# Identification of the Photodegradation Products of the Tricyclic Antidepressant Drugs Clomipramine and Doxepine

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**Objective:** Isolation and identification of the photodegradation products of the tricyclic antidepressant drugs clomipramine and doxepine after irradiation with ultraviolet light.
**Methods:** The photodegradation products were separated by a thin layer cromatographic method, followed by scraping the spots from the chromatoplate and extracting in methanol, which was followed by their identification by mass spectrometry.
**Results:** In the case of clomipramine seven degradation products were separated and the corresponding m/z values were determined, while analyzing doxepine there have been separated eight degradation products, of which six were identified by their m/z values. The results obtained for clomipramine are in accordance with literature data, except for desmethyl-clomipramine, for which we could not find any reference.
**Conclusions:** The m/z values indicate that the possible degradation products for clomipramine are imipramine, HO-imipramine, desmethyl-clomipramine and HO-imipramine-N-oxide. In the case of doxepine we could identify two possible photodegradation products, HO-doxepine and doxepine-N-oxide.

**Keywords:** tricyclic antidepressants, photodegradation products, identification

## Introduction

Tricyclic antidepressants are a group of pharmaceutical substances with marked photosensitivity and phototoxic potential [1–6]. Previous studies have shown that these substances undergo significant decomposition under ultraviolet (UV) irradiation [7–9] and have phototoxic effect on erythrocytes, but in varying degrees [10]. It was reported that degradation products of these drugs play an important role in the occurrence of their photosensitivity and phototoxicity [1,3,4]. Clomipramine and its degradation products were investigated in several studies [1], but as far as we know, no studies have been conducted until now to identify photodegradation products of doxepine.

Our research objective was the isolation and identification of photodegradation products of clomipramine hydrochloride and doxepine hydrochloride.

## Methods

Clomipramine hydrochloride (Sigma-Aldrich, Germany) and doxepine hydrochloride (Dipharma, Italy) were high purity standards. Other chemical reagents were also of analytical grade purity.

### Preparation and irradiation of the solutions

Aliquots of 50 mL aqueous solution were prepared at a concentration of 0.1% and then irradiated with a 20W ultraviolet lamp (UV-A) from a distance of 20 cm, at a temperature of 25°C, for 4 hours.

### Separation of photodegradation products

Separation of degradation products was carried out by a thin-layer chromatographic method, developed in our previous research, which presented a good separation of degradation products between them and the parent substance [8]. To prepare the mobile phase we used the following solvents (chromatographic quality): ethyl-acetate, acetone and concentrated ammonia with the following composition: 80+20+5. The chosen stationary phase was a 20×20 cm pre-coated silicagel GF254 HPTLC plate (Merck, Germany).

The chromatographic separation was performed with a Camag thin-layer chromatographic (TLC) system, which consisted of a Camag Linomat IV automatic sampler, a Hamilton microsyringe (Hamilton, USA), a Camag normal development chamber and a Camag fluorescence inspection lamp (Camag, Switzerland). The separated spots were examined in UV light at 254 and 366 nm, using winCATS Planar Chromatography Manager. Amounts of 100 µL sample were spotted on the chromatoplate. The spots migrated at a distance of 15 cm from the starting line, for about one hour and 20 minutes, at room temperature.

### Isolation of photodegradation products

After drying the plates at room temperature, the spots of the investigated substances and degradation products were scraped and extracted in 1 mL methanol. The solutions obtained were subjected to centrifugation for 10 minutes at 4000 rotations/min. The supernatant was filtered through a 0.45 µm syringe filter.

### Identification of photodegradation products

Mass spectra of the photodegradation products of doxepine

**Table I. Rf values and MS analysis results for clomipramine hydrochloride**

| Compound | Rf values | m/z values | Compound | Rf values | m/z values |
|---|---|---|---|---|---|
| Clomi. | 0.63 | 315.1 | Clomi. | x | |
| $C_{254A}$ | 0.54 | 282.3 | $C_{366A}$ | 0.54 | 282.3 |
| $C_{254B}$ | 0.29 | 297.2 | $C_{366B}$ | 0.51 | 304.3 |
| $C_{254C}$ | 0.12 | 288.3 | $C_{366C}$ | 0.29 | 297.2 |
| $C_{254D}$ | 0.05 | 313.2 | $C_{366D}$ | 0.12 | 288.3 |
| | | | $C_{366E}$ | 0.10 | 227.0 |
| | | | $C_{366F}$ | 0.06 | 227.0 |
| | | | $C_{366G}$ | 0.05 | 313.2 |

**Table II. Rf values and MS analysis results for doxepine hydrochloride**

| Compound | Rf values | m/z values | Compound | Rf values | m/z values |
|---|---|---|---|---|---|
| Doxep. | 0.49 | 280.2 | Doxep. | 0.49 | 280.2 |
| $D_{254A}$ | 0.33 | 353.3 | $D_{366A}$ | 0.41 | 588.2 |
| $D_{254B}$ | 0.28 | 353.3 | $D_{366B}$ | 0.33 | 353.3 |
| $D_{254C}$ | 0.23 | 353.3 | $D_{366C}$ | 0.28 | 353.3 |
| $D_{254D}$ | 0.19 | 298.2 | $D_{366D}$ | 0.23 | 353.3 |
| $D_{254E}$ | 0.10 | 312.3 | $D_{366E}$ | 0.19 | 298.2 |
| $D_{254F}$ | 0.08 | – | $D_{366F}$ | 0.10 | 312.3 |
| $D_{254G}$ | 0.05 | – | $D_{366G}$ | 0.08 | – |
| | | | $D_{366H}$ | 0.05 | - |

and clomipramine was recorded using an Agilent 6410 Series Triple Quad mass spectrometer with electrospray ionization (ESI) operating in positive mode. The resulted ions were detected in MS2 Scan mode between 50–1000 m/z, with Unit Resolution, Fragmentor 135, Dwell 200. Nebulizator gas, nitrogen pressure was set at 50 psi, with Gas Flow 10 L/ min, Dry temperature 350 oC, Voltage 4000V.

## Results

### a) Clomipramine hydrochloride

On the chromatoplate obtained after migration of clomipramine hydrochloride irradiated solution there were observed several degradation products at 254 nm and 366 nm, their labeling was based on their Rf value, using the first letter of the name of the parent substance, the detection wavelength and block letters. Rf values and data obtained from mass spectrometric (MS) analysis of parent substance and degradation products are listed in Table I.

### b) Doxepine hydrochloride

In the case of doxepine hydrochloride can also be seen more degradation products. Analyzing the chromatoplate under UV light at 254 nm can be observed seven and at 366 nm eight degradation products. Rf values and MS analysis results are presented in Table II, the labeling name of the compounds being made like for clomipramine hydrochloride.

## Discussion

Based on the results obtained from mass spectrometric analysis we tried to identify the degradation products. Analyzing data recorded for clomipramine irradiated solutions, the $C_{254A}$-$C_{366A}$, $C_{254B}$-$C_{366C}$, $C_{254C}$-$C_{366D}$, $C_{254D}$-$C_{366F}$ product pairs have the same Rf and m/z values, so we may find that it is the same product in every pair. On the basis of the m/z values with high probability we can identify certain substances, as presented in Figure 1. The m/z values represent the protonated form of the molecules (M+H) because of the positive electrospray ionization (Figure 2).

- $C_{254A}$ – imipramine;
- $C_{254B}$ – OH-imipramine;
- $C_{254C}$ – desmethyl-clomipramine;
- $C_{254D}$ – OH-imipramine-N-oxide.

In this regard, the results obtained are in accordance with literature data, where there are described the same degradation products [1]. In the case of desmethyl-clomipramine we found no bibliographical references. Contrary to the literature we didn't find clomipramine-N-oxide. The other

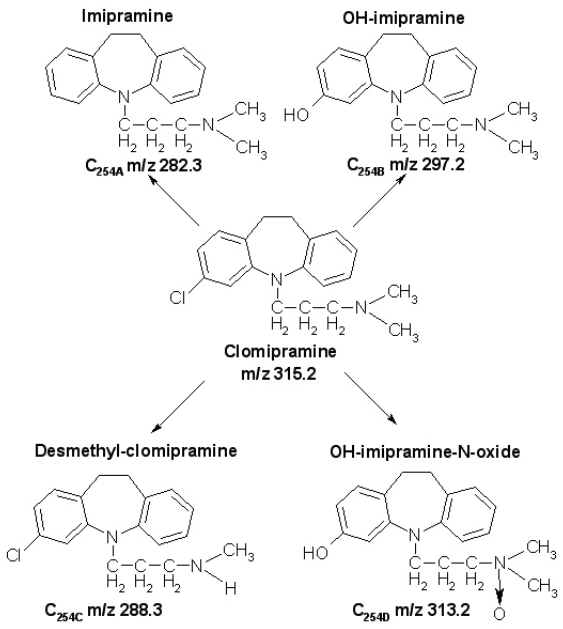


Fig. 1. Photodegradation products of clomipramine hydrochloride

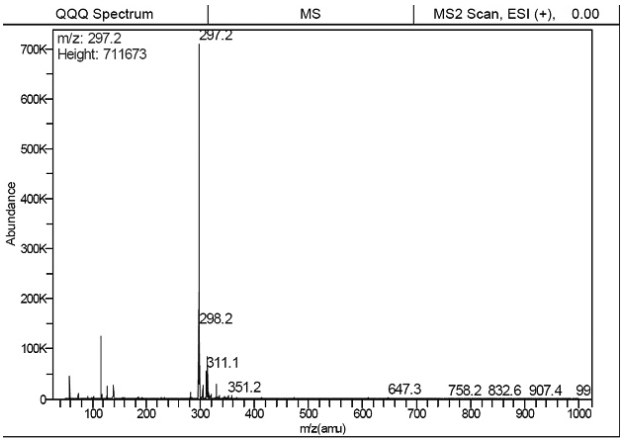


Fig. 2. Mass spectrum of clomipramine photodegradation product: Imipramine-OH ($C_{254B}$, $C_{366C}$), m/z 297.2

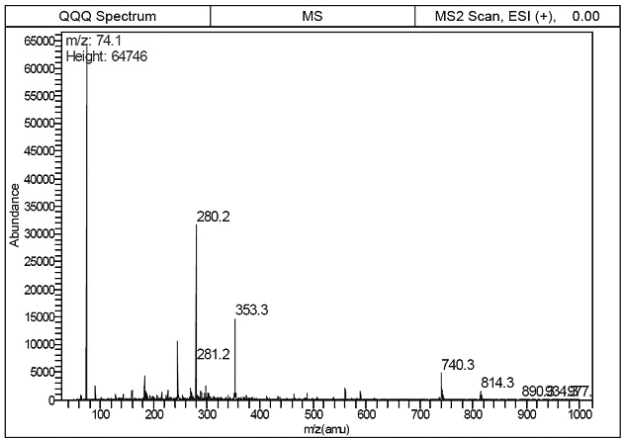


Fig. 3. Mass spectrum of doxepine photodegradation product $D_{254A}$, m/z 353.3 (transformed also to doxepine m/z 280.2)

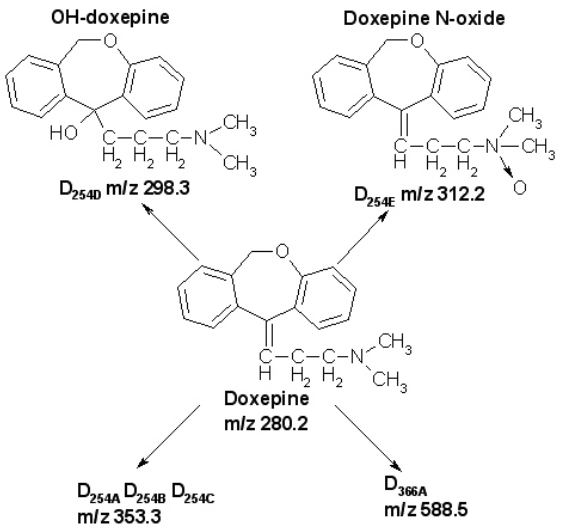


Fig. 4. Photodegradation products of doxepine hydrochloride

degradation products failed to conclusive identification on the basis of m/z values.

Data recorded for doxepine irradiated solutions show that $D_{254A}$-$D_{366B}$, $D_{254B}$-$D_{366C}$, $D_{254C}$-$D_{366D}$, $D_{254D}$-$D_{366E}$, $D_{254E}$-$D_{366F}$, $D_{254F}$-$D_{366G}$ and $D_{254G}$-$D_{366H}$ product pairs hide the same substance. $D_{254A}$, $D_{254B}$ and $D_{254C}$ products are probably isomers of the same unidentified substance, which transforms to doxepine with increasing collision energy (Figure 3). On the basis of m/z value of $D_{254D}$ it can be said that it may be probably OH-doxepine. Like at clomipramine products, it is possible an N-oxide formation, which on the basis of m/z value is the $D_{254E}$ product: doxepine- N-oxide. It is probable that the $D_{366A}$ product with m/z value of 588.8 is a doxepine-dimer formed by a Diels-Alder reaction (Figure 4).

## Conclusion

After irradiation with UV light of the tricyclic antidepressant drug, clomipramine and doxepine solutions, there were separated, isolated and identified as potential photodegradation products imipramine, HO-imipramine, desmethyl-clomipramine and HO-imipramine-N-oxide for clomipramine. In the case of doxepine we could identify two possible photodegradation products, HO-doxepine and doxepine-N-oxide.

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