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Case Report

Efficient photooxidation processes for the removal of sildenafil from aqueous environments: A comparative study

Angelica R. Zizzamia [a], Carmen Tesoro [a], Giuliana Bianco [a,d], Sabino A. Bufo [a,b], Rosanna Ciriello [a,d], Monica Brienza [a,d], Laura Scrano [c,d], Filomena Lelario [a,d,*]

[a] Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, Potenza, 85100, Italy
[b] Department of Geography, Environmental Management and Energy Studies, University of Johannesburg, Johannesburg, 2092, South Africa
[c] Department of European Cultures, University of Basilicata, Matera, 75100, Italy
[d] Spin-off MITIGAM S.R.L.S., University of Basilicata, Potenza, 85100, Italy

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ABSTRACT

The erectile dysfunction drug sildenafil has attracted a great deal of attention in recent years due to its widespread legal and illegal use around the world and its increasing use by young people for recreational rather than medical purposes. Due to sildenafil's high stability in various environmental conditions and its accumulation or phototransformation in receiving waters, this dangerous trend poses a significant risk to both human health and the environment. Therefore, in-depth studies are needed to find innovative methods for completely removing sildenafil from the aquatic environment while limiting the formation of more toxic derivatives. This study investigated the efficacy of photooxidation processes for removing sildenafil and its potentially toxic derivatives from water. Distilled water and synthetic wastewater were treated with three different oxidants: peroxymonosulfate (PMS), persulfate (PS) hydrogen peroxide (H_2O_2), and a heterogeneous catalyst, TiO_2. The investigation also considered the formation of potentially toxic phototransformation products, performing a tentative structural identification by LC-ESI-MS and MS^n. The results proved that the Sunlight/PMS system is the most effective for entirely and environmentally friendly removal of this drug and its transformation products from aqueous environments, achieving complete degradation in distilled water and synthetic wastewater after 80 and 130 minutes of irradiation, respectively. Toxicity testing with *Vibrio fisheri* confirmed the non-toxic nature of the phototransformed products. This study highlights the potential of Sunlight/PMS photooxidation as a promising strategy for mitigating the environmental risks associated with sildenafil contamination.

1. Introduction

The Fourth International Consultation on Sexual Medicine defined Erectile dysfunction (ED) as "the constant or recurrent inability to achieve and maintain a penile erection sufficient for sexual satisfaction" [1]. Phosphodiesterase 5 inhibitors are one of the solutions to ED. The Food and Drug Administration (FDA) of USD indicates the four most used and approved inhibitors: sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra®), and avanafil (Spedra®) [2]. Sildenafil (structure in Fig. 1) has the molecular formula $C_{22}H_{30}N_6O_4S$, a molecular mass of 474.6 g/mol, a poor aqueous solubility (5 - 10 mg L^{-1}) [3] and is available in the market as sildenafil citrate ($C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$).

In recent years, sildenafil has received particular attention from the scientific community due to the high number of sales and the consequent presence in the environment, particularly in wastewater [4,5]. According to a study carried out in 2023 [6], sales of sildenafil are incredibly high, even among young people aged 20 - 30 years, who you cannot believe are facing real impotence problems. Perhaps people perceive sildenafil and similar drugs as substances for fun purposes rather than under medical prescription to treat an actual pathology. As a result, leaching of these pharmaceuticals into the aquatic environment is expected [7]. These so-called "emerging contaminants" reach the wastewater purification plants in a metabolised or non-metabolised form, but many of them escape conventional activated sludge treatments and can reach the streams of surface water and spread into the environment. Wastewater treatment plants cannot remove all kinds of organic contaminants [8]; consequently, their molecules are conveyed into all water compartments, accumulating in a concentration range between ng/L

* Corresponding author. Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, Potenza, 85100, Italy.
E-mail address: filomena.lelario@unibas.it (F. Lelario).

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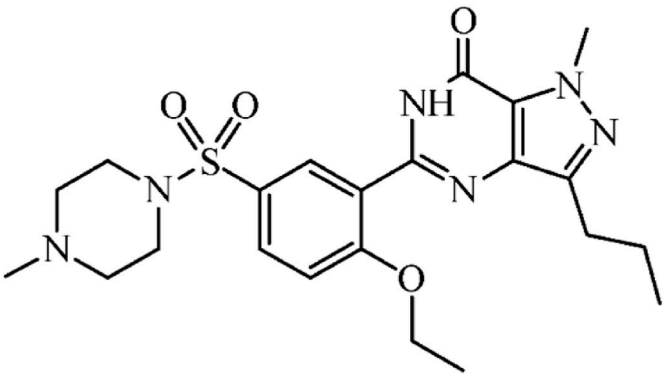


Fig. 1. Structure of sildenafil.

and μg/L. Nieto et al. (2010) reported the occurrence of this drug in sewage treatment plant influents, effluents and sludge in Spain at ng/L concentrations [9]. In Germany, Schroeder et al. (2010) reported its concentration in municipal wastewater around 35 ng/L [10]. Causanilles et al. found a threefold increase in sildenafil concentrations in urban wastewater in Korea between 2012 and 2015, possibly because sildenafil has lost patent protection and Korean pharmaceutical companies have produced 64 generic versions of sildenafil [11]. Some studies have shown the potentially toxic effects of sildenafil and its metabolites on aquatic organisms [12]. These studies raised concerns about the effect of these compounds on the aquatic ecosystem, especially due to unknown loads from their non-prescribed consumption. The predicted no-effect concentration of sildenafil was estimated to be 640 ng/L, but additional studies are required to fully understand their impact on aquatic ecosystems [13]. Besides, in the environment, these molecules undergo various physical and chemical processes, classified into abiotic (photolysis, hydrolysis) and biotic (biodegradation) reactions, leading to the formation of new transformation products, often more toxic than the starting ones, which may persist in the environment or be further degraded. More needs to be known about the emerging contaminant sildenafil, its metabolites and its potential environmental health effects. In recent decades, awareness that sewage treatment implants cannot effectively remove all pollutants has increased [14]. For this reason, numerous studies have been conducted to identify additional technologies that may remove recalcitrant compounds altogether [15]. Among these innovative processes, advanced oxidation processes (AOPs) represent an alternative and promising treatment of wastewater containing recalcitrant organic compounds [16]. These processes are often essential contributors to the removal of pollutants and exogenous substances from the environment because they generate *in situ* strong oxidising radical species such as hydroxyl radicals ($OH^{\bullet}$), which interact with the molecules of the organic pollutants, leading to the progressive degradation of the contaminants [7]. In fact, there are several previous studies of photochemical degradation of sildenafil: the study performed by Eichhorn et al. (2012) describes the photolysis of sildenafil and its human metabolite N-desmethyl sildenafil under simulated solar radiation and has been proposed intermediates and transformation products [17]. These results, in addition, were confirmed also by Herbert et al. (2015), where sildenafil and vardenafil were degraded under the action of simulated natural sunlight [18]. Medana et al. (2011) utilized a photocatalytic process (TiO_2+UV radiation) to generate chemically modified products, which were assumed to be similar to those formed by the metabolic system of horses [19]. The authors identified the main reaction intermediates and products generated after 10 min of reaction and proposed a degradation route. The results of this study are very attractive for our research since the photocatalytic degradation of sildenafil involves the production of hydroxyl radicals, which are also involved in the solar photodegradation using TiO_2 and hydrogen peroxide. Among the technologies AOPs, less investigated for this molecule are the sulphate radical technologies driven by the activation of peroxymonosulfate (PMS) and persulfate (PS).

Nevertheless, evaluating advanced treatments with appropriate toxicity tests is essential for a global assessment of water quality after purification [20]. Consequently, a series of acute and chronic toxicity bioassays for aquatic organisms has been developed to establish compounds' toxicity levels [21]. From an analytical point of view, using a detection technique that can separate and quantify the various degradation products is crucial. For this purpose, the most suitable is high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) [9,18].

This study evaluates the effectiveness of different AOPs for removing sildenafil from aqueous solutions. The investigation concerned the solar phototransformation of sildenafil in distilled water and synthetic wastewater (SWW) in the presence of the oxidants PMS, PS and H_2O_2 and the presence of the catalyst TiO_2, giving particular attention to potentially toxic phototransformation products. For detecting and identifying sildenafil and its photoproducts, LC–ESI–MS and MS^n were the analytical techniques of choice; besides, the *Vibrio fischeri* test was helpful for the ecotoxicity estimation of transformation products.

2. Materials and methods

2.1. Chemicals

The standard sildenafil (Certified Reference Material), potassium peroxymonosulfate (PMS, available as the triple potassium salt $KHSO_5{\cdot}0.5\ KHSO_4{\cdot}0.5\ K_2SO_4$ under the trade name of Oxone®), sodium persulfate (PS, reagent grade ≥ 98%), hydrogen peroxide (H_2O_2, 30%) were purchased from Sigma Aldrich (St. Louis, USA). All chemicals were used as received without further purification. In the case of heterogeneous photocatalysis, TiO_2 (Degussa P-25) was obtained as a gift from Evonik (Hanau, Germany). All the solutions aqueous samples were prepared with a Milli-Q grade ultrapure water system produced using a Milli-Q RG system from Millipore (Bedford, MA, USA). The exact composition of synthetic wastewater (SWW) is as follows: $NaHCO_3$ (96 mg L^{-1}), NaCl (7 mg L^{-1}), $CaSO_4{\cdot}2H_2O$ (60 mg L^{-1}), urea (6 mg L^{-1}), $MgSO_4$ (60 mg L^{-1}), KCl (4 mg L^{-1}), K_2HPO_4 (0.28 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (4 mg L^{-1}), peptone (32 mg L^{-1}), and $MgSO_4{\cdot}7H_2O$ (2 mg L^{-1}) [22].

2.2. Experimental devices for oxidation processes

The photocatalytic experiments were carried out using a cylindrical reactor (250 mL) covered with a quartz plate and placed in a solar simulator (Heraeus-Atlas Suntest CPS+, Chicago, USA) equipped with a xenon arc lamp (1.8 KW) as an irradiation source, with a light output of 400 Wm^{-2} and a spectral wavelength range of 290–800 nm. The temperature was kept constant (26 ± 0.1 °C) by an air conditioning system and the solutions were kept under continuous stirring to ensure optimal mixing. Thanks to a quartz filter that protected the lamp, and taking into account the borosilicate filtration effect, the wavelength spectrum applied was between 300 and 580 nm. All the experiments were performed at the initial concentration of 3 mg L^{-1} for kinetics study and 10 mg L^{-1} for the identification of photoproducts of sildenafil in distilled water and in synthetic wastewater at pH 8 and in buffer solutions at pH 7.3 and pH 5, to evaluate the influence of pH on the degradation. Potassium peroxymonosulfate (PMS) 100 mM stock solution was prepared by dissolving about 1.5 g of PMS in 50 mL of distilled water. Potassium persulfate 100 mM stock solution was prepared by dissolving about 1.2 g of PS in 50 mL of distilled water, and finally, the hydrogen peroxide solutions were prepared by diluting a 0.98 M stock solution. For each medium, the oxidation efficiency was evaluated by part of H_2O_2, PMS, and PS at 400, 800, and 1600 μM and by part of TiO_2 at the concentration of 0.2 g L^{-1}. SWW tests were conducted using PS, PMS and TiO_2 at the same concentrations. To remove the TiO_2 powder from the samples collected during the photocatalytic process, the solution to be analysed

was filtered through a 0.2 mm PTFE filter.

2.3. Analytical procedures

The degradation curves of sildenafil were obtained by using a high-performance liquid chromatography (HPLC) system (Agilent Technologies 1200 series, USA) equipped with a Luna C_{18} column (150 mm x 4.6 mm, 5 μm) and a diode array detector (DAD), set at λ=254 nm. The optimal eluent phase used consists of a two-phase gradient, ultrapure water with 0.1% formic acid (solvent A) and acetonitrile (solvent B): 0-3 minutes, 20% B; 3-7 minutes from 20% to 60% B; 7-13 minutes, 60% B; 13–15 minutes, 60%–100% B; 15-20 minutes, 100% B; 20-22 minutes, 100%–20% B, 22-25 minutes, 20% B. Flow was 0.800 mL/min and injection volume was 20 μL. Mass spectrometric data were acquired in the positive ion mode scanning *m/z* 90–1200 using an LC system coupled with an LTQ-mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Separation was carried out on the same chromatographic column Luna C_{18}, working in gradient elution at a flow rate of 0.8 mL/min by splitting 3:1 post column.

Low-resolution MS^n experiments were performed by collisional induced dissociation (CID). Mass spectra were imported, elaborated, and plotted by SigmaPlot 10.0 (Systat Software, Inc., London, UK). Structures of recognised molecules and moieties were drawn by ChemDraw Ultra 12.0 (CambridgeSoft Corporation, Cambridge, MA). The calibration curve of sildenafil used for the quantitative analysis of the unknown samples was obtained by injecting into the chromatograph standard solutions at different concentrations in the 0.25–10 mg L^{-1} range and fitting the analytical results. The LOD, the most minor concentration of sildenafil that can be detected, equal to 0.1 mg L^{-1}, and the LOQ, the most minor concentration of sildenafil that can be quantified, equal to 0.3 mg/L, were calculated.

2.4. Toxicity tests

Toxicity tests on *V. fisheri* were carried out by placing the bacteria and the samples in contact with the wells of a microplate and evaluating the results after 5, 10 and 30 minutes. Three samples were tested: non-irradiated, a mixture containing sildenafil and the maximum number of photoproducts formed during the processes studied, and a third photo-degraded sample containing neither the sildenafil nor the photoproducts.

3. Results and discussion

3.1. Degradation tests

Degradation tests in the presence of Sunlight or Sunlight/TiO_2, Sunlight/H_2O_2, Sunlight/PMS and Sunlight/PS were carried out to determine the potential of advanced oxidation processes (AOPs) for removing sildenafil and its transformation products from aqueous solutions. Tests were also carried out in the dark under the same experimental conditions to assess the possible presence of hydrolysis phenomena. All experiments were carried out in triplicate, the solution was irradiated for sufficient time for both sildenafil and its photoproducts to disappear. A comparison of the degradation curves in Fig. 2 shows that photolysis is faster in distilled water than in buffer solution. In distilled water, the concentration of sildenafil decreases quite rapidly with time, halving after approximately 90 minutes, but is no longer quantifiable after 240 minutes. In contrast, in phosphate buffer (pH 7.3) and acetate buffer (pH 5), a half-life of about 350 minutes and an 80% degradation time of more than 420 minutes are observed. In SWW, no significant sildenafil degradation is evident after 6 hours of irradiation. The greatest recalcitrance to degradation in SWW could be due to the presence of ionic species, which would have inhibited the photochemical reaction, reducing the absorption of radiation by sildenafil [18].

In the case of heterogeneous photocatalysis in the presence of TO_2, a

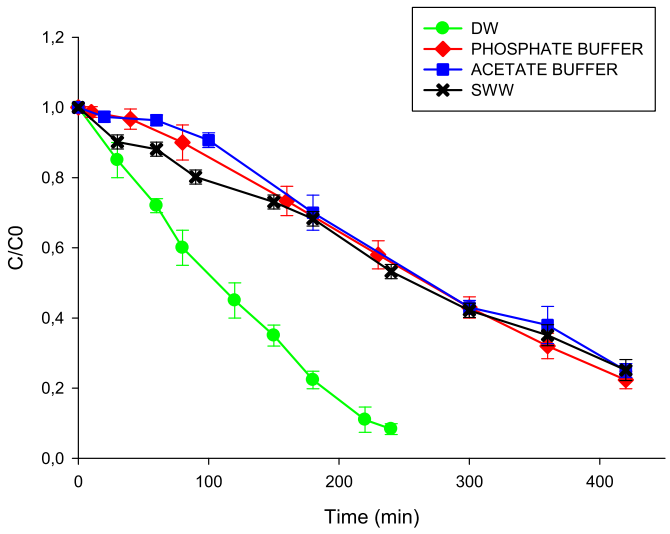


Fig. 2. Degradation curves of sildenafil subjected to photolysis in solutions with different pH: distilled water (DW, pH 6), phosphate buffer (pH 7.3) and acetate buffer (pH 5) and simulated wastewater (SWW, pH ±8); C = concentration at time t, Co = initial concentration (3 mg L^{-1}).

first experiment was carried out by stirring the TiO_2 suspension in the dark for more than 24 hours. Under these conditions, the concentration of sildenafil did not change significantly, ruling out any hydrolysis reaction or immobilisation of the organic molecules on the TiO_2 surface. A 3 mg L^{-1} solution of sildenafil was then irradiated in the presence of a 0.2 gL^{-1} suspension of TiO_2 under continuous magnetic stirring. The photocatalytic reaction rapidly degraded the organic molecules with a half-life of about 50 min, which agrees with the literature data [19]. The initial concentration of sildenafil was reduced below the LOD in approximately 120 min, as shown in Fig. 3. After the same time, the degradation in SWW was about 80%. The parabolic trend of the photocatalysis curves is typical of an initial adsorption phase that does not strongly immobilise the organic molecules, allowing a rapid photoreaction.

As a valid alternative to the use of TiO_2, in terms of effectiveness, low cost, and eco-compatibility, in recent years, the use of processes based on photodegradation in the presence of oxidants such as H_2O_2, PS, and PMS has caught on. Unlike heterogeneous photocatalysis, this process does not require an additional catalyst recovery phase. Despite the differences between the methods, even significant ones, the common

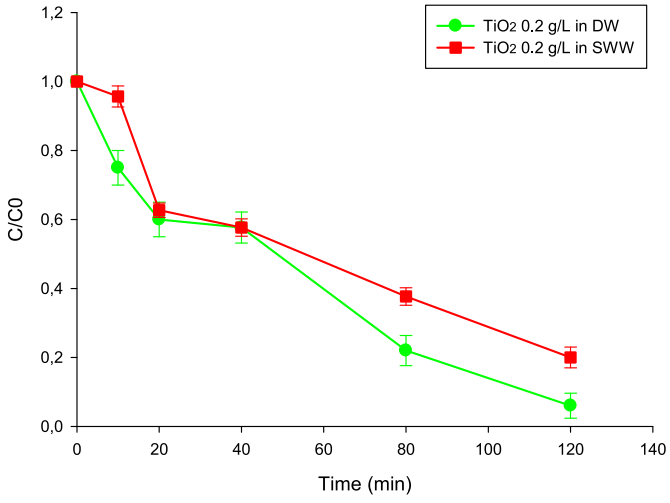


Fig. 3. Photodegradation curves of sildenafil in distilled water (DW) and SWW in the presence of the catalyst TiO_2 (data shown based on three replicates).

element consists of using and *in situ* generating reactive species (radicals) capable of degrading oxidisable organic pollutants, including many emerging pollutants. It is essential to underline the influence of the initial concentration of pollutants and the amount of oxidant used because they affect the number of reactive species formed. For this reason, in this work, experiments were conducted keeping the sildenafil concentration constant (3 mgL^{-1}) and varying the concentrations of the three oxidants tested in the 100–1600 μM range. Maximum effectiveness of all processes was observed using an oxidant concentration equal to 800 μM, but already 100 μM was sufficient to ensure a partial degradation of sildenafil and its photoproducts. Fig. 4 shows the degradation curves of sildenafil relating to the photodegradation processes carried out in 800 μM solutions of H_2O_2, PS, PMS, and PS+PMS (400 μM each).

At the oxidant concentration of 800 μM, PMS is more effective for sildenafil degradation. The half-life is less than 50 minutes, and more than 90% degradation occurs after about 80 minutes. The single PS 800 μM use is comparable to the PS + PMS mix. Fig. 5 shows the chromatograms of some of the samples taken during the photolysis test in distilled water (a), in the presence of 800 μM PMS (b) and darkness conditions (c). Under these experimental conditions, the peak of sildenafil and that of its main transformation product (eluted immediately after sildenafil), the N-oxide sildenafil, rapidly decreased from the sample taken at 60 min to those taken at 90 and 120 min. During the Sunlight/PMS experiment, oxidation and photooxidation reactions coexist, and the removal efficiency observed under these conditions is due to the sum of both phenomena. The best system was Sunlight/PMS 800 μM. Heterogeneous catalysis via TiO_2 gave a slightly lower degradation efficiency, in addition to the fact that this catalyst requires a filtration step to remove it. In the corresponding dark test (Fig. 5c) with PMS 800 mM, there was a quantitative conversion of sildenafil to the corresponding N-oxide, an inactive form of sildenafil and one of its best-known metabolites and photoproduct [17]. No appreciable degradation of sildenafil was observed in the PS and H_2O_2 dark tests.

Further photodegradation reactions were monitored for sildenafil solutions prepared in synthetic wastewater until the drug was utterly degraded. Among the oxidising agents tested, PMS 800 μM proved far more efficient than PS 800 μM.Fig. 6 shows that in the presence of 800 μM PMS the sildenafil concentration is no longer detectable after 130 minutes, whereas with 800 μM PS the drug is completely degraded after 430 minutes. Sildenafil was only about 70% degraded in H_2O_2 after the same time. These last experiments are probably affected by the wastewater composition; nevertheless, they confirm the results obtained with

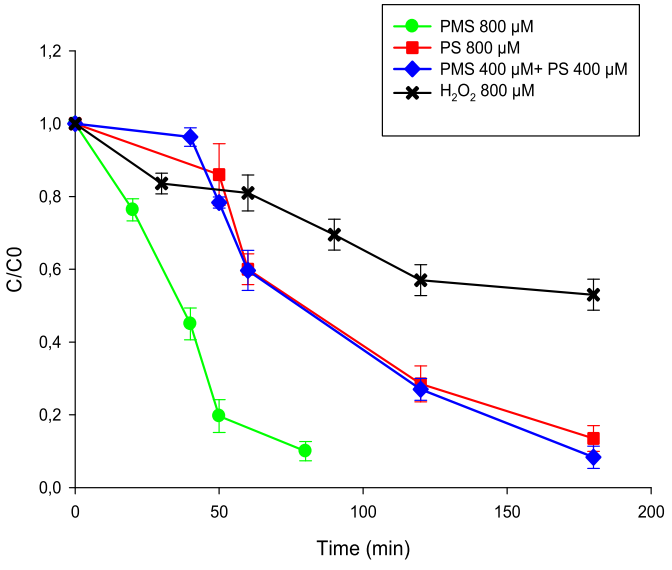


Fig. 4. Sildenafil degradation curves under Sunlight/PMS, Sunlight/PS, Sunlight/(PMS + PS), and Sunlight/H_2O_2 systems.

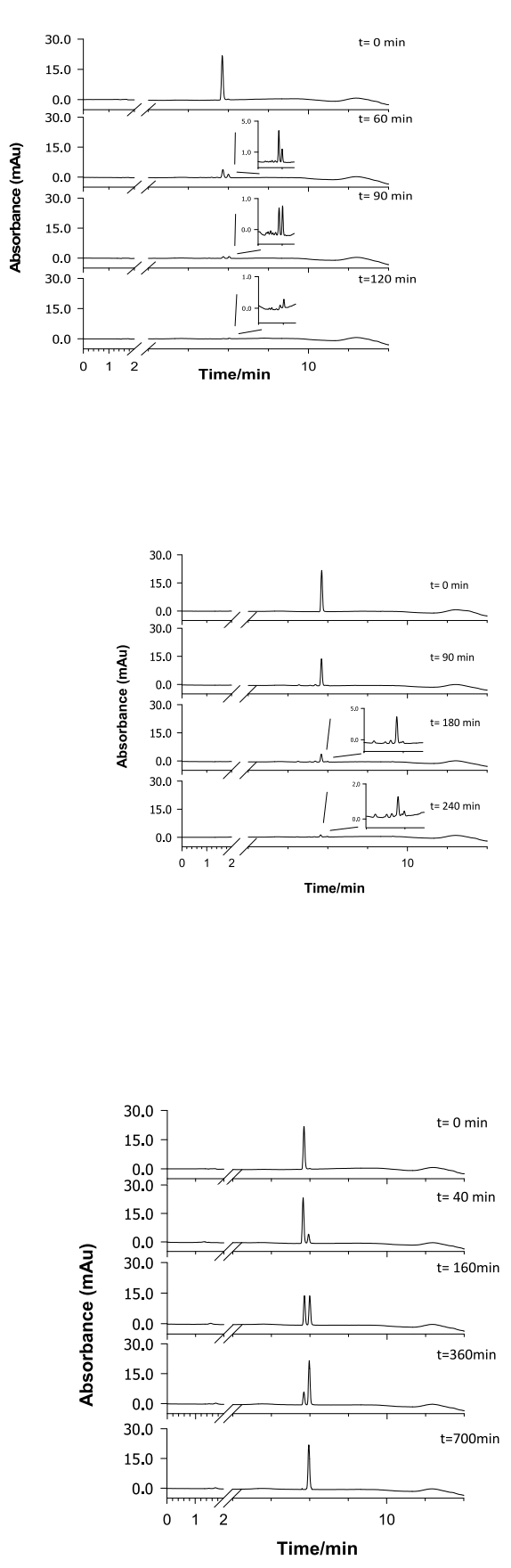


Fig. 5. Chromatograms of a 3 mg/L sildenafil solution at different times of degradation by Sunlight (a), Sunlight/PMS 800 μM (b) and PMS 800 μM (c).

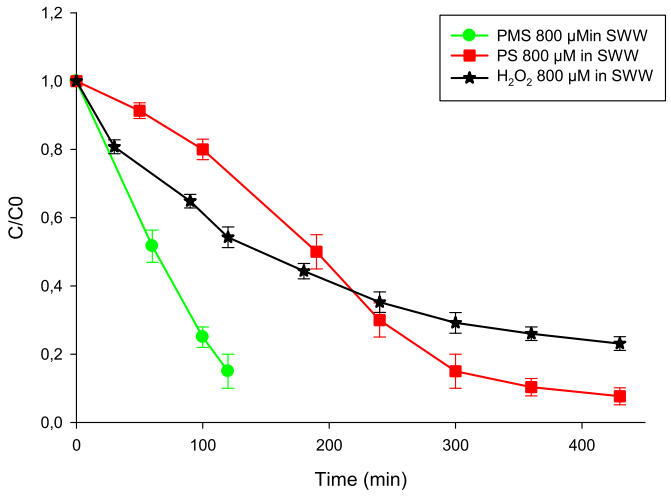


Fig. 6. Sildenafil degradation curves in SWW subjected to photodegradation in the presence of 800µM PMS, 800µM PS and 800µM H_2O_2.

the tests in distilled water and validate the efficiency of PMS as the best oxidant among the three under investigation.

3.2. Characterisation of sildenafil and its photoproducts by LC-ESI-LTQ-MS and CID-MS^n

Retention time (t_r) and UV spectrum are not sufficient parameters for the correct identification of the constituents of complex systems, especially in the case of unknown compounds. For this reason, a part of this work concerned the LC-ESI-MS study in a linear ion trap (LTQ) and in full-scan mode with positive ionisation of sildenafil and its photoproducts formed during the photolysis and photocatalysis processes. MS^n experiments were also performed to analyse their fragmentation patterns and obtain more information about their structure. The MS^n spectra of the $[M+H]^+$ ion of sildenafil (*m/z* 475, $C_{22}H_{30}N_6O_4S$), obtained by applying collisional energies equal to 20–35% of the maximum possible value, were shown in Fig. 7. Fig. 7*a* depicts that the fragmentation of the sildenafil molecular ion led to the formation of ten intense fragment ions at *m/z* values of 475, 447, 391, 377, 329, 313, 311, 299, 283, and 163. The most intense peak occurs at *m/z* 377 $[C_{17}H_{20}N_4O_4S + H]^+$, which can be attributed to the neutral loss of 98 Da (group methylpiperazine, $C_5H_{10}N_2$) due to the easy cleavage of the weak S-N bond. The nature of this fragment was confirmed by MS^3 of the ion at *m/z* 377 and MS^4 of the ion at *m/z* 331 (Fig. 7*b* and *d*), which generated the characteristic fragments shown in Figs. 7 and 8.

The MS^3 spectrum of the ion at *m/z* 311 (Fig. 7*c*) and the spectrum MS^4 of the ion at *m/z* 283 (Fig. 7*e*) reveals that the first byproduct (*m/z* 311) is attributable to the cleavage of the C–S bond and the second one (*m/z* 283) comes from the loss of the ethyl group on the ethoxy substituent of the phenyl ring. The hypothesised structures are in agreement with what was reported by Eichhorn et al. [17], who highlighted these two ions as devoid of the piperazine and sulfonamide groups, as well as the ion at *m/z* 313, also originating from the breaking of the C-S bond. The three fragments at *m/z* 311, 299, and 283 are a common feature in the mass spectra of most of the photoproducts observed in this study and can be used as fingerprints to differentiate the structural changes induced by light in the phenyl pyrazolopyrimidinone group from those occurring in the piperazine ring. The complementary ion of the fragment at *m/z* 311 is the signal at *m/z* 163, obtained after the cleavage of the bond between the aromatic and the sulfonamide moieties. The

a)

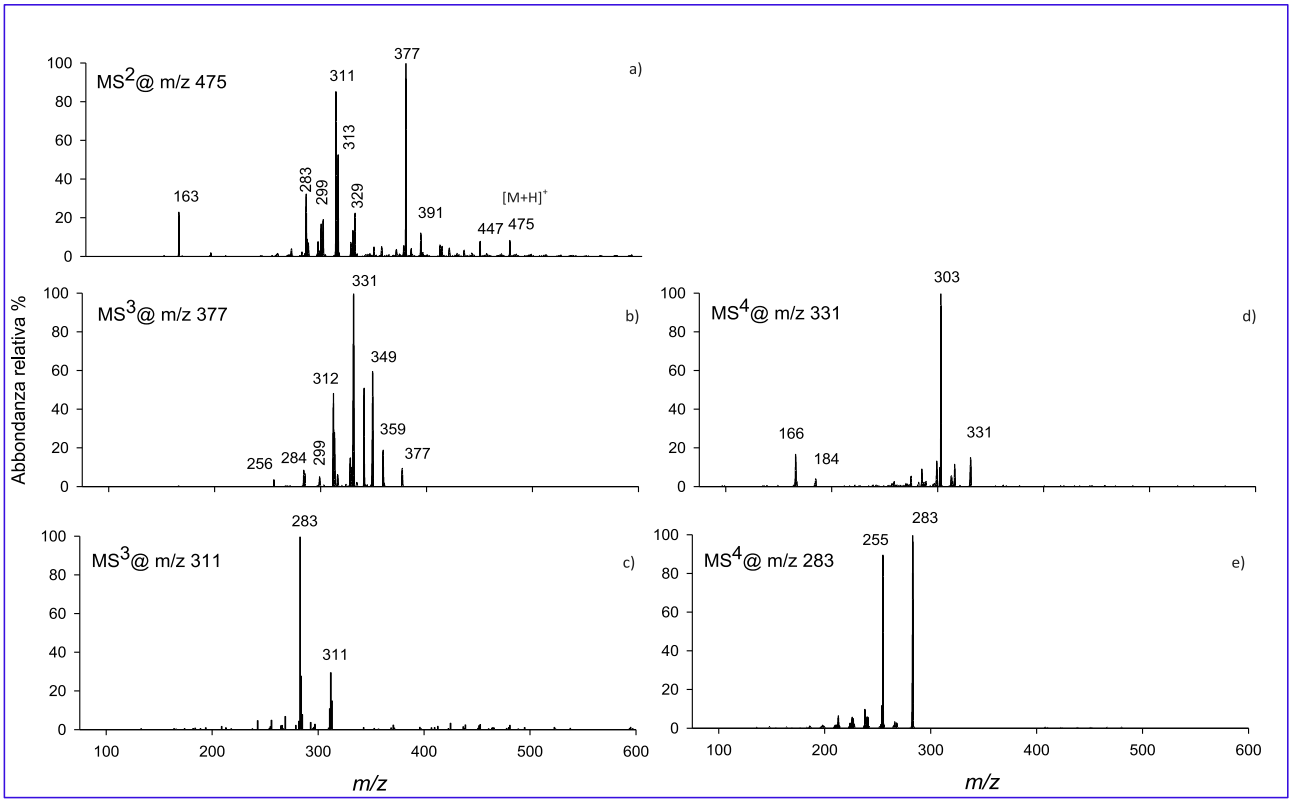


Fig. 7. CID-MS^n spectra (*a-e*) in positive ion mode of sildenafil, $[M+H]^+$ at *m/z* 475, generated by applying relative collisional energies between 20% and 35% (a) and proposed fragment structures (b).

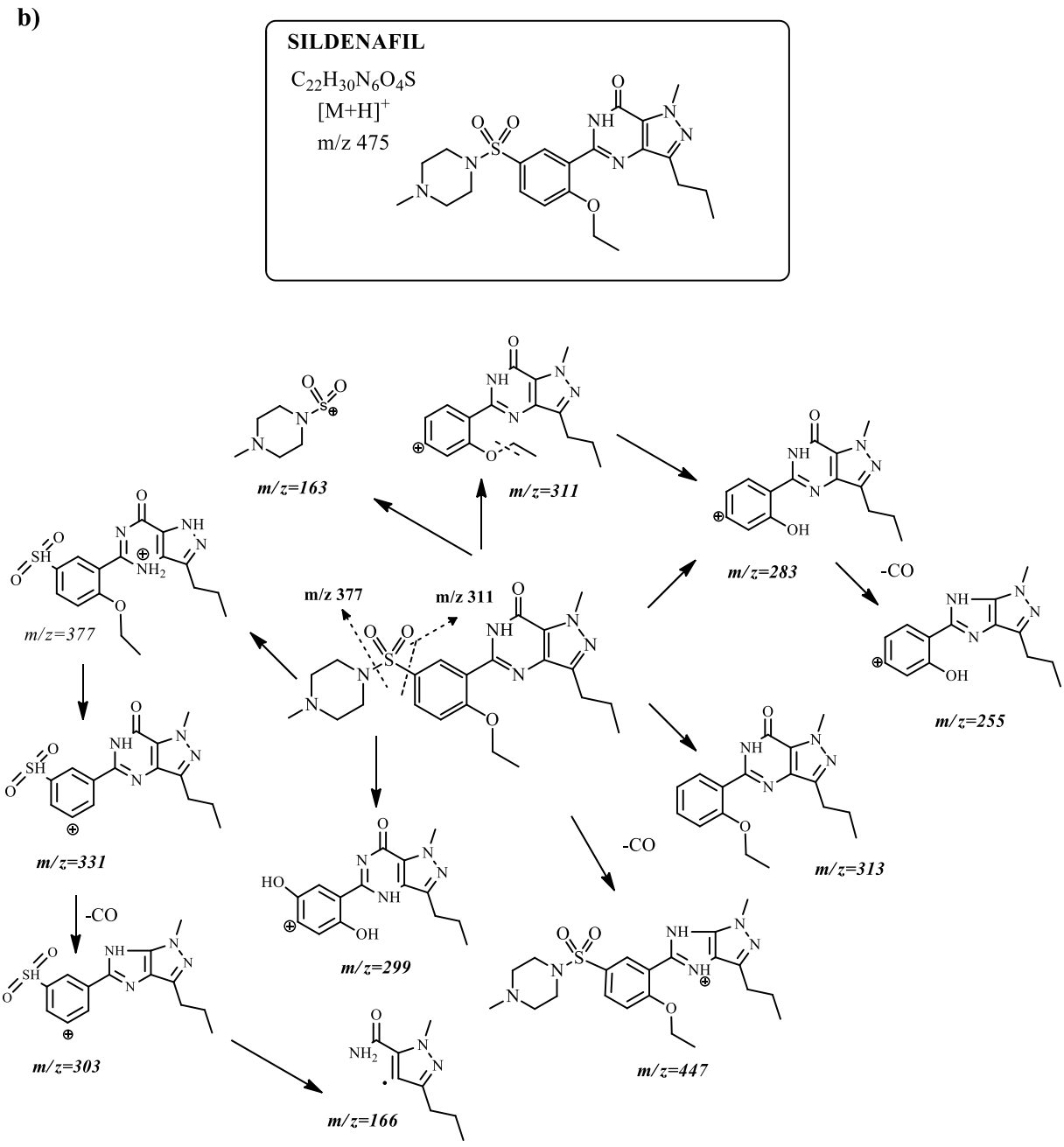


Fig. 7. (*continued*).

rationalisation of the fragment at *m/z* 299 ($C_{15}H_{15}N_4O_3$) is less immediate but could be attributed to the breaking of the C–O bond in the ethoxy group and to the rearrangement of the sulfonamide group involving the displacement of an oxygen atom to the aromatic ring and the transfer of hydrogen to the outgoing group. A secondary pathway of *m/z* 475 ion fragmentation is characterised by the neutral loss of 28 Da (CO) with the less intense fragment formation at *m/z* 447.

Table 1 shows all proposed structures of photoproducts detected in AOPs experiments under investigation and the corresponding product ions. Two isobaric compounds eluting after sildenafil have been identified as having an *m/z* ratio of 491 (compounds 2 and 3, eluting at 8.1 min and 10.4 min, respectively); the difference of 16 Da from the *m/z* ratio of 475 of sildenafil is compatible with the formation of two isomeric hydroxy derivatives. This hypothesis is confirmed by analysing fragments identified in the MS^n spectra. The formation of some characteristic fragments allows us to determine the position of the OH group in the molecules (Fig. 8).

Based on the fragmentation paths and the data reported in the literature [17], the hypothesised structures are shown in Fig. 8 for more informative fragments identified through their MS^n spectra. According to literature data, some of the products identified in the sildenafil photodegradation investigations are also products of human metabolism [23] and *in vivo* studies in mice and horses [19]. Among these, N-desmethyl sildenafil (Table 1, compound 4) is a compound that originates from sildenafil with the loss of the methyl group bonded to the N of the piperazine ring. MS^2 fragments in positive mode (Table 1) of N-desmethyl sildenafil, $[M+H]^+$ at *m/z* 461, eluting at a retention time of 7.7 minutes, are very similar to those of sildenafil, thus confirming the structure proposed for this compound [24]. The most relevant ions are the fragments obtained from breaking the bond on the piperazine fraction and the C-S bond. The presence of the characteristic peaks at *m/z* 377, 311, 299, and 283 confirms that the structural modification in this

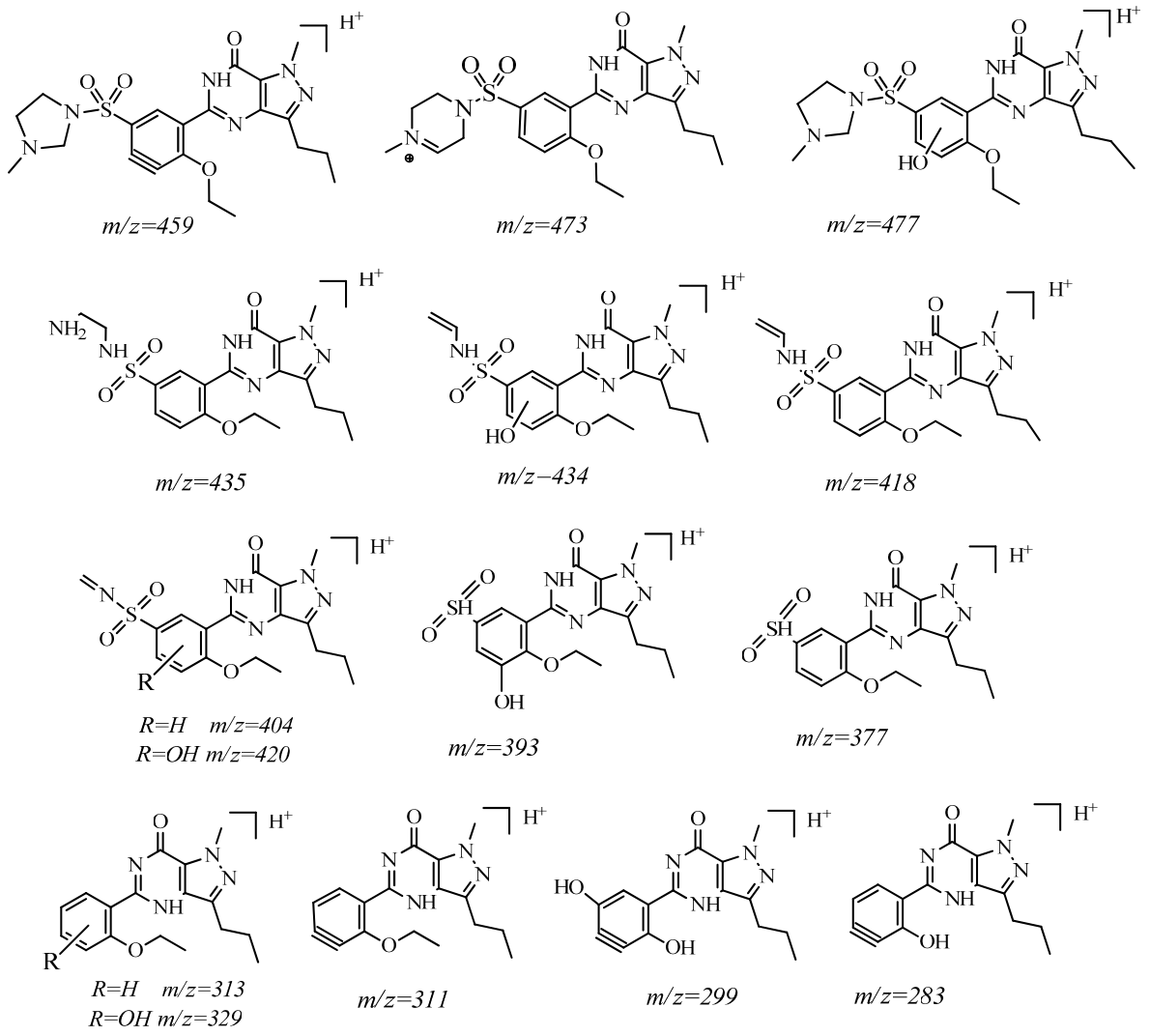


Fig. 8. Proposed structures for more informative fragments of photoproducts identified in this study, based on ESI-CID-MS^n spectra.

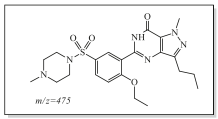
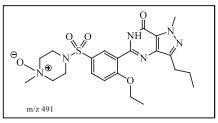
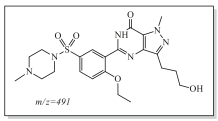
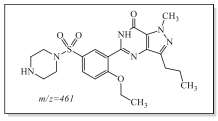
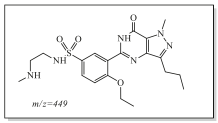
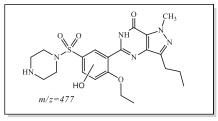
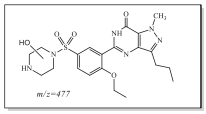
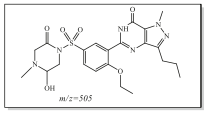
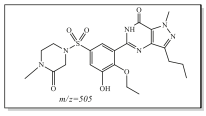
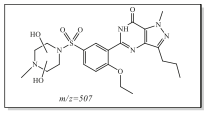
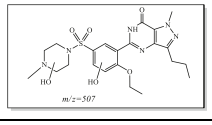
compound with sunlight irradiation concerns the piperazine ring.

Another interesting byproduct of sildenafil is the compound that elutes at the retention time of 7.1 minutes, whose molecular ion shows an *m/z* ratio of 449, analogous in structure (Table 1, compound 5) to the major metabolite identified in pharmacokinetic studies on humans and also recognised in raw and treated wastewater [19]. Compared with sildenafil, its molecular mass is reduced by 26 Da, corresponding to the elimination of a C_2H_2 group, therefore compatible with the formation of dealkylsildenafil. The MS^2 spectrum shows two fairly intense characteristic ions at *m/z* 311 and 313, indicative of a structure in which the piperazine ring is modified, while the two most intense peaks, the base peak at *m/z* 418 and the peak at *m/z* 392, can be traced back to a breaking of the piperazine ring due to subsequent neutral loss of NH_2CH_3 (31 Da) and C_2H_2 (26 Da). The hypothesised structure is confirmed by the MS^3 (418) spectrum, which shows the formation of the three intense ions characteristic at *m/z* 377, 361, and 311.

Table 1 shows the fragments produced by two other isobaric photoproducts, at *m/z* 477, eluting at t_r=7.4 minutes and t_r =10 minutes (compounds 6 and 7, respectively), whose tentative structures are given in the same table. The MS^2 fragments of the compound eluting at 7.4 minutes show that the structural change induced by the irradiation of the sample does not occur on the piperazine ring. The key fragment is the one at *m/z* 434 (Fig. 8), which is not present in the spectrum of the other compound at *m/z* 477, which elutes at 10 min, where instead the fragment appears at *m/z* 418. Both are due to the cleavage of the piperazine ring due to the loss of a neutral fragment of 31 Da (NH_2CH_3) but differ by 16 Da due to the presence of an additional OH group in the fragment produced by the compound eluting at 7.4 minutes.

The two photoproducts that show a protonate ion at *m/z* 505, compatible with the molecular formula $C_{22}H_{29}N_6O_6S$, and a mass difference compared to sildenafil of 30 Da, are attributable to the combination of a double hydroxylation and an oxidation process, with the formation of a ketone group. Table 1 (compounds 8 and 9) shows the two hypothesised structures. Data from the MS^2 (505) and MS^3 (418) spectra of compound 8 show the formation of characteristic ions of sildenafil at *m/z* 283, 311, 377 and 418 by the loss of the piperazine moiety, suggesting both hydroxylation and oxidation involving the piperazine ring. Instead, the base peak at *m/z* 487 is due to the loss of an H_2O molecule from the same ring. Differently, the MS^2 (505) and MS^3 (459) spectra of compound 2 show the formation of more intense fragments at *m/z* 459, due to the simultaneous loss of a water molecule and a CO group, at *m/z* 393 due to the loss of the ketopiperazine ring, and at *m/z* 477, due to the loss of a CO molecule for the cleavage of the piperazine ring. Furthermore, the presence of the ion at *m/z* 416

Table 1
Main photoproducts detected and corresponding product ions [17].

Compound	Precursor ion $[M+H]^+$ (*m/z*)	Structure	Main MS/MS product ions (*m/z*)[a]
1	475		447; 377; 331: 329; 313; 311; 303; 299; 283; 255; 166; 163
2	491		447; 473; 404; 377; 313; 311; 283; 255
3	491		463; 445; 435; 420; 393; 377; 329; 311
4	461		377; 329; 313; 311; 299; 283
5	449		432; 418; 392; 377; 361; 313; 311; 285
6	477		459; 434; 395; 390; 377; 362; 311; 298
7	477		459; 449; 418; 392; 377; 311;
8	505		487; 477; 463; 418; 391; 377; 326; 311
9	505		487; 477; 459; 393; 311; 299; 283
10	507		489;461; 447;377; 349
11	507		479; 461;395

indicates the presence of a hydroxyl group on the aromatic ring.

Less intense but detectable in the mixture of photoproducts eluted before sildenafil, there are two isobaric compounds whose $[M+H]^+$ ions are at *m/z* 507, consistent with double sildenafil hydroxylation. The fragments and structures hypothesised for these two compounds, which elute at t_r=7.2 minutes and t_r =7.7 minutes and not reported in the literature, are shown in Table 1 (compounds 10 and 11). Compound 11 (tr = 7.2 min) shows a fragmentation pathway similar to that of sildenafil N-oxide, with the formation of a few fragments at *m/z* 479, 461 and 395, due respectively to the loss of one molecule of CO, two molecules of H_2O, and S-N bond cleavage with loss of the hydroxylated piperazine ring and formation of a sulphonic acid (Fig. 8). This loss is similar to that observed for the photoproduct at *m/z* 505 in the formation of the fragment at *m/z* 393. The presence of these fragments is consistent with the presence of a hydroxyl group on the piperazine ring and one on the aromatic moiety. The low intensity of the chromatographic peak of this photoproduct did not allow the acquisition of the MS^3 spectrum. For the other isobaric compound at *m/z* 507 (compound 10), it was possible to obtain both the MS^2 and MS^3(489) spectra. The appearance of a base peak at *m/z* 377 strongly indicates the presence of the two hydroxyl groups on the piperazine ring. The simple subsequent loss of an H_2O molecule (-18 Da) and a CO molecule (-28 Da) with the formation of the fragment ions at fragment ions at *m/z* 489 and 461, respectively, analogous to the fragment at *m/z* 459 (with the double bond instead of the triple bond) and the contraction of the piperazine ring confirm this hypothesis. The presence of the peak at *m/z* 325 in the MS^3 spectrum (data not shown) is also consistent with this hypothesis, as it is due to a fragment similar to that characteristic of sildenafil at *m/z* 299 plus a C_2H_2 alkyl group (26 Da), probably due to the rearrangement of the sulphonamide group involving the transfer of an oxygen atom to the aromatic ring and hydrogen transfer to the leaving group.

All photoproducts in this study, except the two compounds at *m/z* 507, were found in previous investigations [17, 23-24]. The results of the photodegradation studies of sildenafil in the presence of the three oxidising agents provide clear evidence that the piperazine ring is highly susceptible to hydroxyl radical attack and is readily degraded by simulated sunlight irradiation. It should be emphasised that under the adopted experimental conditions, but also in the natural aquatic environment, sildenafil, by the action of sunlight, undergoes reactions that are in part similar to those in the human body. Several human metabolites derive from oxygenation and N-dealkylation reactions in the piperazine ring and show structures analogous to those hypothesised for some photoproducts. Based on these considerations, the studies conducted for removing sildenafil and its byproducts released into the environment through wastewater discharges [7] appear of considerable importance.

3.3. Toxicity tests

In this paper, the ecotoxicity in the aquatic compartment of sildenafil and its transformation products is determined using the Microtox® toxicity test. This test provides a rapid means of determining the acute toxicity of aqueous compounds by measurement of the decrease in light output from the luminescent bacterium *Vibrio fischeri*. Light emission is directly proportional to bacterial metabolic activity and inhibition of enzymatic activity results in a corresponding decrease in luminescence. Tests on *V. fischeri* is one of the most widely used ecotoxicological bioassays due to its high reproducibility and sensitivity to different compounds [25]. In the presence of pollutant agents, the natural bioluminescence of *V. fischeri* is reduced and the toxicity is expressed as EC_{50} counted according to the standard procedure ISO 11348-3 [26,27]. Table 2 shows the percentage inhibition results (I%) obtained after 5, 15, and 30 minutes of contact. The tests were carried out on fresh sildenafil solution in water (T_0), after 120- and 300-min irradiation time (T_{120} and T_{300}, respectively) and sildenafil solution with 800 μM PMS at PS after 0, 40-, and 100- minutes of light irradiation.

The data obtained show low toxicity for 15 and 30 minutes of contact of *V. fisheri* with a fresh sildenafil starting solution (T_0). On the other hand, the sample taken after 120 minutes of irradiation (T_{120}), corresponding to the maximum number of photoproducts in the solution, and the sample T_{300}, taken at the end of the degradation process, when no signals attributable to sildenafil and its photoproducts were detectable

Table 2
Results of the *Vibrio fischeri* acute toxicity test [26] after 5, 15, and 30 minutes of contact with aqueous sildenafil samples photodegraded under light irradiation without oxidant or in the presence of PMS 800 μM.

SAMPLES	5-min*	15-min*	30-min*
SILD 3mg/L T_0	19.4%	22.55%	23.1%
SILD 3 mg/L T_{120}	15.24%	15.37%	15.68%
SILD 3 mg/L T_{300}	13.67%	14.97%	15.62%
SILD 3mg/L + PMS 800 μM T_{PMS-0}	19.4%	22.55%	23.1%
SILD 3mg/L + PMS 800 μM T_{PMS-40}	16.5%	16.6%	16.7%
SILD 3mg/L + PMS 800 μM $T_{PMS-100}$	12.1%	12.2%	12.4%

Percentage inhibition I%	EC_{50}	[a]Judgment
<20%		Absence of acute toxicity
>=20% < 50%		Weakly toxic
>=50%	100–10	Toxic
>50%	<10-1	Very toxic
>50%	<1	Extremely toxic

[a] Toxicity legend table according to UNI EN ISO 11348–3:2009.

in the acquired chromatogram, do not show acute toxicity. The same considerations can be made for the Sunlight/PMS system. Samples collected at 40 min (T_{40}) and 100 min (T_{100}) do not show acute toxicity. The inhibition percentage of the bioluminescence of *V. fischeri* decreased during the irradiation time, confirming the effectiveness of the proposed photodegradation processes. Sildenafil and its photoproducts have also undergone an *in silico* toxicological evaluation based on the chemical structure/toxicity relationship. The Ecosar program provides the degree of acute toxicity of the drug and its photoproducts, based on the LC_{50}, on daphnids after 48 hours of contact [28]: preliminary results obtained (data not shown) indicate that only sildenafil and two of its photoproducts, the one at *m/z* 461 and the one at *m/z* 449, are potentially toxic.

4. Conclusions and future perspectives

This study examined the effectiveness of various photooxidation processes for degrading sildenafil, a drug of increasing environmental concern. While simulated solar light exhibited limited efficacy, both Sunlight/TiO_2 and Sunlight/oxidant systems demonstrated promising results. Notably, among the various systems tested, the Sunlight/PMS system achieved complete degradation of sildenafil in both distilled water and synthetic wastewater within 80 and 130 minutes, respectively. This highlights the potential of Sunlight/PMS photooxidation as a viable technique for sildenafil removal. Further analysis using LC-LTQ-MS revealed the formation of photoproducts resembling human sildenafil metabolites, emphasising the importance of their environmental impact assessment. However, toxicity studies with *Vibrio fisheri* confirmed the overall environmental friendliness of the proposed remediation process. By addressing these aspects, this study paves the way for implementing sustainable and efficient solutions to mitigate the environmental burden of sildenafil contamination. Future research should focus on optimising the Sunlight/PMS system by exploring more environmentally friendly catalysts with efficient and cost-effective recovery and disposal strategies

CRediT authorship contribution statement

Angelica R. Zizzamia: Formal analysis, Investigation, Methodology, Writing – original draft. **Carmen Tesoro:** Data curation, Methodology, Writing – original draft, Investigation. **Giuliana Bianco:** Writing – review & editing, Supervision. **Sabino A. Bufo:** Writing – review & editing, Data curation, Methodology. **Rosanna Ciriello:** Writing – review & editing, Data curation. **Monica Brienza:** Writing – review & editing, Data curation, Conceptualization. **Laura Scrano:** Writing – review & editing, Resources. **Filomena Lelario:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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