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A study of photodegradation of quetiapine by the use of LC-MS/MS method

Research Article

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Abstract: Photodegradation of quetiapine under UVC irradiation in methanol solution was investigated and structural elucidation of its photodegradation products was performed with the use of the reversed phase UHPLC system coupled with accurate mass hybrid ESI-Q-TOF mass spectrometer. During one run all essential data for the determination of photodegradation kinetics and for the structural elucidation of the products was collected with the use of auto MS/MS mode. Five degradation products were found and their masses and formulas were obtained with high accuracy (0.26-5.02 ppm). For all the analyzed compounds, MS/MS fragmentation spectra were also obtained allowing structural elucidation of the unknown degradation products and indicating photodegradation pathways of quetiapine. The main photodegradation product was identified as 2-[2-[4-(5-oxidodibenzo[b,f][1,4]thiazepin-11-yl)-1-piperazinyl] ethoxy]-ethanol and the photodegradation reaction yields the first-order kinetics with the rate constant k = 0.1094 h^{-1}.

Keywords: *UHPLC • Q-TOF • Photodegradation • Quetiapine • UV irradiation*

1. Introduction

Quetiapine 2-[2-(4-benzo[b][1,4]benzothiazepin-6-ylpiperazin-1-yl)ethoxy]ethanol is a second generation antipsychotic drug whose pharmacological activity is based on the selective binding 5-HT_2 and D_2 receptors, however the serotonin receptors are blocked twice as much as the dopamine receptors [1]. This drug is well tolerated and characterized by fewer extrapyramidal symptoms (EPS) than the conventional antipsychotic drugs and it is also effective in hard to treat patients and is a suitable first-line option for the treatment of schizophrenia [2].

Photostability investigation is an integral part of the stability study of drugs and must be considered during the development and registration process of pharmaceuticals [3-5]. Photodegradation of drugs is a very important subject of stability testing because this kind of process can result in the loss of the activity of a drug and also in adverse effects due to the formation of toxic degradation products. The knowledge of what exact compounds are formed from the drug during this process can be very useful for the manufacturing, storage and administration of pharmaceuticals and may significantly improve the safety of therapy [6,7].

The majority of papers concerning the analysis of quetiapine described the determination of this drug in biological materials with the use of: voltametric [8], TLC [9], GC-MS [10-14] and LC methods with UV [10,15-21], DAD [22] and MS [23-27] detection. There are a few papers concerning the determination of this drug in pharmaceuticals by UV spectrophotometry [28-31], capillary zone electrophoresis [28,32], potentiometric [33], polarographic [34] and HPTLC [35] methods. Two publications describe the identification of potential impurities of quetiapine by the use of spectroscopic methods [36,37].

Several papers report the use of chromatographic methods for the stability-indicating assay of quetiapine [38-42], however no comprehensive photodegradation study was performed and what is more, the contrary information about photostability of quetiapine is presented in this case. Raju *et al*. [40] claim that quetiapine is stable after 10 days irradiation (sunlight simulation) while Dhaneshwar *et al*. [41] in similar conditions (in methanolic solution) observed its degradation after 5 days but no identification of photodegradation products was performed in this case. On the other hand Trivedi and Patel [42] report that quatiepine in methanol solution is stable under UVC (254 nm) irradiation.

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Taking into account the above facts, it is necessary to carry out the complete photodegradation study of quetiapine including the structure elucidation of the formed photoproducts. For this purpose a new analytical method with the use of new generation hydrid MS/MS spectrometer combined with ultra-high-pressure liquid chromatography (UHPLC) was developed.

2. Experimental procedure

2.1. Materials

Quetiapine fumarate with 99.8% purity was obtained from AstraZeneca UK Ltd. (Macclesfield, UK). Gradient grade acetonitrile and hypergrade methanol for LC-MS were purchased from Merck (Darmstadt, Germany). Water for LC was obtained from Honeywell Burdick & Jackson (Muskegon, USA).

Quetiapine fumarate solution for photodegradation tests (at concentration 2.6×10^{-5} M as a base) was prepared in hypergrade methanol.

2.2. Photodegradation conditions

Quetiapine methanolic solution was placed in quartz caped cell (l = 1 cm) and irradiated with UVC radiation. As a UVC source Haland HA-05 (Warsaw, Poland) ultraviolet laboratory lamp equipped with one 6W quartz ultraviolet tube emitting mercury spectrum with 254 nm principal line was used. The distance between the UVC lamp and the sample was 10 cm and the temperature in the chamber was controlled and kept below 25°C. The UVC intensity was controlled with a radiometer and average irradiation intensity was 390 μW cm^{-2}. The dark control sample was used by exposing the quetiapine sample in quartz cell wrapped in aluminum foil for the same period of time.

2.3. Liquid chromatography-mass spectrometry (LC-MS/MS)

LC-MS/MS analysis was performed with the use of Agilent Accurate-Mass Q-TOF LC/MS G6520B system with dual electrospray (DESI) source and Infinity 1290 ultra-high-pressure liquid chromatography system consisting of: binary pump G4220A, FC/ALS thermostat G1330B, autosampler G4226A, DAD detector G4212A, TCC G1316C module and Zorbax Eclipse-C18 (2.1×50 mm, dp=1.8 μm) Rapid Resolution HD column (Agilent Technologies, Santa Clara, USA). A mixture of acetonitrile (A) and water (B) was used as a mobile phase. The gradient elution was carried out at constant flow 0.4 mL min^{-1} from 30%A (70%B) 0 - 2 min and then 30%A to 80%A 2-10 min. 1 min post time (30%A) was performed to return to initial conditions. The injection volume was 1 μL and the column temperature was maintained at 40°C. Mass Hunter workstation software in version B.04.00 was used for the control of the system, data acquisition, qualitative and quantitative analysis.

Q-TOF detector was tuned in a positive mode with the use of Agilent ESI-L tuning mix in high resolution mode (4 GHz). Next, the main parameters were optimized and the following settings were applied: gas temp.: 270°C, drying gas: 10 L min^{-1}, nebulizer pressure: 40 psig, capillary voltage: 3000 V, fragmentor voltage: 200 V, skimmer voltage: 65 V, octopole 1 RF voltage: 250 V.

In order to make the qualitative and quantitative analysis in one run, data acquisition was performed in auto MS/MS mode with spectral parameters: mass range: 40-950 *m/z* and acquisition rate: 1.40 spectra s^{-1} (for MS and MS/MS data). In this mode the maximum data for structure elucidation was collected, and it was not necessary to repeat the analysis in different modes. Ions for the fragmentation are selected automatically and MS/MS spectra are recorded simultaneously with MS spectra. The MS/MS spectra were used for the structural elucidation of the obtained photodegradation products and MS spectra in the form of extracted ion chromatogram (EIC) were used for the quantification of quetiapine concentration.

Collision energy was also calculated with auto algorithm with formula: 5 V (slope)*(*m/z*)/100 + 6.8 V (offset) and in this case it ranged: 14.6 V – 26.8 V. Maximum 2 precursors per cycle were selected with an active exclusion mode after 1 spectra for 0.1 min.

To ensure accuracy in masses measurements, reference mass correction was used. Masses 121.0508 and 922.0097 were used as lock masses.

Diode array detector (200 – 400 nm) was also used for the analysis monitoring.

2.4. Quantitative analysis and photodegradation kinetics

The method calibration for the determination of the concentration of quetiapine in tested samples was performed with the use of MS detection and extracted ion chromatograms (EIC) for *m/z* 384.1740. The calibration curve was obtained by plotting the peak area against the amount of the drug in the range: 1.3×10^{-7} to 3.1×10^{-5} M and studied by fitting the results to linear least-squares regression. All calibration standards were analyzed five times and the average calibration curve with statistic parameters was calculated (sec. 3.3.).

The obtained calibration curve was used for the determination of photodegradation kinetics of quetiapine

in methanol solution. During the irradiation procedure 100 µL of the tested solution was collected and analyzed by LC-MS/MS system in the time: 0, 0.5, 1, 2, 3, 4, 5 and 6 h of exposing to UVC radiation. The photodegradation kinetics parameters: rate constant (k) and $t_{1/2}$ were calculated with the use of equation:

$$\ln c = \ln c_0 - kt,$$

where c_0 is the concentration in the time 0, c is the remaining concentration, k the rate constant (h^{-1}) and t is the time (h).

The dark control sample concentration was measured in the same time period.

3. Results and discussion

3.1. Identification of the photoproducts

In order to forced degradation of quetiapine in methanol solution UVC radiation was used and after 6 h of irradiation near 50% of the analyzed drug was degradated. Although, the used LC-MS/MS system was coupled with DAD detector, only two degradation products (D1 and D3) was well registered with the use of this standard UV-VIS detection (Fig. 1A). In this situation for the purpose of quantitative assay of quetiapine and qualitative analysis of its photoproducts in one run auto MS/MS mode of Q-TOF detector was used.

As shown in Table 1, the molecular ion of quetiapine was found with very high accuracy (0.26 ppm) and the chemical formula was calculated in this case ($C_{21}H_{26}N_3O_2S$). Furthermore, five degradation products (D1-D5) were found and their masses as well as formulas were also calculated.

As shown in Fig. 2, quetiapine gave MS/MS fragmentation to three main ions: *m/z* 279.0939, 253.0789 and 221.1066 which are formed as a result of the piperazine ring breaking. Basing on this information the structure elucidation of the main photodegradation product (D1) was started. The measured mass (*m/z* 400.1682) and generated formula ($C_{21}H_{26}N_3O_3S$) for this compound suggest that this photoproduct is the result of oxidation process. MS/MS spectrum of this product (Fig. 3) is similar to quetiapine and all the characteristic fragmentation ions for this compound are observed. The main difference in this case is the explicit presence of *m/z* 352.1995 fragmentation ion which confirms that additional oxygen atom is connected with sulphur from dibenzothiazepine ring.

The measured mass of the second photodegradantion product D2 (*m/z* 175.1436) enabled the generation of $C_8H_{19}N_2O_2$ formula which corresponds to piperazinylethoxyethanol fragment of quetiapine. The obtained MS/MS fragmentation spectrum of this product (Fig. 4) exactly confirmed the proposed structure in this case.

The third photodegradation product (D3) was also identified as a result of photolytic process of the main compound. As shown in Fig. 5, its fragmentation spectrum responds to benzo[b][1,4]benzothiazepine structure which is the main heterocyclic ring of quetiapine.

In Fig. 6, MS/MS spectrum of D4 photodegradation product and the proposed fragmentation pathway are shown. The measured accurate mass in this case (*m/z* 159.1120) enabled the generation of $C_7H_{14}N_2O_2$ formula with DBE (double bond equivalent) 2. The main fragmentation ion *m/z* 71.0341 suggested that the imidazole ring with an additional double bound is the main structure of this product.

The measured mass of the last photodegradantion product D5 (*m/z* 191.1383) was 16 Da higher than D2 photoproduct and the oxidative derivative of this product was considered. The obtained MS/MS spectrum (Fig. 7) confirmed that this product is formed basis on piperazinylethoxyethanol fragment of quetiapine and the significant attendance of *m/z* 173.1276 fragmentation ion suggested that this photoproduct is its N-oxide derivative.

3.2. Proposed photodegradation pathway

The main photodegradation product (D1) was found as a result of photooxidation process. In this case S-oxide derivative of quetiapine (2-[2-[4-(5-oxidodibenzo[b,f]

Table 1. Exact mass measurements and elemental composition of quetiapine and its photodegradation products (D1-D5) using Q-TOF MS method.

Compound	t_R (min)	Measured mass (*m/z*)	Theoretical mass (*m/z*)	Mass error (ppm)	Molecular formula $[M+H]^+$	DBE
D5	0.39	191.1383	191.1390	3.66	$C_8H_{19}N_2O_3$	1
D2	0.43	175.1436	175.1441	2.85	$C_8H_{19}N_2O_2$	1
D4	0.49	159.1120	159.1128	5.02	$C_9H_{15}N_2O_2$	2
D1	1.47	400.1682	400.1689	1.74	$C_{21}H_{26}N_3O_3S$	11
Quetiapine	4.32	384.1739	384.1740	0.26	$C_{21}H_{26}N_3O_2S$	11
D3	4.96	212.0518	212.0528	4.71	$C_{13}H_{10}NS$	10

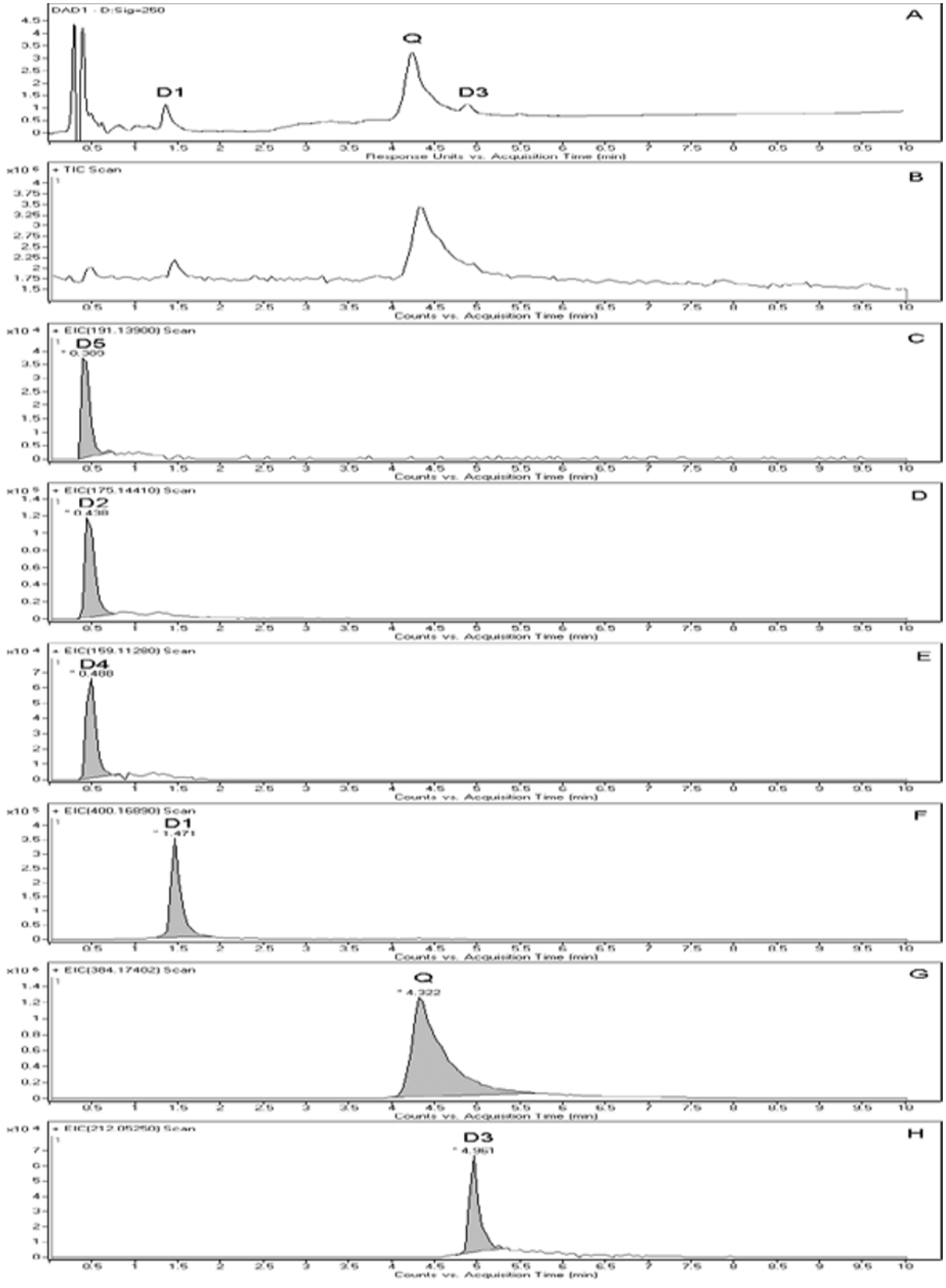

Figure 1. UHPLC DAD chromatogram (A), Q-TOF total ion chromatogram (B) and extracted ion chromatograms (C-H) obtained from quetiapine (Q) methanolic solution after 6 h UVC irradiation; D1-D5 – photodegradation products.

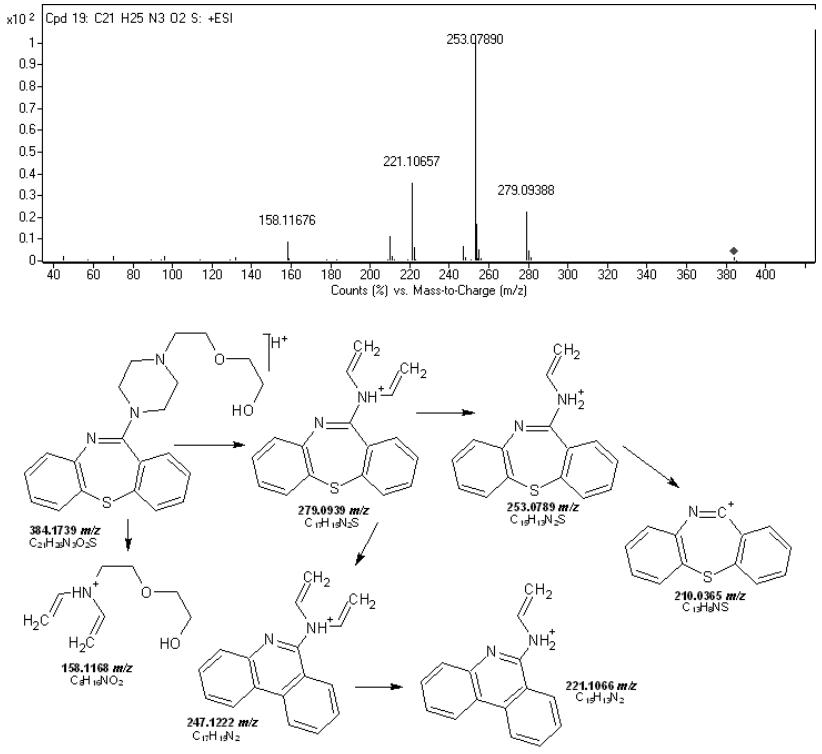

Figure 2. Q-TOF MS/MS spectrum and fragmentation pathway of quetiapine.

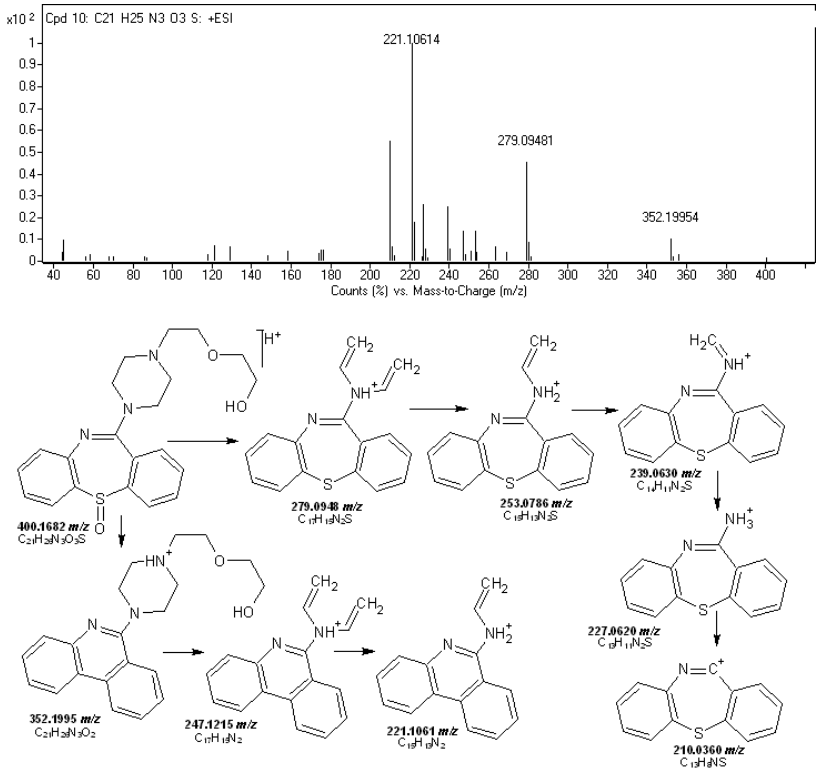

Figure 3. Q-TOF MS/MS spectrum and fragmentation pathway of photodegradation product D1 (t_R = 1.47 min).

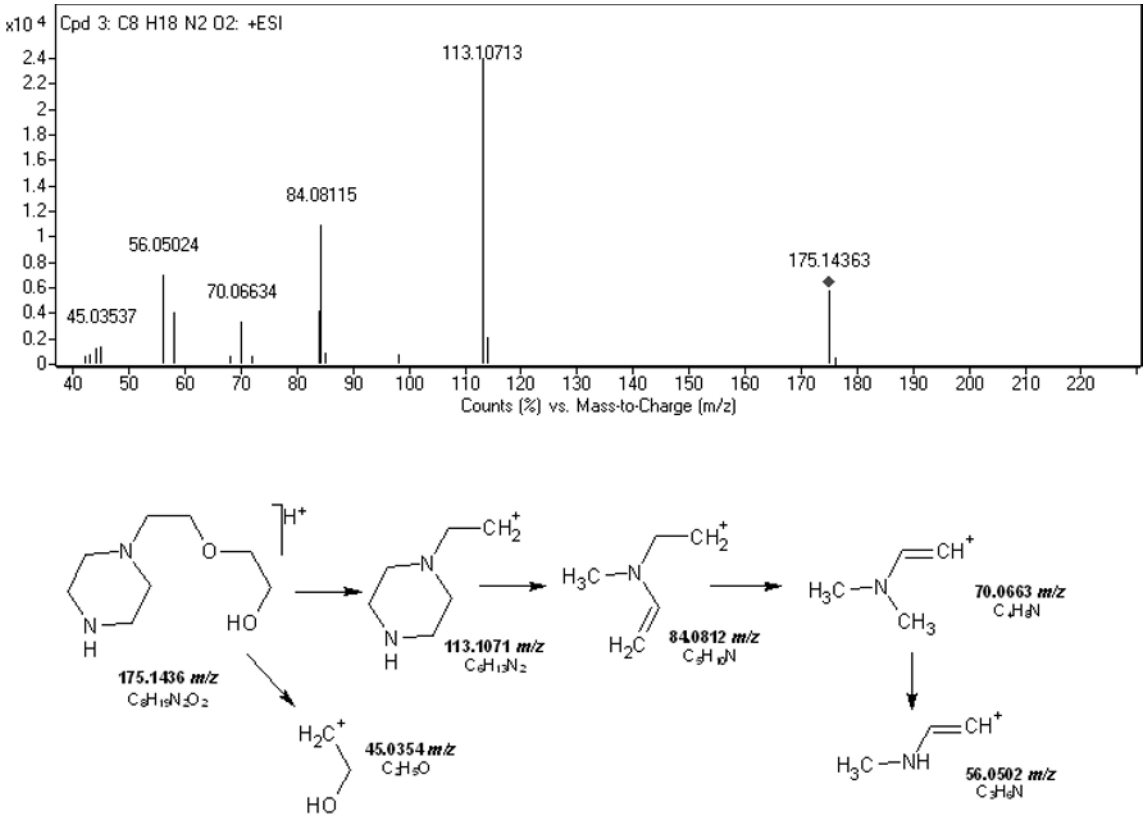

Figure 4. Q-TOF MS/MS spectrum and fragmentation pathway of photodegradation product D2 (t_R = 0.43 min).

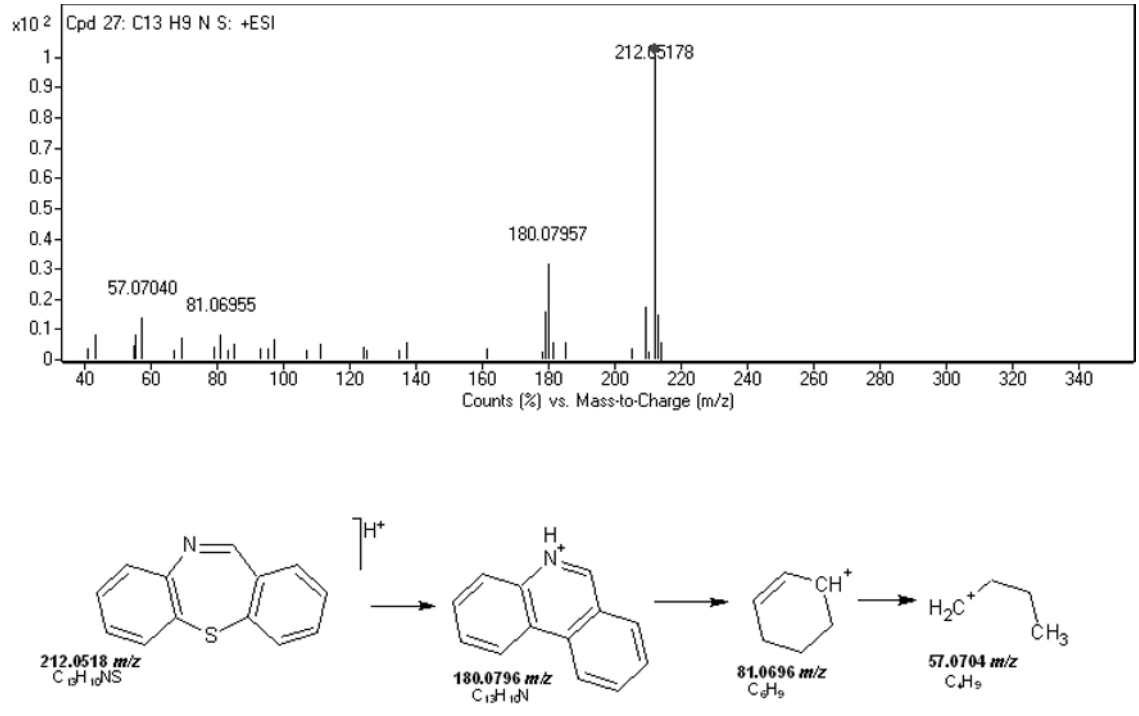

Figure 5. Q-TOF MS/MS spectrum and fragmentation pathway of photodegradation product D3 (t_R = 4.96 min).

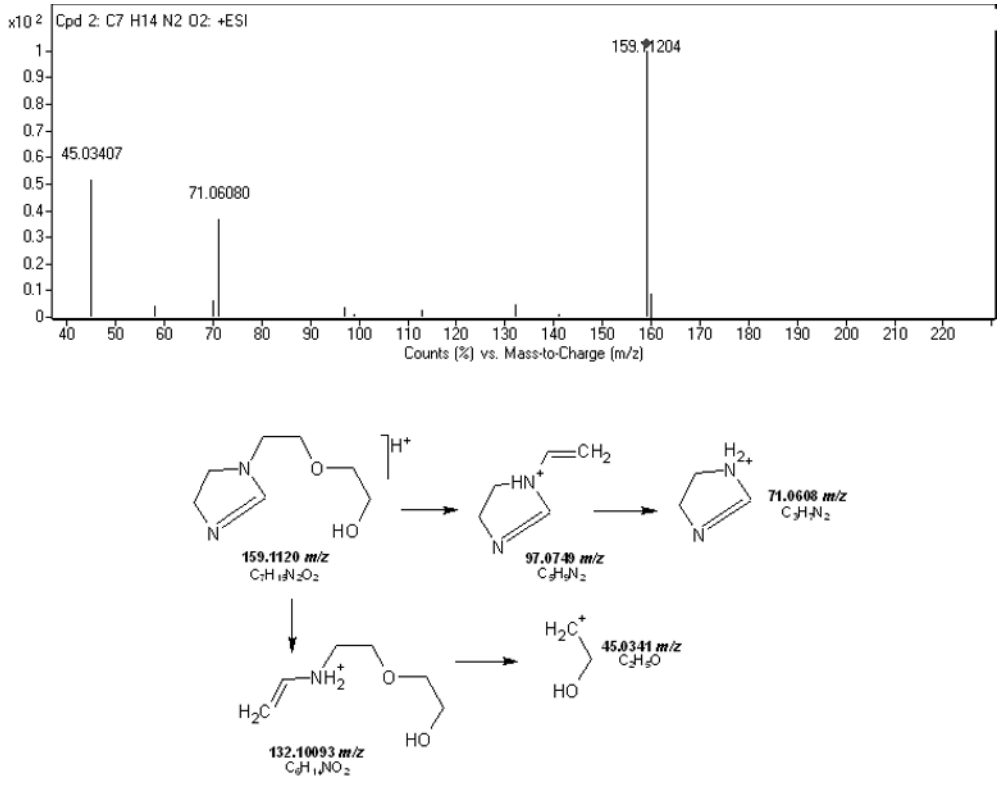

Figure 6. Q-TOF MS/MS spectrum and fragmentation pathway of photodegradation product D4 (t_R = 0.49 min).

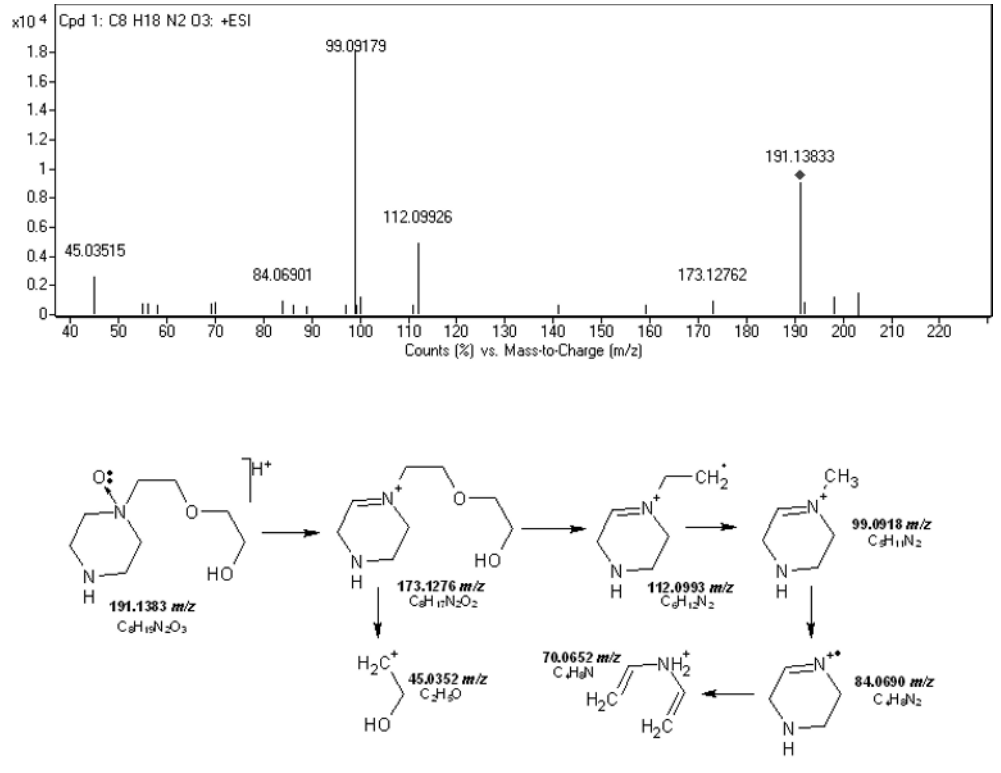

Figure 7. Q-TOF MS/MS spectrum and fragmentation pathway of photodegradation product D5 (t_R = 0.39 min).

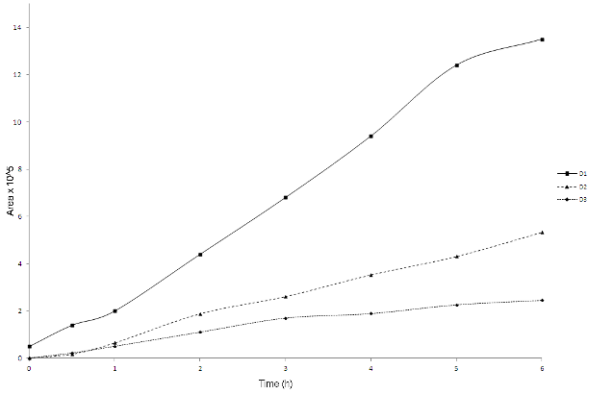

Figure 8. Evaluation profile of the main photodegradation products (D1 – D3).

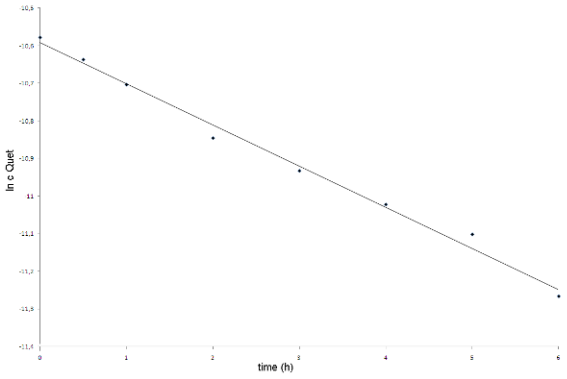

Figure 9. First-order kinetics of the photodegradation of quetiapine in methanol solution.

[1,4]thiazepin-11-yl)-1-piperazinyl]ethoxy]-ethanol) was formed whereas the next two photoproducts were found as an the effect of quetiapine photolysis – D2 (2-[2-(piperazin-1-yl)ethoxy]ethanol) and D3 (benzo[b][1,4]benzothiazepine). As shown in Fig. 8, the preferred degradation pathway of quetiapine is based on the oxidation reaction of dibenzothiazepine ring and the photolysis process is less relevant in this case.

The other two photodegradation products D4 (2-[2-(4,5-dihydro-1H-imidazol-1-yl)ethoxy]ethanol) and D5 (2-[2-(1-oxidopiperazin-1-yl)ethoxy]ethanol) were formed in a slight amount and are the result of the secondary reaction of D2 photodegradation product.

3.3. Quantitative study of the photodegradation process

The calibration of the quantitative analysis method for the determination of quetiapine was performed on MS detector with the use of EIC (*m/z* 384.1740) in the range: 1.3×10^{-7} to 3.1×10^{-5} M. The obtained calibration curve: $y = 1.14\times10^{12}$ ($\pm6.5\times10^{10}$)x + 443876 (±130346) was linear over the concentration range ($r = 0.9995$) and the limits of detection (LOD) and quantification (LOQ) were 2.6×10^{-8} M and 7.2×10^{-8} M respectively. RSD values were in the range: 0.61-1.89%. The obtained results were used to calculate the concentration of quetiapine at proper time intervals during UVC irradiation. As shown in Fig. 9, the decomposition of quetiapine yields the first-order kinetics reaction according to the equation:

$$\ln c = \ln c_0 - kt.$$

The rate constant value, correlation coefficient and the half-life time of photodegradation process were in this case respectively:

$k = 0.1094\ h^{-1}$, $r = 0.9957$, $t_{1/2} = 6.34$ h.

The quantitative analysis of the dark control sample of quetiapine confirmed the absence of the photodegradation process in this case.

4. Conclusions

Photodegradation of quetiapine in methanolic solution yields a first-order kinetic reaction and five degradation products are formed. The main photodegradation product was found as S-oxide derivative of quetiapine.

UHPLC-ESI-Q-TOF system was found to be a powerful analytical tool for the fast and accurate photodegradation study of quetiapine. During one run all essential data for the quantitative analysis and for the structural elucidation of the photodegradation products was collected.

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