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MASS SPECTROMETRY APPLICATION FOR THE DETECTION OF SILDENAFIL IN AQUEOUS PHASES

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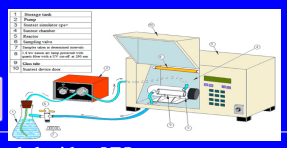
INTRODUCTION

In recent years, sildenafil, a drug for erectile dysfunction commonly marketed as Viagra, has attracted a great deal of attention due to its widespread use [1], its commercialization through legal and illegal routes and the growing tendency of young people to use this drug for recreational rather than medical purposes. The abuse of this substance and the fact that Wastewater treatment plants (WWTP) cannot remove all types of contaminants that enter the sewer legitimates thinking that they can pose a severe threat to ecosystems and human health [2]. Once released into the environment, this pollutant may be subject to a series of transformations due to solar radiation or oxidant agents present in the water, the unambiguous analytical determination of the active drug and the identification of its transformation products are therefore indispensable to try understanding if the quantity found of this drug in wastewater and surface water is linked to actual medical use or abuse and to verify whether tertiary purification treatments of wastewater are effective in the removal.

This study is focused on the evaluation of the effectiveness of photodegradation processes in distilled water and synthetic wastewater (SWW) in presence of three different oxidants, peroxymonosulphate (PMS), peroxidisulphate (PS) and hydrogen peroxide (H_2O_2) for the removal of this pollutant from aqueous phase and the identification of sildenafil and its photoproducts by LC–ESI–MS and MS^n. In addition, experiments were conducted to assess the toxicity of photoproducts.

MATERIALS AND METHODS



- All experiments were performed using a LC system coupled with a LTQ-mass spectrometer (Thermo Fisher Scientific, Bremen, Germany); separation was carried out on a chromatographic column Luna C18, Phenomenex (150 x 4.6 mm, 5 µm), working in gradient eluition at a flow rate of 0.800 mL/min, splitted 3:1 post column.
- Standard solutions of sildenafil (10 mg/L) were prepared in distilled water and SWW, addionated of variable amount of oxidants (100-1600 µM) and undergone to photodegradation processes in a CPS+ Solar Simulator equipped with a xenon lamp.
- Mass spectrometric data were acquired in the positive ion mode while scanning m/z 90-1200 at rate of 2 scan/s.
- Low resolution MS^n experiments were performed by collisional induced dissociation (CID). On the CID-MS^n the most abundant ion was automatically selected as precursor ion and fragmented up to the MS^5 stage (Fig. 1), each successive most abundant fragment ion being selected again as precursor ion for the next step.
- Different energies ranging from 25% to 35% of arbitrary units were applied (100% corresponding to a 35eV excitation voltage), as it ensures fragments ions peaks intense enough and it get precursor ion peak less than 20% relative intensity.
- Data acquired and processed by Xcalibur software package (version 2.0 SR1 Thermo Scientific).
- Mass spectra were imported, elaborated and plotted by SigmaPlot 10.0 (Systat Software, Inc., London, UK) and chemicals structures were drew by ChemDraw Ultra 12.0 (CambridgeSoft Corporation, Cambridge, MA).

RESULTS AND DISCUSSION

PHOTODEGRADATION TESTS

The highest efficiency for all processes under investigation was observed with PMS 800 µM: the total degradation of sildenafil and its photoproducts was recorded after approximately 80 minutes in distilled water and 130 minutes in SWW (Fig. 1 a and b).

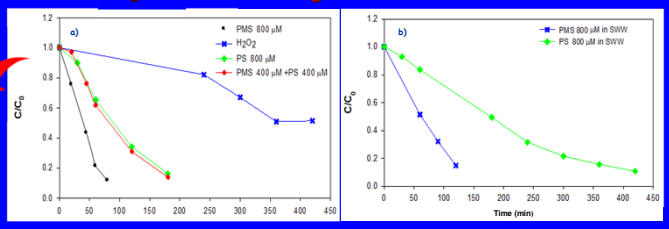


Fig 1. Photodegradation curves of sildenafil in distilled water (a) and SWW (b)

In the corresponding dark test (Fig. 2) with PMS 800 µM, the quantitative conversion of sildenafil into the corresponding N-oxide, an inactive form of sildenafil and one of its most well-known metabolites and photoproduct [3], occurred.

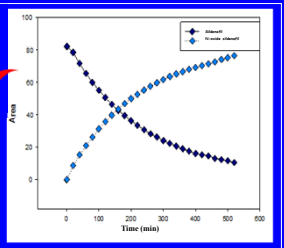


Fig 2. Degradation curve of sildenafil and formation curve of N-oxide sildenafil in dark conditions.

LC-ESI- LTQ- MS AND CID-MS^n

Analyses aimed to:
- Confirm the effectiveness of processes tested in the removal of sildenafil and its photoproducts;
- Characterize the photoproducts;
- Assess the presence of analogues of human metabolites.

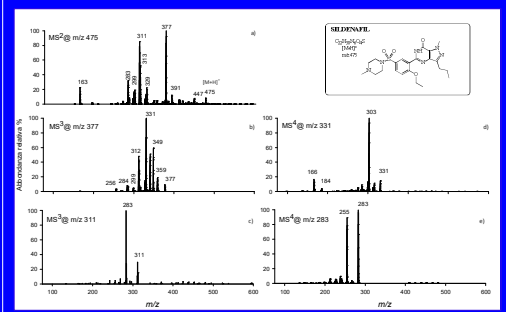


Fig 3. Mass spectra of sildenafil by CID-MS^n *a-e*). Relative collision energies in CID ranging from 25% to 35% were applied.

The main intermediates identified (Table 1) showed similar structures as the major sildenafil metabolites found *in vivo* studies [4], and as products of human metabolism [5]. Their formation involved reaction of:
- Mono-hydroxylation on the aliphatic or piperazine moiety;
- Di-hydroxylation on the piperazine moiety
- N-demethylation and N-N deethylation on the piperazine or pyrazole ring

Compound	Precursor ion	Structure	Main MS/MS product ions
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		443; 377; 329; 313; 311; 299; 283.
5	449		432; 418; 392; 361; 313; 311; 285.
6	477		459; 434; 395;390; 377; 362; 311; 298.
7	477		459; 449;418; 392; 377; 311.
8	505		505; 487; 477; 463; 418; 391; 326; 311.
9	505		487; 416; 389; 377; 311; 299; 283.
10	507		489; 461; 447; 377; 349.
11	507		479; 461; 395.

Table 1. Main photoproducts detected and corresponding product ions

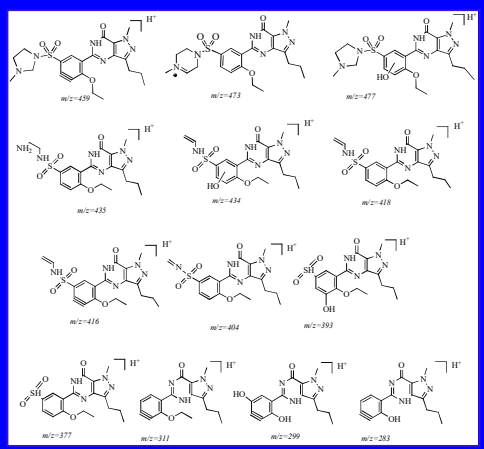


Fig 4. Proposed fragmentation structures for product ions of sildenafil and its photoproducts, based on CID-MS^n spectra.

Main fragmentation pathways of all photoproducts involved (Figures 3-4):
- Demethylation;
- Cleavage of the C–S bond and loss of the ethyl group from the ethoxy substituent of the phenyl ring;
- Loss of the piperazine ring;

The three fragments at m/z 311, 299 and 283 are common in the mass spectra of most of the photoproducts observed in this study and can be used as a fingerprint to differentiate the light-induced structural changes in the phenyl pyrazolopyrimidinone group from those in the piperazine ring.

All photoproducts found in this study, except the two compounds at m/z 507, were found in previous studies [6]. Toxicity tests on *Daphnia magna* and *Vibrio fisheri* showed that photoproducts are less toxic than Sildenafil, but the use of an excess of PMS during photochemical processes should be avoided

CONCLUSIONS

Data showed that the most effective photochemical process in distilled and synthetic wastewater was the PMS/UV system. The structures of the main photoproducts were determined. Toxicity tests showed lower toxicity of photoproducts than sildenafil. This study represents a starting point to optimize identification of structures related to human metabolites and forensic analyses.

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