

# Using ESI-QToF-MS for the identification of phototransformation products of sildenafil (Viagra) and its human metabolite N-demethyl-sildenafil generated in a sunlight simulator



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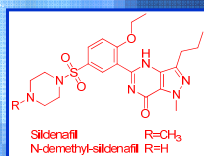
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## Abstract

This study describes the photolysis of sildenafil and its human metabolite N-demethylsildenafil under simulated solar radiation (Xenon lamp). Following chromatographic separation of the irradiated samples on a sub-2  $\mu$ m RP-C18 column, a number of photoproducts were detected for both test compounds. The application of (+)ESI-QToF-MS allowed to propose plausible chemical structures for the photoproducts taking into account the characteristic fragmentation patterns and the accurate mass measurements. These mass spectral data provided sound evidence for the susceptibility of the piperazine ring towards photochemical degradation. A gradual breakdown of this heterocyclic structure gave rise to a series of products, which in part were identical for SDF and DM-SDF. The sulfonic acid was identified as key intermediate in the photolysis pathway. In both drug/metabolite molecules phototransformation processes taking place beyond the sulfonamide group were deemed to be of minor relevance.

## Rationale

The presence of a large number of pharmaceuticals in surface waters has been extensively documented in the last decade. However, the spectrum of bioactive compounds detectable in the aquatic environment is steadily growing, not least because of advances of analytical instrumentation and novel drugs reaching the market. For instance, sildenafil (SDF) a phosphodiesterase type V inhibitor, has been recently determined along with its major human metabolite (N-demethyl-sildenafil; DM-SDF) in wastewater effluents at concentrations up to 20  $\mu$ g L<sup>-1</sup>. While these compounds are detected at low concentration in the effluents, their concentration are expected to be lower in the surface waters due to dilution, its biological activity may give cause of concern. Once emitted in natural waters, the photolytic transformation under the influence of natural sunlight may play a key role in the elimination process. A number of studies have been dedicated to assessing the importance of this process for numerous drugs, yet few of them set out to elucidate the structures of the resulting photoproducts.



Because of these, the identification of biotic and abiotic transformations is particularly important to assess environmental distributions. At the same time at least seven illegal analogs of Sildenafil have also been identified in herbal formulations mainly targeted to overcome patent limitations (Singh et al., 2009). In many cases, these designer analogs are rather similar to the patented substance with small modifications in the side chains aimed to retain effectiveness. Tracking the legal or illegal analogs then becomes a function of how well-characterized the degradation pathway is when the parent compound is no longer present in the sample.

## Experimental section

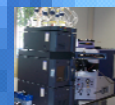
- Filter to 0.22  $\mu$ m
- 20 ppm SILDENAFIL
- 500 W/cm<sup>2</sup>
- 1-mL sample
- 60 hour exposure
- UPLC-ESI-MS



- UPLC-QToF (Waters Micro)
- ACQUITY C-18 (50 x 2.1 mm x 1.7  $\mu$ m)
- ACN:0.05%FA 9:1  $\rightarrow$  1:9 at 300  $\mu$ l min<sup>-1</sup>
- ESI+ 3000V, CV 25, 330°C, 50/600LH-1
- FS HRMS 5000 FWHM (170-550 Da)
- Lock Mass Val-Tyr-Val at [M+H]<sup>+</sup> 380.2185
- MS/MS HR [TP+H]<sup>+</sup>  $\rightarrow$  50 to [TP+H]<sup>+</sup>-10Da

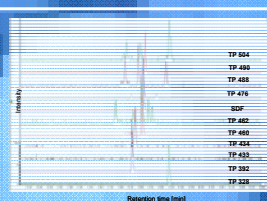


Irradiation: Suntest CPS

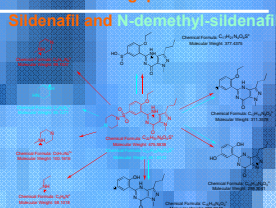


Structural elucidation: UPLC-ESI(+)-QToF-MS

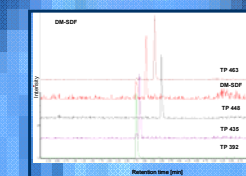
## Sildenafil Transformation Products



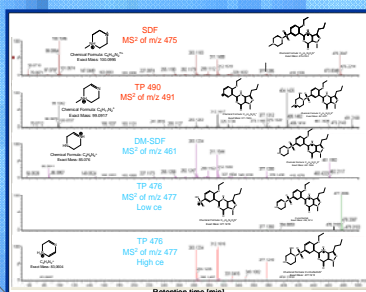
## Mass frag. pattern of Sildenafil and N-demethyl-sildenafil



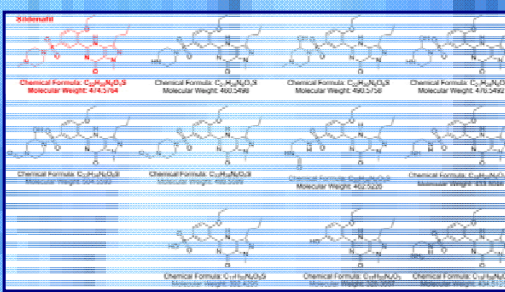
## N-demethyl-sildenafil Transformation Products



## MS/MS Spectra of Sildenafil and TPs



## Identification of the phototransformation products of SDF and DM-SDF



SDF (R=CH <sub>3</sub> )	Demethyl-SDF (R=H)
Parent compound (m/z 476)	Parent compound (m/z 461)
TP504 (m/z 500)	not possible
TP490 (m/z 491)	TP476 (m/z 477)
TP488-B (m/z 489)	not possible
TP488-C (m/z 489)	not possible
TP462 (m/z 463)	TP462 (m/z 463)
TP392	-
TP448 (m/z 449)	not detected
not detected	TP448-B (m/z 449)
TP434 (m/z 435)	TP434 (m/z 435)
TP392 (m/z 393)	TP392 (m/z 393)
TP328 (m/z 329)	not detected

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