ⁻¹ LOQ: 1.4 ng L ⁻¹ <i>Drinking water</i> LOD: 1.0 pg L ⁻¹ LOQ: 1.3 ng L ⁻¹	[107]
Homosildenafil, Hydroxyhomosildenafil, Norneosildenafil, Thiosildenafil	Artificial freshwater & wastewater samples	Irradiation of tests solution in artificial freshwater with an initial concentration of 10 mg L ⁻¹ and 1 µg L ⁻¹ Wastewater samples Liquid-liquid extraction	UHPL-ESI-MS/MS	Not reported	[108]

Table 9. Cont.

Investigated Compound	Sample Matrix	Sample Preparation	Detection Method	LOD/LOQ	Reference
Sildenafil and Vardenafil	Phosphate buffer (pH = 7.4)	Irradiation of tests solution in 5 mM phosphate buffer diluted with distilled water, with an initial concentration of 10 mg L ⁻¹	LC-ESI-MS/MS	Not reported	[109]
Sildenafil, Tadalafil, Vardenafil and their metabolites	Spiked wastewater samples	Homogenisation and filtration through 0.2 µm cellulose filter	HPLC-(QqQ)-MS/MS	Sildenafil LOD: 1.8 ng L ⁻¹ LOQ: 6 ng L ⁻¹ Tadalafil LOD: 2.3 ng L ⁻¹ LOQ: 7.5 ng L ⁻¹ Vardenafil LOD: 7.2 ng L ⁻¹ LOQ: 24 ng L ⁻¹	[110]

9. Conclusions and Future Perspectives

The potential effects of PDE-5 inhibitors on the environment are not still apparent. However, ED medicines will prove to have very high usage rates in the near future, mainly because they are very diffuse and popular drugs. The availability of EDs without prescription over the Internet and their use for ludic activity can yield an increased potential for environmental exposure and conceivable non-target effects.

Risk is connected principally to the accumulation, persistence and toxicity of pharmaceutical compounds diffused in the environment rather than the actual quantity released. Given their bioactive nature, even small amounts of this kind of drug could impact delicate organisms; in addition, synergic action with other contaminants might have additive effects once released into the environment, especially if they insist on the same receptors of biological organisms.

Since these substances are rapidly becoming one of the most widespread and extensively used drugs at the world level, improper disposal in the environment is a reason of general concern.

More detailed knowledge of biotic and abiotic processes, able to interfere with these pollutants, will notably improve the used remediation strategies.

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