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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.

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1. Introduction

Toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-1,3-oxazo lidin-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.

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
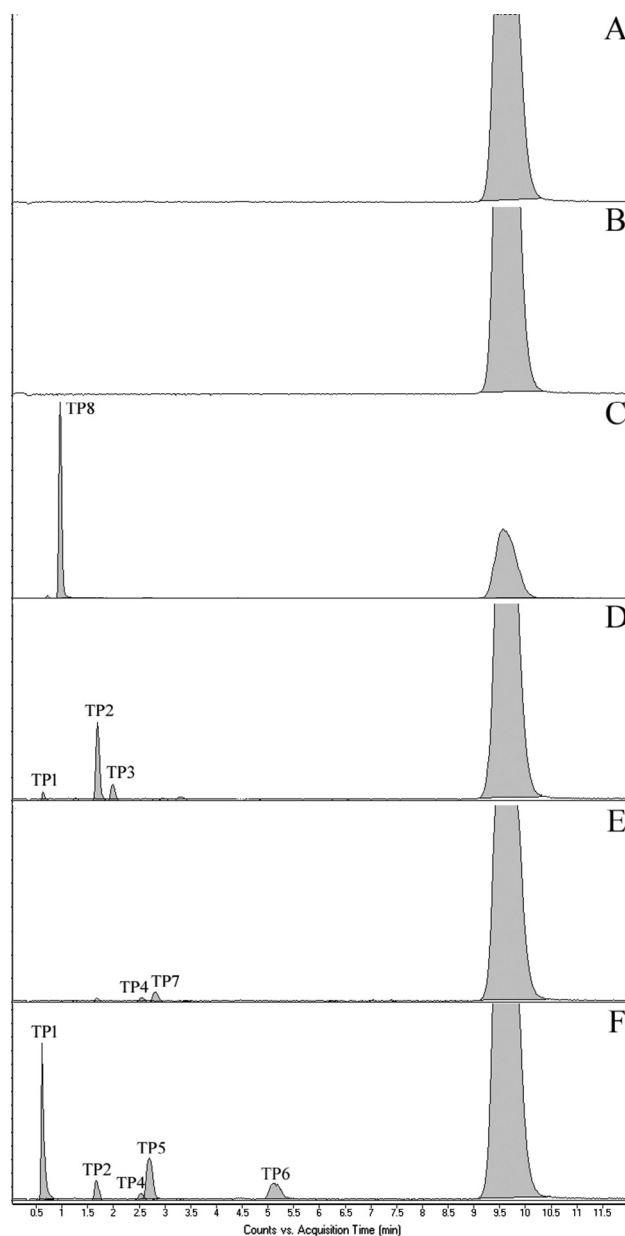
Table 1
Stress conditions applied to toloxatone degradation.

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$_2$O	80 °C	2
Oxidation	0.01% H$_2$O$_2$	80 °C	2
Photolysis (UV–VIS)	H$_2$O	Room temp.	48
Photolysis (UVC)	H$_2$O	Room temp.	2

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.

applied: maximum 1 charge state of the analyzed ions, more than 5000 counts for the compound filter, isotope model: common organic molecules with peak spacing tolerance 0.0025 m/z.

The multivariate chemometric analysis were performed under Mass Profiler Professional (MPP) software version 12.61 (Agilent and Strand Life Sciences Pvt. Ltd.). The data was normalized and aligned before the principal component analysis (PCA) was performed. This procedure allowed to evaluate qualitative differences in the registered degradation profiles of toloxatone.

2.5. In silico toxicity estimation

Acute toxicity to rodents, mutagenicity and carcinogenicity of elucidated transformation products, as well as the parent compound, were calculated with the use of following software: ACD/Percepta 14.0.0 (ACD/Labs, 2015 Release), and Vega 1.1.1. Then multivariate chemometric analysis was performed, in order to compare toxicity of the photoproducts and toxicity assessment methods. Data pre-processing and PCA analysis were performed with the use of R 3.2.3 software (GNU project). The obtained data was centered and scaled before the chemometric analysis.

3. Results and discussion

3.1. Optimization of the LC-ESI-MS/MS method

Chromatographic conditions were optimized on UHPLC reversed-phase C18 column. Various organic modifiers, buffers and elution systems including gradient elution were tested. Finally, taking into account high polarity of the parent compound as well as its transformation products, a simple isocratic elution with a mixture of acetonitrile and water (10:90, v/v) with addition of

Table 2
Q-TOF accurate mass elemental composition and MS/MS fragmentation of the analyzed compounds.

Name	Observed in stress condition	Retention time [min]	Measured mass [m/z]	Theoretical mass [m/z]	Mass error [ppm]	Molecular formula [M+H]$^+$	MS/MS fragmentation [m/z]	Fragmentation ion formula [M+H]$^+$
Toloxatone	–	9.44	208.09640	208.09682	−2.02	$C_{11}H_{14}NO_3$	164.10366	$C_{10}H_{14}NO$
							152.07060	$C_8H_{10}NO_2$
							145.09508	$C_{10}H_{12}N$
							134.06004	C_8H_8NO
							120.08078	$C_8H_{10}N$
TP1	H_2O_2, UVC	0.57	108.08070	108.08078	−0.74	$C_7H_{10}N$	93.05787	C_6H_7N
							91.05297	C_7H_7
							83.05866	C_5H_9N
							66.98539	C_4H_5N
							65.03889	C_4H_3N
TP2	H_2O_2, UVC	1.65	224.09181	224.09173	0.36	$C_{11}H_{14}NO_4$	180.10190	$C_{10}H_{14}NO_2$
							168.06353	$C_8H_{10}NO_3$
							136.07549	$C_8H_{10}NO$
							108.05501	C_7H_8O
							69.06978	C_5H_9
TP3	H_2O_2	1.96	240.08595	240.08665	−2.93	$C_{11}H_{14}NO_5$	222.07457	$C_{11}H_{12}NO_4$
							206.07968	$C_{11}H_{12}NO_3$
							194.08350	$C_{10}H_{12}NO_3$
							148.07569	$C_9H_{10}NO$
							134.05898	C_8H_8NO
							122.06004	C_7H_8NO
							106.06482	C_7H_8N
							77.03952	C_6H_5
TP4	UVC, UV–VIS	2.54	222.07635	222.07608	1.22	$C_{11}H_{12}NO_4$	178.04828	$C_9H_8NO_3$
							160.07337	$C_{10}H_{10}NO$
							134.05936	C_8H_8NO
							106.06541	C_7H_8N
TP5	UVC	2.75	208.09620	208.09682	−2.98	$C_{11}H_{14}NO_3$	190.08630	$C_{11}H_{12}NO_2$
							172.07440	$C_{11}H_{10}NO$
							160.07510	$C_{10}H_{10}NO$
							144.07800	$C_{10}H_{10}N$
							132.08000	$C_9H_{10}N$
							120.07900	$C_8H_{10}N$
							105.06960	C_8H_9
TP6	UVC	5.04	208.09620	208.09682	−2.98	$C_{11}H_{14}NO_3$	190.08460	$C_{11}H_{12}NO_2$
							72.07680	$C_{11}H_{10}NO$
							160.07440	$C_{10}H_{10}NO$
							144.07930	$C_{10}H_{10}N$
							132.07970	$C_9H_{10}N$
							120.07870	$C_8H_{10}N$
							105.06830	C_8H_9
TP7	UV–VIS	2.81	224.09171	224.09173	−0.09	$C_{11}H_{14}NO_4$	206.07974	$C_{11}H_{12}NO_3$
							134.09441	$C_9H_{12}N$
							118.06396	C_8H_8N
							83.08265	C_6H_{11}
							69.06885	C_5H_9
TP8	NaOH	1.01	182.11687	182.11756	−3.79	$C_{10}H_{16}NO_2$	164.10613	$C_{10}H_{14}NO$
							146.09675	$C_{10}H_{12}N$
							134.09491	$C_9H_{12}N$
							120.07984	$C_8H_{10}N$
							108.08078	$C_7H_{10}N$

0.1% solution of formic acid as a mobile phase was chosen. In these conditions in a quite short time of analysis (11 min) a good separation of the analyzed products was obtained (Fig. 1).

The MS conditions were optimized based on our previous study (Trawiński et al., 2017) and in the beginning electrospray ionization (ESI) was selected as a more effective ion source for this research. In TOF (MS) mode negative and positive ionization was tested and only in positive ionization all transformation products were registered. In the case of MS/MS experiments the collision energy (CID) was primarily optimized and finally for all the analyzed compounds CID was ranged from 8.7 to 20.0 eV.

3.2. Identification of forced degradation products

Under applied stress conditions eight unique degradation products (TPs) of toloxatone were formed. Most of them were characteristic for one condition, with the exception of TP1, TP2 and TP4. Two former products were common for H_2O_2 and UVC conditions. TP3 was formed only as a consequence of impact of oxidative environment. TP4, another product formed in more than one environment, was detected in both irradiated samples. Nevertheless it should be noticed that, besides of this product, TPs formation profile was dependent on applied radiation spectrum. TP5 and TP6 were formed only in UVC irradiated sample, while presence of

TP7 was characteristic for UV–VIS stressed sample. TP8 was sole product of basic hydrolysis. None of the TPs was formed in acidic and neutral hydrolysis experiments.

In order to elucidate probable structures of formed TPs MS and MS/MS spectra were collected. As shown in Table 2 masses of toloxatone, as well as its transformation products were collected with good accuracy (0.09–3.79 ppm), which enabled determination of molecular formulae, and, along with information obtained from fragmentation spectra, the TPs structures.

As shown in Fig. 2 fragmentation pattern of toloxatone (m/z 208.09640, $C_{11}H_{14}NO_3$) consisted of two paths. The first one began with elimination of a carbon dioxide molecule, which resulted in formation of m/z 160.10366 ($C_{10}H_{14}NO$) ion, followed by loss of water (m/z 145.09508, $C_{10}H_{12}N$). Elimination of propanol molecule, and formation of m/z 152.07060 ($C_8H_{10}NO_2$) was first stage of the second path. Then elimination of water took place, and ion of m/z 134.06004 (C_8H_8NO) was formed. Two mentioned paths eventually gave rise to formation of the most abundant ion in the spectrum – m/z 120.08078 ($C_8H_{10}N$).

TP1, identified as m-toluidine (m/z 108.07808, $C_7H_{10}N$), was formed as a consequence of the oxazolidinone ring decomposition. The most abundant peak in its MS/MS spectrum (Fig. 3) represented an ion radical (m/z 93.05787, C_6H_7N) formed after loss of a methane molecule. Then it started to gradually decompose,
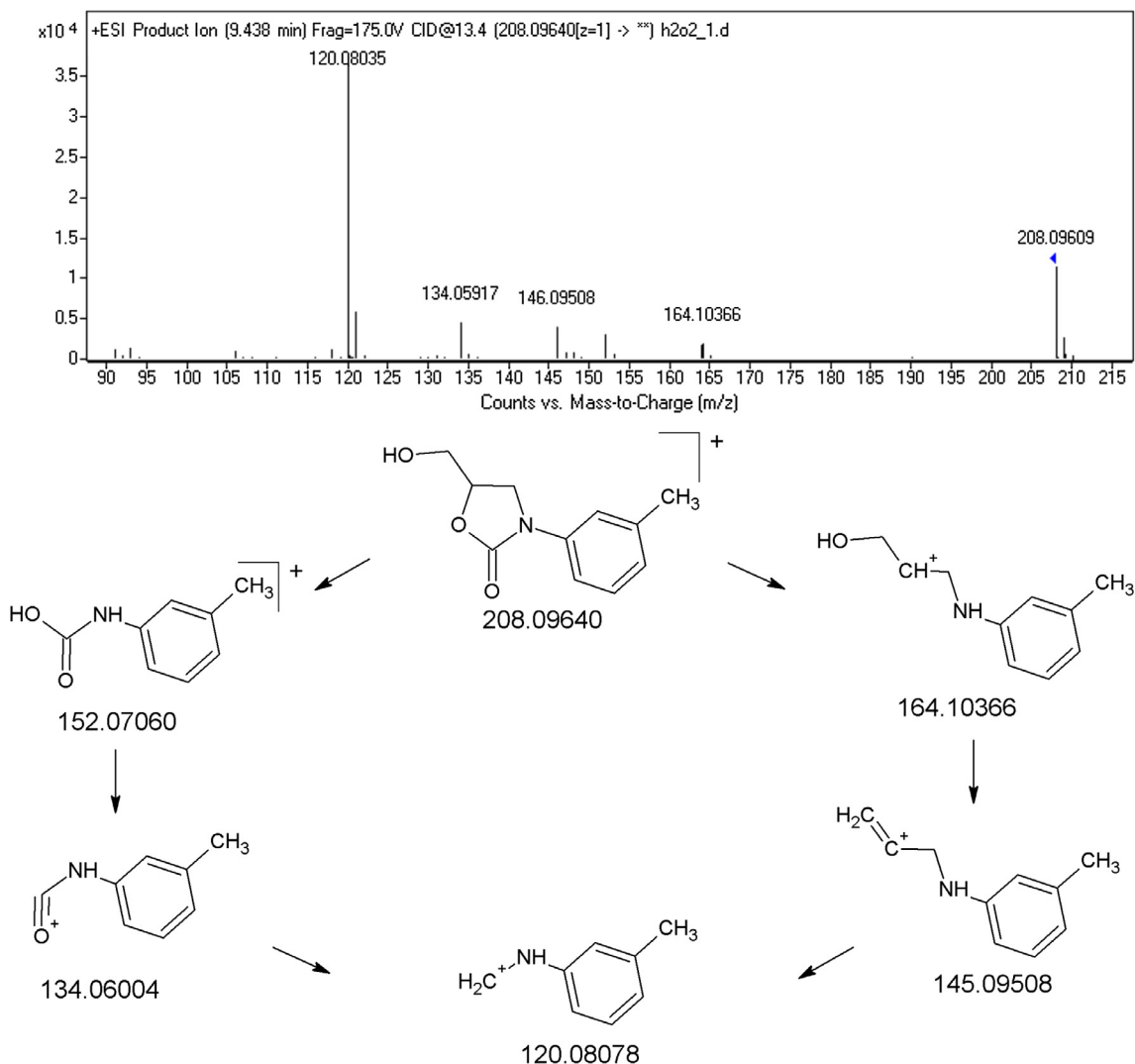

Fig. 2. Q-TOF MS/MS spectrum of toloxatone and corresponding fragmentation pathway.

giving m/z 83.05866 (C_5H_9N) ion radical and products of cyclization: m/z 66.98539 (C_4H_5N) and m/z 65.03889 (C_4H_3N) ion radicals. Ion of m/z 91.05297 (C_7H_7) was a tropylium cation.

Accurate mass (m/z 224.09118) and corresponding molecular formula ($C_{11}H_{14}NO_4$) of TP2 suggested that this compound was a product of toloxatone oxidation. Its fragmentation pathway (Fig. 4) began, similarly to toloxatone, with loss of a carbon dioxide molecule (m/z 180.10190, $C_{10}H_{14}NO2$), and then elimination of ethanol, which resulted in formation of m/z 136.07549 ($C_8H_{10}NO$) ion (the most abundant peak in the spectrum). This fragmentation ion was also formed via ion of m/z 168.06353 ($C_8H_{10}NO_3$) which was a result of elimination of propanol from the parent molecule. Further decomposition involved loss of methanamine (m/z 108.05501, C_7H_8O) and cleavage of an aromatic ring (m/z 69.06978, C_5H_9). Based on these evidences, location of an additional hydroxyl group was restricted to an aromatic ring. Despite the data obtained from the MS/MS spectrum did not allow for unequivocal determination of its position, para relative to the oxazolidinone ring was chosen, as probably the most favored.

The case of TP3 (m/z 240.08595, $C_{11}H_{14}NO_5$) was similar to TP2, however two additional oxygen atoms were attached to the parent compound. Its fragmentation (Fig. 5) began with an unusually effortless loss of water, with formation of m/z 222.07457 ($C_{11}H_{12}NO_4$) ion, which suggested that one oxygen formed an N-oxide group. Then gradual decomposition of a hydroxymethyl group took place, and ions of m/z 206.07968 ($C_{11}H_{12}NO_3$) and 194.08350 ($C_{10}H_{12}NO_3$) were formed. Further stages of decomposition were similar to those observed in the case of TP2: elimination of a carbon dioxide molecule (m/z 148.07569, $C_9H_{10}NO$) and methyl group (m/z 134.05898, C_8H_8NO). The latter product then gave two derivatives of a tropylium cation: containing amino and hydroxyl groups (m/z 122.06004, C_7H_8NO), and dehydroxylated (m/z 106.06482, C7H8N). Therefore TP3 was probably a product of further oxidation of TP2. Ion of m/z 77.03952 (C_6H_5) was a phenyl cation.

TP4 (m/z 222.07635, $C_{11}H_{12}NO_4$), similarly to TP2, contained one additional oxygen atom. Nonetheless in this case double bond equivalent (DBE) value was lower than toloxatone (7 versus 6),
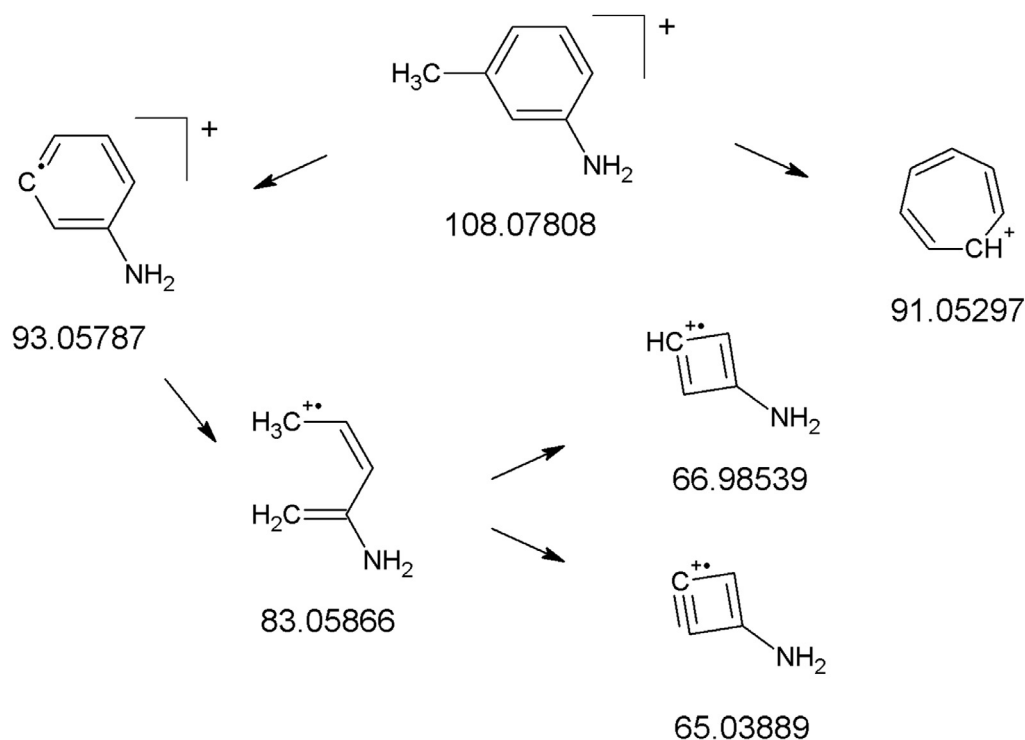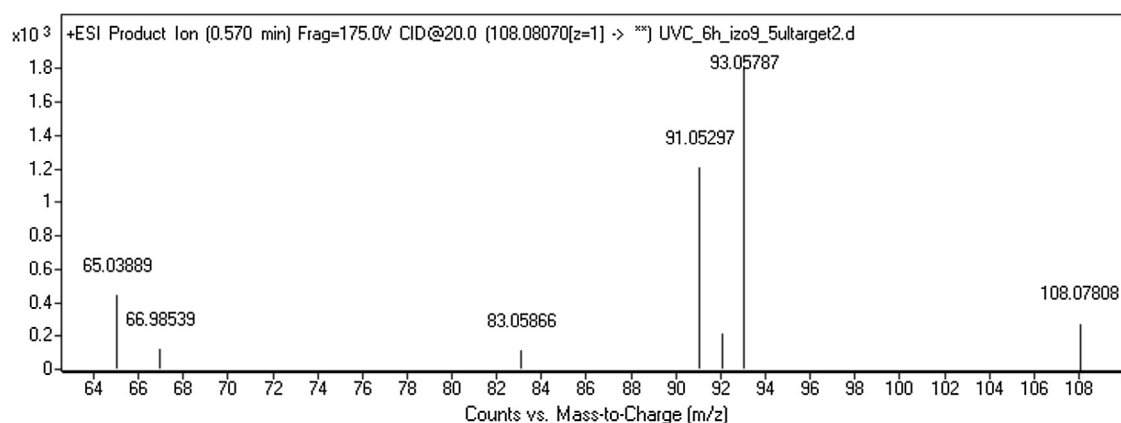

Fig. 3. Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP1.

which suggested presence of oxo group. Fragmentation of this product (Fig. 6) started with elimination of ethanol (m/z 178.04828, $C_9H_8NO_3$) or carbon dioxide along with water molecules (m/z 160.07337, $C_{10}H_{10}NO$). These two fragmentation ions formed then m/z 134.05936 (C_8H_8NO) ion, which then decomposed forming amino derivative of tropylium cation (m/z 106.06541, C_7H_8N).

TP5 (m/z 208.09620) and TP6 (m/z 208.09626) possessed the same molecular formula ($C_{11}H_{14}NO_3$) were both structural isomers of toloxatone. None significant differences between MS/MS spectra of these products occurred, however their fragmentation patterns did not resemble pattern of the parent compound. These observations suggested that TP5 and TP6 were almost identical products, but they remarkably differed from toloxatone. Presence of ions representing C_8H_9 formula (m/z 105.06960, Fig. 7. and 105.06830, Fig. 8) indicated that nitrogen atom in the oxazolidinone ring was replaced by a carbon atom. Therefore TP5 and TP6 were probably products of toloxatone oxazolidinone ring rearrangement. Fragmentation pathway began with loss of water molecule in both cases (m/z 190.08630 and 190.08460, $C_{11}H_{12}NO_2$ ions), which might suggest that the oxo group of the parent compound underwent the transformation into an alcohol group, accompanied with a migration of a double bond. Then elimination of water (m/z 172.07440 and 172.07680, $C_{11}H_{10}NO$) or methanol (m/z 160.07510 and 160.07440, $C_{10}H_{10}NO$) took place. These ions afterwards formed ions of m/z 144.07800 and 144.07930 ($C_{10}H_{10}N$),

which underwent gradual decomposition (via $C_9H_{10}N$ and $C_8H_{10}N$) forming eventually discussed C_8H_9 ion. As it was mentioned, due to lack of significant differences between fragmentation spectra of TP5 and TP6, determination of location of an additional double bond was impossible. Thus calculated logP values for two most probable structures were taken into the account. Calculations were done with the use of ALOGPS 2.1 software (VCCLAB platform, http://www.vcclab.org/). Average values of logP values were: 0.86 for imine derivative (TP5, shorter t_R), and 1.15 for enol derivative (TP6. longer t_R).

TP7 (m/z 224.09123, $C_{11}H_{14}NO_3$), similarly to TP2, was a product of the addition of one oxygen atom to the parent molecule. In this case it was probably attached to the oxazolidinone nitrogen atom. This assumption was based on presence of m/z 206.07974 ($C_{11}H_{12}NO_3$) ion, which was a product of loss of a water molecule. None of remaining fragmentation ions contained additional oxygen atom (Fig. 9). The next stage of fragmentation was elimination of methanol and cleavage of oxazolidinone ring (m/z 162.09378, $C_{10}H_{12}NO$ ion), followed by elimination of a carbon oxide molecule (m/z 134.09441, $C_9H_{12}N$ ion), and further decomposition (ion of m/z 118.06396, C_8H_8N). Two low mass ions (m/z 83.082365 and 69.06885) represented residuals of an aromatic ring (C_6H_{11} and C_5H_9 respectively).

Accurate mass and corresponding molecular formula of TP8 (m/z 182.11662 and $C_{10}H_{16}NO_2$ respectively) suggested that loss
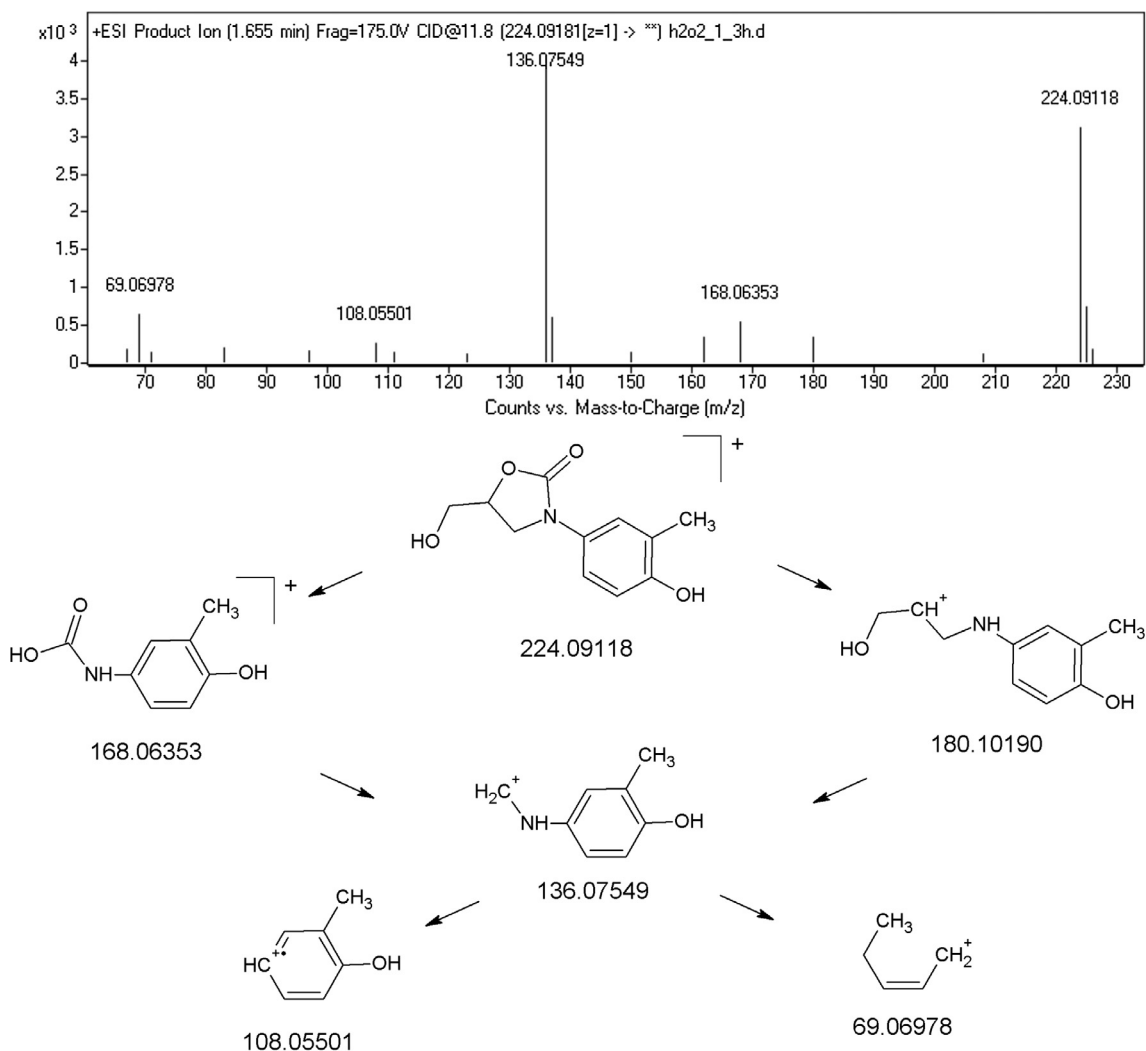

Fig. 4. Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP2.

of carbon oxide took place in case of this product. This assumption was supported by its fragmentation spectrum (Fig. 10), consisting of four main peaks, representing fragments similar to those observed in the case of toloxatone. Firstly elimination of a water molecule took place, and ion of m/z 164.10613 ($C_{10}H_{14}NO$) was formed. Then next water molecule was eliminated, which resulted in formation of m/z 146.09675 ($C_{10}H_{12}N$) ion, and was followed by gradual decomposition of propenyl chain (ions of m/z 134.09491, $C_9H_{12}N$ and 120.07984, $C_8H_{10}N$). The last stage was formation of m-toluidine (m/z 108.08078, $C_7H_{10}N$).

The suggested transformation pathway of toloxatone in the investigated stress conditions was shown in Fig. 11.

3.3. Chemometric study

In order to perform the multivariate chemometric analysis all the obtained chromatographic profiles (21 chromatograms) registered in TOF (MS) mode were aligned with MPP software giving 62 entities. After a build-in MPP filtration including sample abun-

dance, setting the fold change (FC) threshold on the level not less than 4 and one-way ANOVA statistical test (p = 0.05), 31 entities were finally selected for the chemometric study. The PCA analysis based on this data showed a visible categorization of all the analyzed groups of the forced degradation samples (Fig. 12). Basic (NaOH) stressed samples stood out from the other samples while neutral (H_2O) and acidic (HCl) stressed samples were very close to each other and to control samples without degradation (STD). It should be also noticed that the samples subjected to UVC irradiation visibly stood out from the ICH photolytic conditions (UV–VIS) and are placed between oxidative (H_2O_2) and UV–VIS stressed samples. In the presented PCA analysis the first three components (PC) explained 70.7% of the total variance.

3.4. Computational estimation of toxicity

In order to estimate and compare toxicity of toloxatone as well as its TPs, carcinogenic and mutagenic potential, and acute toxicity to rodents was calculated.
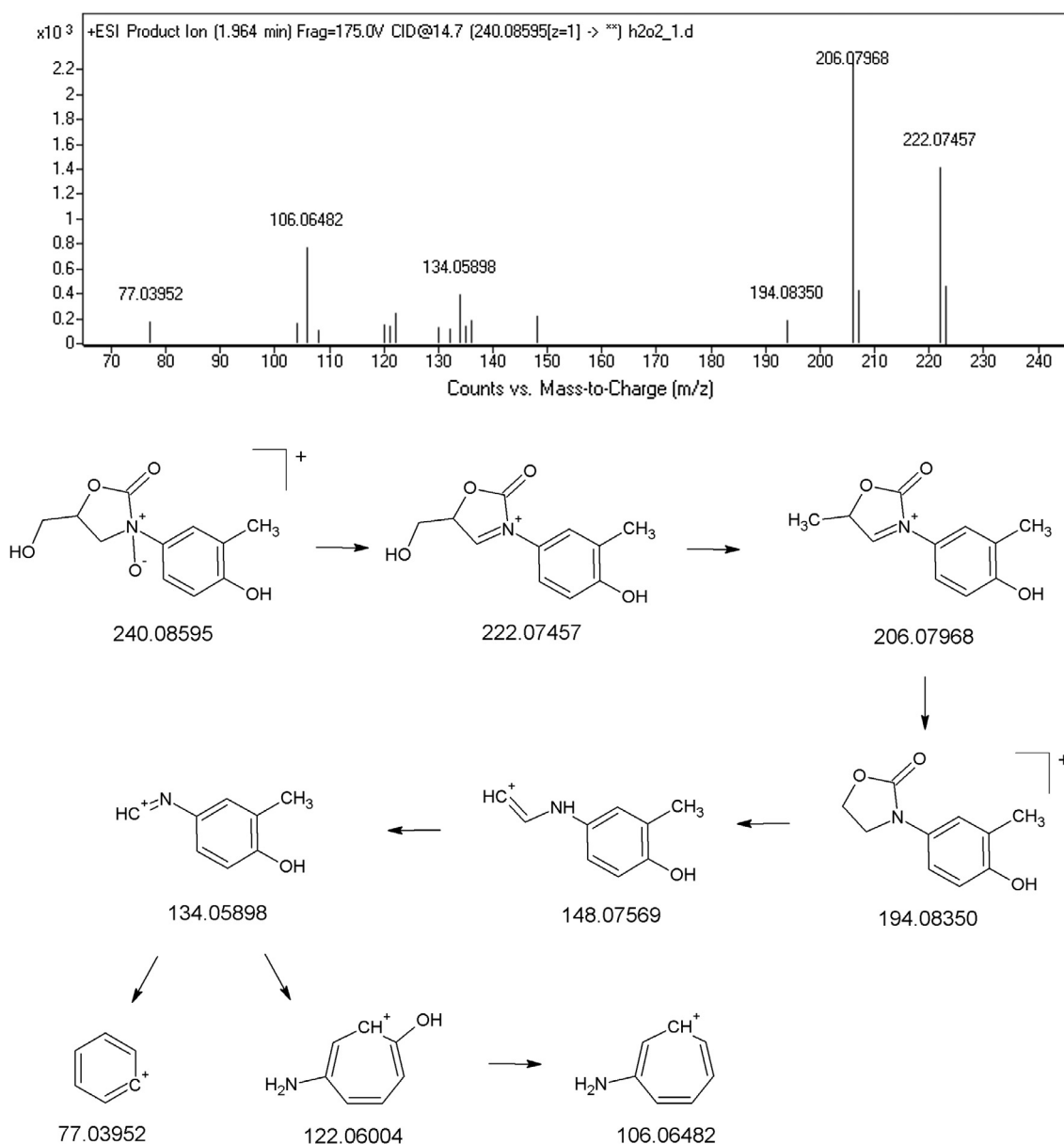

Fig. 5. Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP3.
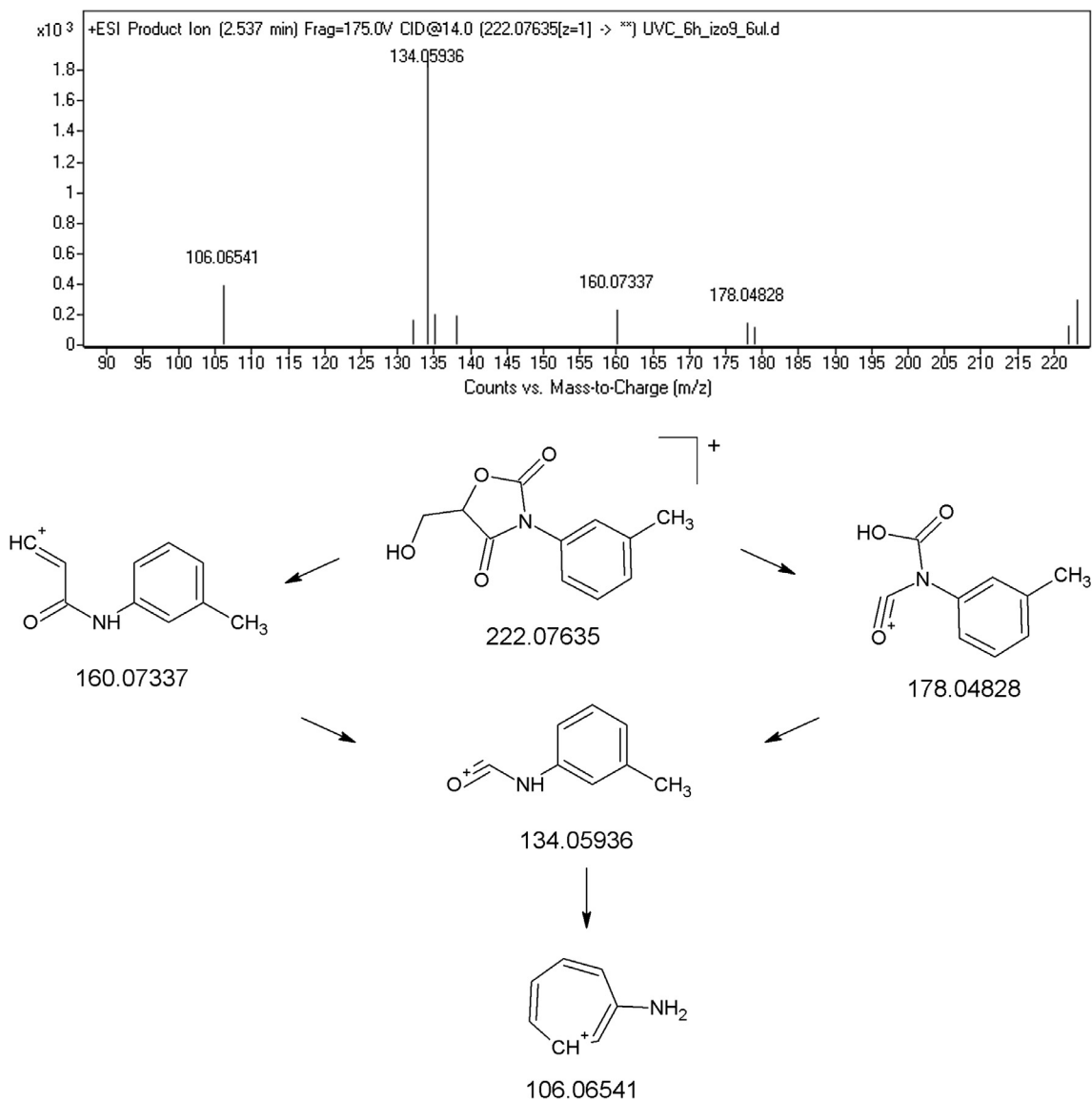
Fig. 6. Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP4.

Probability of carcinogenic activity was estimated with the use of CAESAR 2.1.9 model provided by Vega software (version 1.1.1). According to this model, most of identified compounds possessed lower carcinogenic potential than the parent compound (probability equaled 0.71, Supplementary data – Table S1). Higher potential was predicted only for one compound (TP5).

Mutagenicity (probability of positive Ames test) was predicted by model included in Percepta software (ACD/Labs). Similarly as in the case of carcinogenicity, majority of TPs was marked by lower mutagenic potential than toloxatone (probability equaled 0.33). Only two compounds – TP1 and TP5 – possessed higher mutagenic potential. Nevertheless even in their case the probability did not exceed 0.5 (Supplementary data – Table S2).

Percepta software was also used to estimate acute toxicity to rodents. In this case six models were applied, four for mice (intraperitoneal, intravenous, subcutaneous and oral), and two for rats (intraperitoneal and oral). In order to facilitate interpretation of calculated results (Supplementary data – Table S3), principal component analysis of obtained matrix was performed. This chemometric tool enables reduction of data dimensionality, and visualization of relationships between samples (compounds) as

well as variables (toxicity endpoints). As was shown in Fig. 13A beside two pairs, Mouse Oral – Rat Oral and Mouse Intravenous – Mouse Subcutaneous, variables were rather weakly correlated. Variables representing Rat Intraperitoneal and Mouse Subcutaneous toxicity were almost orthogonal (cos θ close to 0). Points representing TP2 and TP4 were plotted close to toloxatone, which corresponded to their similar properties. TP6 and TP8 were less toxic according to all of the applied models (variables values increase in parallel with LD_{50}). Three compounds placed in upper-right part of the plot (TP1, TP3 and TP7) were more toxic that the parent compound. TP5, the most outlying compound, was less toxic according to Mouse Intravenous and Mouse Subcutaneous models, but more toxic when other models were taken into account.

The overall toxicity was estimated by performing of PCA on set of three variables, one from each toxicity category. As it can be seen form Fig. 13B TP6 was very similar to the parent compound (high carcinogenic potential, low toxicity to rodents and moderate mutagenicity). Amongst two outlying products, TP5 was highly mutagenic and carcinogenic, while its toxicity to mice was low. The second outlier, TP1, possessed moderate mutagenic and
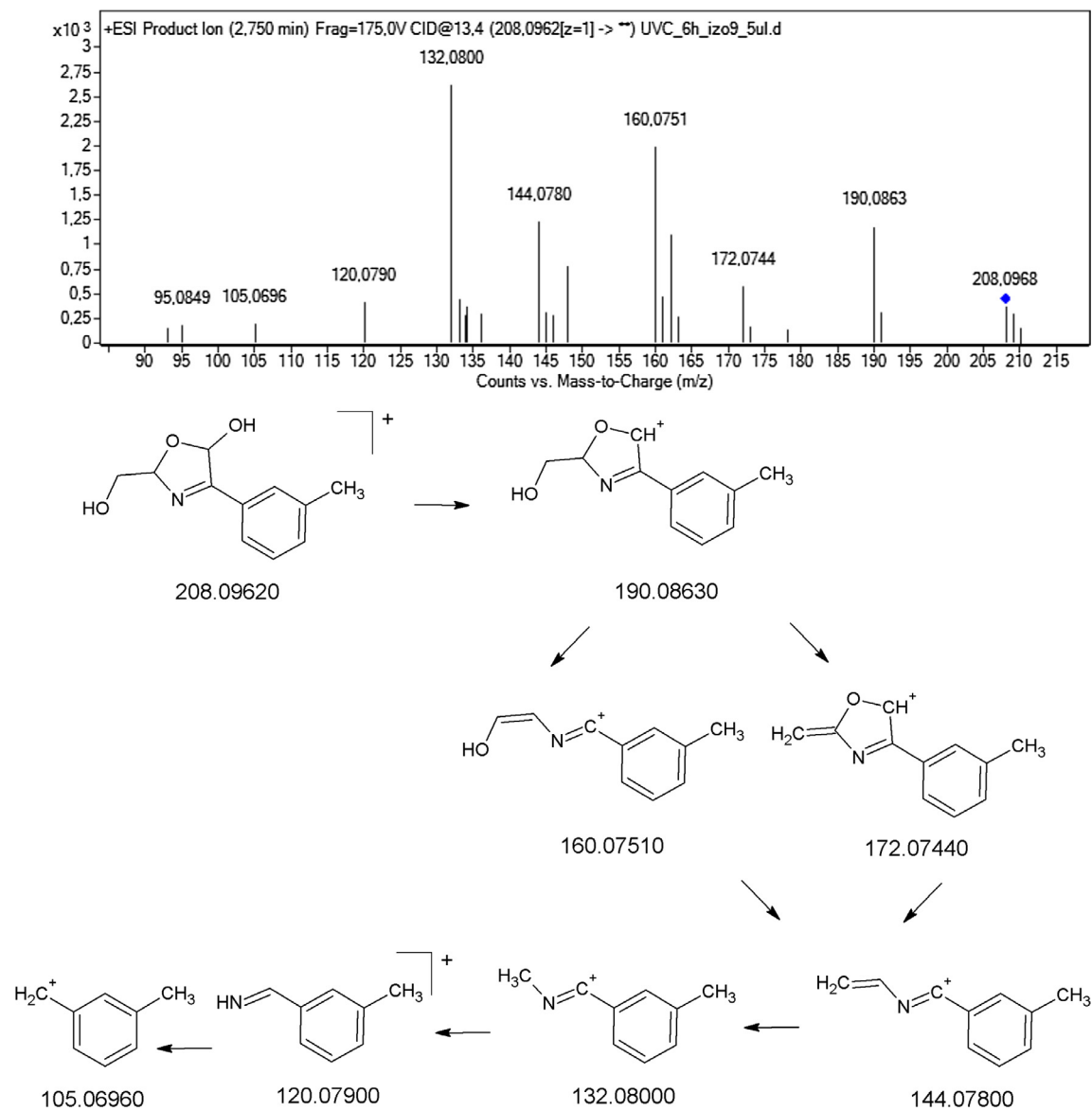
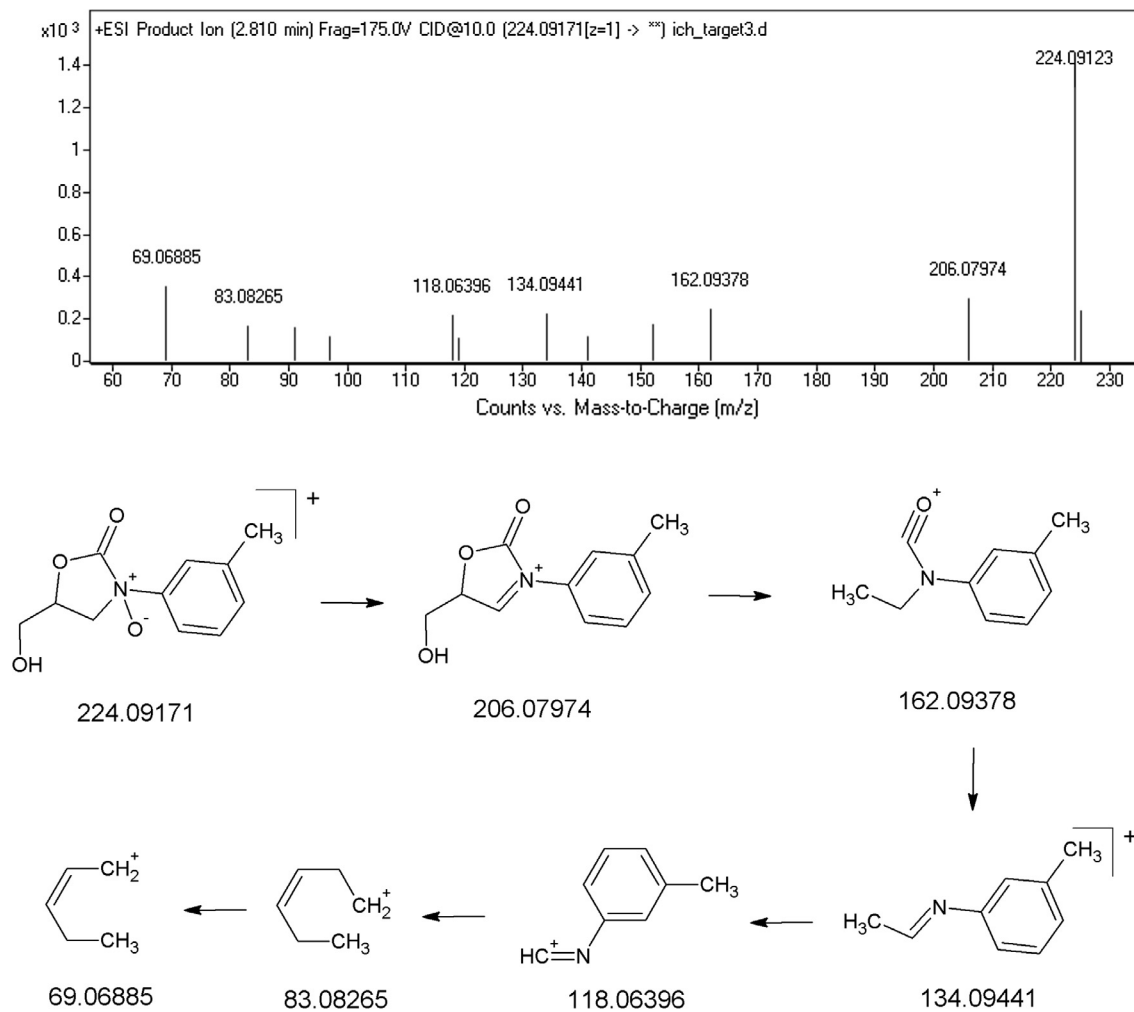**Fig. 7.** Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP5.

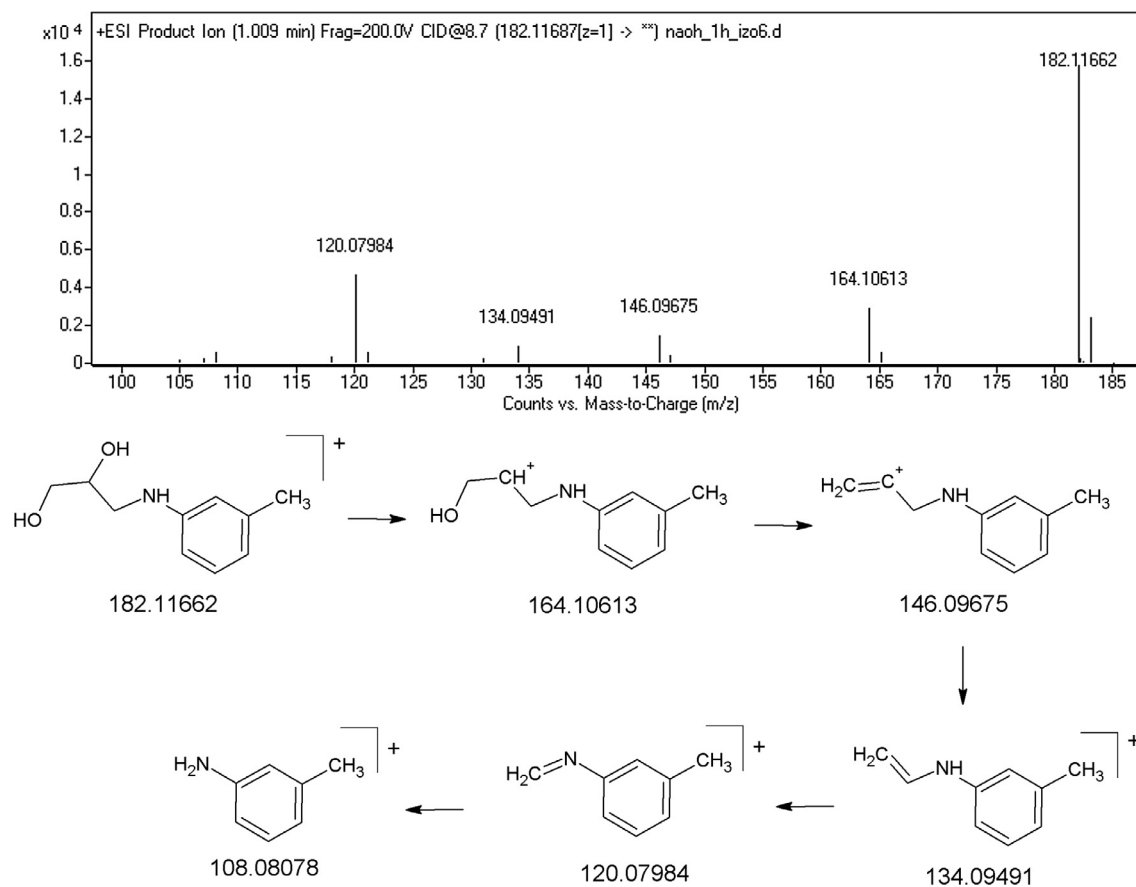**Fig. 8.** Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP6.

Fig. 9. Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP7.

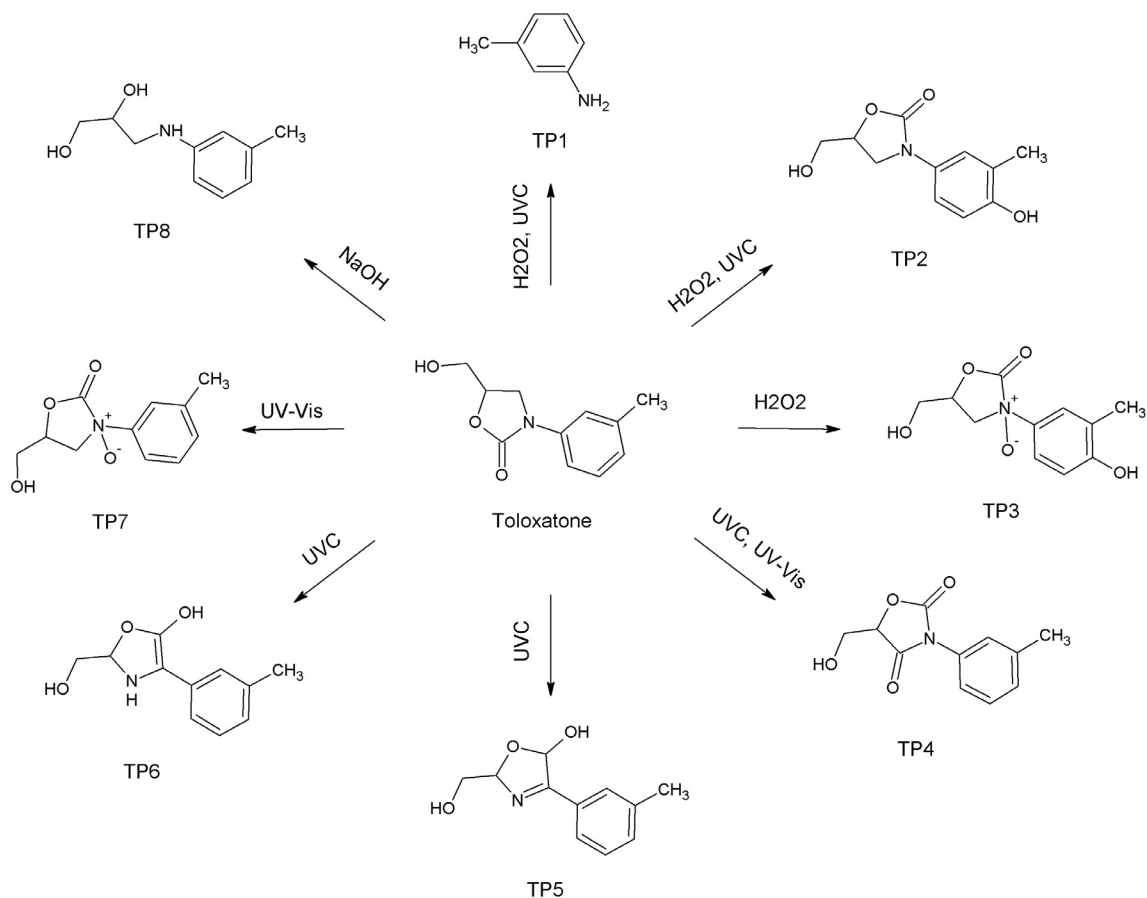**Fig. 10.** Q-TOF MS/MS spectrum and fragmentation pathway of degradation product TP8.

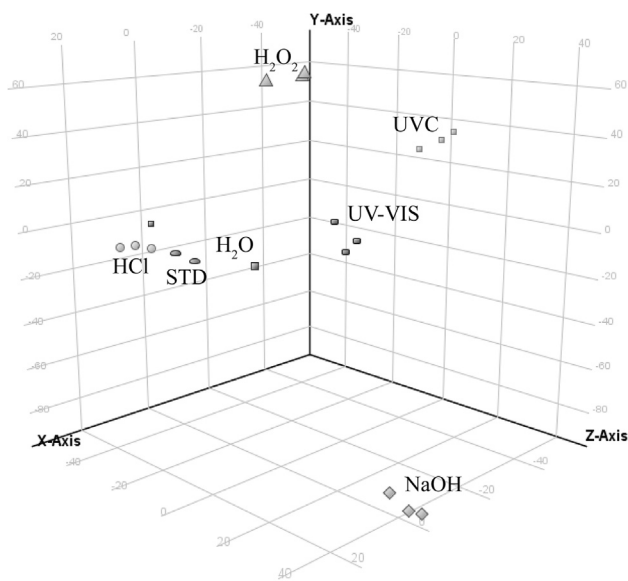**Fig. 11.** Forced degradation pathway of toloxatone in tested conditions.

Fig. 12. The 3D PCA plot of forced degradation profiles of toloxatone in tested conditions.

carcinogenic potential, and very low toxicity to mice. Most TPs were placed in upper part of the plot, which corresponded to their low mutagenic and carcinogenic potential, and low to moderate toxicity to mice.

4. Conclusion

The degradation behavior of toloxatone under hydrolytic – acid, base and neutral, oxidative and photolytic – as per ICH guidelines and UVC stress was studied. It was observed that the tested antidepressant drug is fragile towards basic hydrolysis, oxidative conditions and UVC irradiation. Eight degradation products were found and based on MS/MS fragmentation spectra their structural elucidation was performed. The most effective degradation processes in terms of the number of transformation products was UVC photolysis and five TPs (TP1, TP2, TP4, TP5, TP6) were found in this case. Two of them (TP5 and TP6) were formed only in these conditions. The other three identified transformation products are characteristic for sole degradation conditions – oxidative (TP3), base (TP8) and UV–VIS photolytic (TP7).

The multivariate chemometric analysis (PCA) allowed effortless characterization of the registered degradation profiles which can
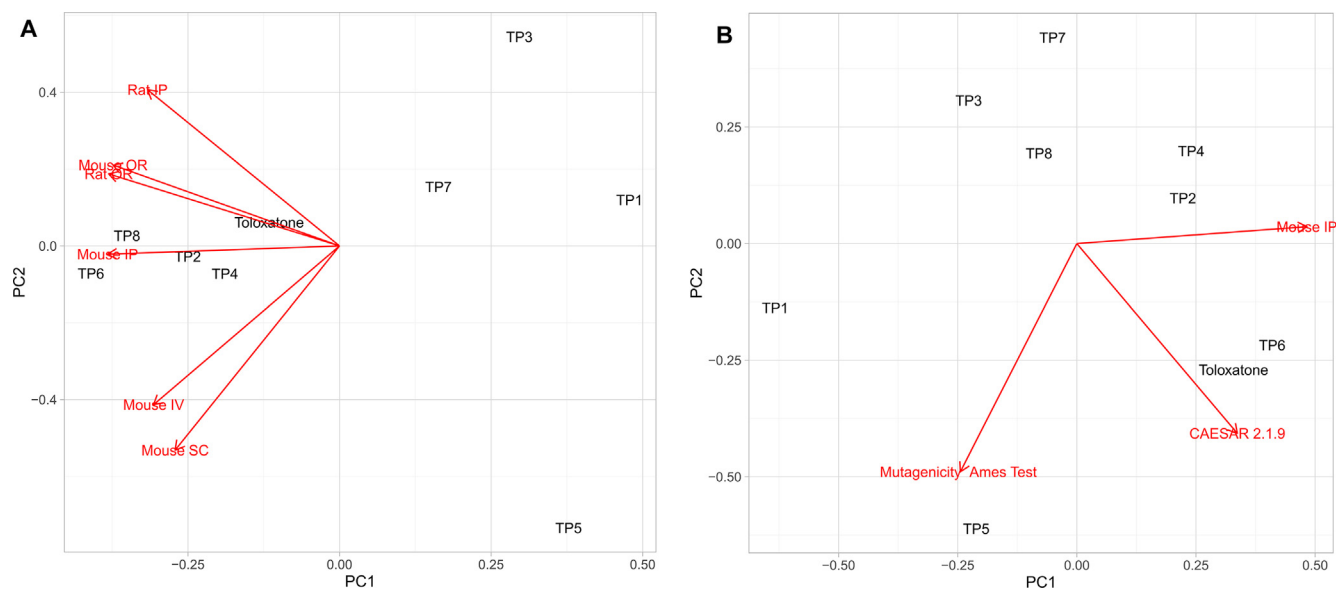
Fig. 13. Comparison of toxicity of forced degradation products by PCA: acute toxicity to rodents, OR – Oral, IV – Intravenous, S.C. – Subcutaneous, IP –Intraperitoneal (A); overall toxicity (B).

be a useful method for a fast preliminary degradation study of drugs.

Additionally, the toxicity of the characterized degradation products was estimated with the use of *in silico* methods and compared with multivariate chemometric method. Two of these products (TP1 and TP5) were found as more toxic that the parent compound and the other transformation products.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jsps.2018.02.012.

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