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Original article

Characterization of forced degradation products of toloxatone by LC-ESI-MS/MS

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ABSTRACT

Forced degradation of toloxatone in solutions under basic, acidic, neutral, photo UV–VIS, photo UVC and oxidative stress conditions was investigated and structural elucidation of its degradation products was performed with the use of UHPLC system coupled ESI-Q-TOF mass spectrometer. Eight degradation products were found and their masses and formulas were obtained with high accuracy (0.09–3.79 ppm). The structure of unknown degradation products were elucidated from MS/MS fragmentation spectra of all analyzed compounds. Additionally, whole signals of decomposed substances were compared chemometrically. It was found that toloxatone is fragile towards basic hydrolysis, oxidative conditions and UVC irradiation. Finally, the toxicity of transformation products was computationally evaluated and compared in multivariate manner.

1. Introduction

Toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-1,3-oxazolidin-2-one) is the third generation of monoamine oxidase inhibitors (MAO) introduced to the market in the late 1980s as an effective agent to major depression. Its pharmacological activity is based on the selective and reversible inhibition of monoamine oxidase type A (RIMA) and is characterized by minimal adverse side effects in comparison to previous two generations of MAO inhibitors (Moureaul et al., 1992; Moureau et al., 1995). It was also reported that the antidepressant efficiency of toloxatone is similar to the most popular RIMA – moclobemide, however, its onset of action is slower (Bonnet, 2003).

In the analytical aspect toloxatone was only studied in the biological materials for the qualification as well as identification of this drug. HPLC with UV detection was the most often used method for the determination of toloxatone in human plasma (Duverneuil et al., 2003; Provost et al., 1992), rabbit plasma and cerebrospinal fluid (Kaltenbach et al., 1999). HPLC with MS/MS detection was used for the qualification of the drug in whole blood (Titier et al., 2007) and GLC method was also used for its determination in plasma (Vajta et al., 1983). The other chromatographic method – TLC was also applied for the identification of toloxatone and its metabolites in urine (Vajta et al., 1984).

It should be noticed that degradation study of drugs is an important part of stability testing of medicines, as decomposed drugs can lose their effectiveness as well as they can gain additional adverse effects. Therefore it is very important to know what transformation products are formed during the degradation process. This data can be very useful for the manufacturing, quality control, storage and administration of pharmaceuticals (ICH guideline Q1B, 1996; ICH guideline Q1A, 2003; Jacobson-Kram and McGovern, 2007).

Hence, it is necessary to perform the forced degradation study of toloxatone including the structure elucidation of the formed products. For this purpose a new analytical method using UHPLC system coupled with accurate hybrid ESI-MS/MS spectrometer was developed. Additionally, the multivariate chemometric analyses (PCA) of the forced degradation profiles of toloxatone as well as *in silico* toxicity of the identified transformation products were performed.

2. Experimental

2.1. Chemicals and reagents

The following chemicals were used: toloxatone (Sigma Aldrich, St Louis, USA), LC-MS grade water (Sigma Aldrich, St Louis, USA) and 30% hydrogen peroxide of trace analysis grade (Sigma Aldrich,

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St Louis, USA), acetonitrile hypergrade for LC-MS (Merck, Darmstadt, Germany, 98% formic acid of mass spectroscopy grade (Fluka, Taufkirchen, Germany). All other analytical grade reagents (hydrochloric acid, sodium hydroxide) were purchased from POCh (Gliwice, Poland).

2.2. LC-ESI-MS/MS analysis

The LC-MS/MS analysis was performed on Agilent Accurate-Mass Q-TOF LC/MS G6520B system with dual electrospray (DESI) ionization 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) HD column (Agilent Technologies, Santa Clara, USA). A mixture of acetonitrile (A) and water (B) with addition of 0.1% solution of formic acid in both media was used as a mobile phase. The isocratic elution was carried out at constant flow 0.5 ml/min at 10%A and 90%B. The injection volume was 5 μl and the column temperature was maintained at 35 °C. MassHunter workstation software in version B.04.00 was used for the control of the system, data acquisition, qualitative and quantitative analysis.

The optimization of the instrument conditions started from the proper tuning of Q-TOF detector in a positive mode with the use of Agilent ESI-L tuning mix in the extended dynamic range (2 GHz). The following instrument settings were applied: gas temp.: 325 °C, drying gas: 9 L/min, nebulizer pressure: 35 psig, capillary voltage: 4000 V, fragmentor voltage: 200 V, skimmer voltage: 65 V, octopole 1 RF voltage: 250 V.

Data acquisition was performed in centroids with the use of TOF (MS) and also targeted MS/MS mode. The spectral parameters for both modes were: mass range: 60–950 *m/z* and the acquisition rate: 1.6 spectra/s. To ensure accuracy in masses measurements, a reference mass correction was used and masses 121.050873 and 922.009798 were used as lock masses.

2.3. Forced degradation studies

Forced degradation studies were performed for the bulk substance using stock solution of toloxatone prepared in water at concentration 200 μg mL^{-1}. The working solutions were prepared by diluting the stock solutions using the proper solvent to obtain the final concentration of 10 μg mL^{-1} and next stressed under hydrolytic, oxidative and photolytic conditions (Table 1). All the hydrolytic and oxidative degradations were performed using 10 ml of working solution placed in hermetically sealed glass vials. For the photodegradation tests the working solutions were placed in a quartz caped cells (*l* = 1 cm) mounted horizontally and irradiated with UV–VIS or UVC radiation. The distance between the lamp and the samples was 10 cm in both cases. A photostability chamber Atlas Suntest CPS+ (Linsengericht, Germany) with full UV–VIS spectrum (D65) was used as an UV–VIS source, according to ICH guidelines. The irradiance was set to 750 W/m^2 which corresponds to the dose of 2700 kJ/m^2/h. As a UVC source a Haland HA-05 (Warsaw, Poland) ultraviolet laboratory lamp equipped with 6 W quartz ultraviolet tube emitting mercury spectrum with 254 nm principal line was used. The average UVC irradiation intensity was 7.5 W/m^2. The dark control samples were also performed for both photostability experiments by exposing the toloxatone sample in a quartz cell wrapped in aluminum foil for the same period of time.

Table 1
Stress conditions applied to toloxatone degradation.

Stress conditions	Diluting solvent	Exposure conditions	Duration (h)
Acid hydrolysis	1 M HCl	80 °C	2
Alkaline hydrolysis	0.01 M NaOH	80 °C	2
Neutral hydrolysis	H_2O	80 °C	2
Oxidation	0.01% H_2O_2	80 °C	2
Photolysis (UV–VIS)	H_2O	Room temp.	48
Photolysis (UVC)	H_2O	Room temp.	2

2.4. Chemometric analysis

Three individual samples were prepared for each stressed condition as well as for not stressed control solution of toloxatone in water (STD) and TOF (MS) mode was used for the registration of their chromatographic/spectral degradation profiles. The MFE (molecular feature extraction) algorithm from the Mass Hunter Qualitative Analysis software version B.06.00 (Agilent) was used for data background ion noise cleaning and to extract the list of the ions characteristic for toloxatone degradation products. The MFE parameters were optimized and the following settings were

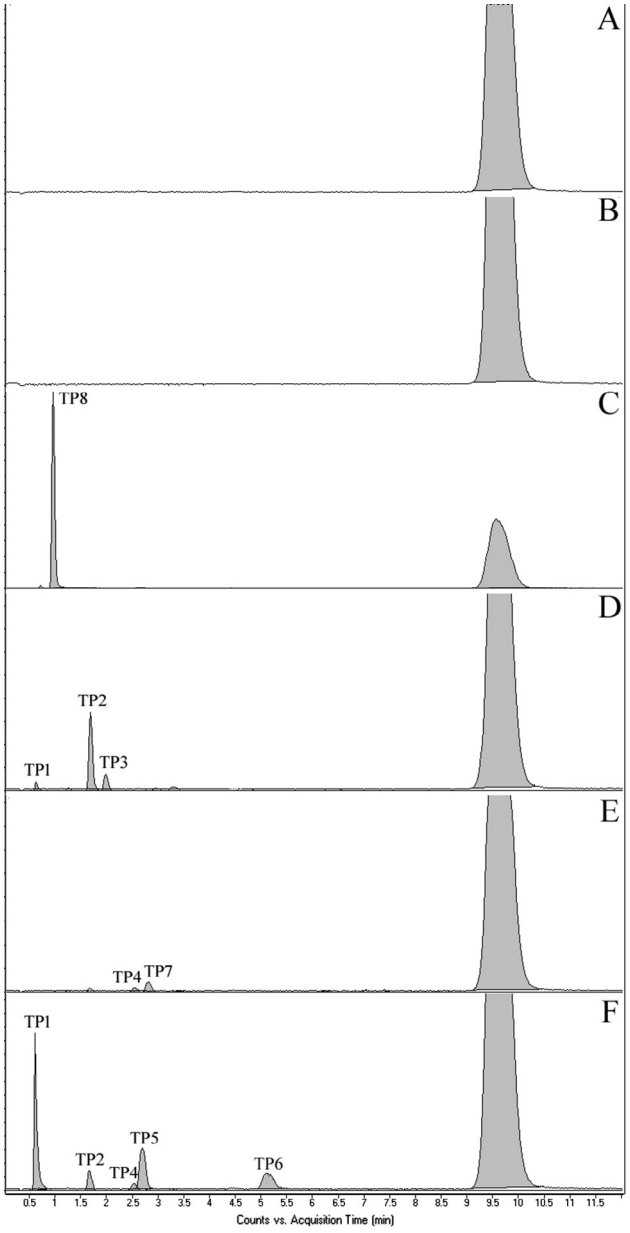

Fig. 1. TOF extracted ion chromatograms obtained under neutral (A), acidic (B), basic (C), oxidative (D), photo UV–VIS (E) and photo UVC (F) stress conditions.

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