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The Force Degradation Study of the Morpholinium 2-((4-(2-Methoxyphenyl)-5-(Pyridin-4-YL)-4*H*-1,2,4-Triazol-3-YL)Thio) Acetate

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ABSTRACT

The aim of the current research was to make force degradation study of the morpholinium 2-((4-(2-methoxyphenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)thio) acetate (APII) for additional confirmation of the selectivity of API and impurity determination method, propose possible structures of degradation products. Agilent 1260 Infinity (degasser, binary pump, autosampler, column thermostat, diode array detector) was used. Mass selective detector was single-quadrupole LC/MS 6120 with electrospray ion source (ESI). The chromatography study was carried out by isocratic elution with a water-acetonitrile mixture (84:16) with the addition of 0.1% methanoic acid. A column Zorbax SB-C18, 30mm x 4.6mm, 1.8um. A column temperature was 40°C. The flow rate was 0.400mL/min. The period of exposition of stress factors was four days. The period of exposition of stress factors was four days. Influence of the such factors as acid, alkali, H_2O_2, temperature, UV radiation on the API in bulk drug, 0.1% solution and 1% "solution for injection" were studied. Dependence of content of APII from a number of days of factors exposition was studied. Most destructive action was during the influence of the H_2O_2 and UV radiation. APII was stable during the storage at laboratory conditions, acid, alkali and temperature (60°C) influence. Some degradation products structure was proposed.
Keywords: 1,2,4-triazoles, LC MS, forced degradation.

INTRODUCTION

Studies of the accelerated degradation of active pharmaceutical ingredients in the bulk drug and dosage forms are necessary to confirm the selectivity of the method of determination in the presence of degradation products and for an elaboration of a stability-indicating assay. Also, this study allows predicting the influence of the environment on the medicinal substance and offering storage conditions. There are requirements for research methods for forced degradation of drugs (ICH guidelines Q1A (R2), 2003). According to the ICH guidelines, stress researches of drug degradation allow make qualification of probable decomposition products, determining the pathway of degradation, establishing the stability of the molecule, confirming the quality of the analytical procedure. Degradation depends on whether an individual substance or drug is being investigated. The necessary factors to study is the effect of temperature, pH, oxidizer action, light influence. Many of review articles discussed such researches (Sehrawat *et al.*, 2010; Blessy *et al.*, 2014; Klick *et al.*, 2005; Reynolds *et al.*, 2002; Rawat *et al.*, 2015).

Numerous articles were devoted to study of the forced degradation of active pharmaceutical ingredients in the bulk drug and dosage forms. Authors (Bhardwaj *et al.*, 2008) studied the effects of stress factors on enalapril maleate according to the ICH guidelines (ICH guidelines Q1A (R2), 2003). Based on studies using LC-MS, a scheme for the decomposition of API under the influence of stress factors was proposed. Scientists (Nageswara *et al.*, 2010) researched the influence of stress factors on ritonavir using LC–MS/MS. Researchers conducted a study on the stress decomposition of ropinirole hydrochloride in its tablets (Parmar *et al.*, 2009).

Morpholinium 2-((4-(2-methoxyphenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)thio) acetate is an active pharmaceutical ingredient (API) of

neuroprotective drug with adaptogenic properties (Kaplaushenko, 2012).

The methods for the determination of this substance were published. However, they are insensitive, low-specific and time-consuming. The HPLC method for APII determination, it is possible to determine only one impurity, the possibility of determination other impurities and stress products was not considered in it. The possibility of quantitative spectrophotometric determination of APII in solutions was considered, but this technique does not allow determination of impurities (Kaplaushenko *et al.*, 2009).

A method by using potentiometric titration for determination of the APII in the bulk drug was elaborated. The technique was not specific, it is impossible to determine degradation products. LC–MS method with APCI ionization for APII and impurities detection was proposed. Degradation products were not qualified. Method was not validated. The normalization method was used for estimation of the content of the API. It is not permit to determine quantity of the API (Kaplaushenko *et al.*, 2010).

Previously new chromatography conditions of API and impurities determination were proposed (Varynskyi *et al.*, 2017, 2018). The method was selective, sensitive, precise, accurate and fast. The complete selectivity study of the method has to include force degradation study. Need to check interference of degradation products and APII. The aim of the current research was to make force degradation study of the morpholinium 2-((4-(2-methoxyphenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (APII) for additional confirmation of the selectivity of API and impurity determination method (Varynskyi *et al.,* 2017, 2018), propose possible structures of degradation products.

MATERIAL AND METHODS

Chemicals and reagents

The APII was synthesized at Physical and Colloidal Chemistry Department. Structure of it was confirmed by Kaplaushenko (2012).

Acetonitrile qualified "HPLC Super Gradient" (Avantor Performance Materials Poland S.A., Poland), methanoic acid was 100% (AppliChem GmbH, Germany), ultra-high pure water (18 MΩ at 25 °C) was prepared by the Direct Q 3UV Millipore (Molsheim, France).

Analytical Instrumentation

Agilent 1260 Infinity (degasser, binary pump, autosampler, column thermostat, diode array detector) OpenLAB CDS Software. Single-Quadrupole LC/MS 6120 with electrospray ion source (ESI).

Chromatography conditions

The chromatography study was carried out by isocratic elution with a water-acetonitrile mixture (84:16) with the addition of 0.1% methanoic acid. A column Zorbax SB-C18, 30 mm x 4.6 mm, 1.8 um. A column temperature was 40°C. The flow rate was 0.400 ml/min.

Mass spectrometry conditions

Temperature of drying gas was 138 °C. Drying gas (nitrogen) flow rate was 10L/min. Nebulizing gas (N_2) pressure was 55 psig. Mass spectra were obtained at m/z 100-2000. Fragmentation of molecular ions was studied at fragmentor voltage: 100, 150, 200 V, positive polarity.

Forced degradation conditions

Samples were taken every day, prepared for injection and injected into the HPLC system. Volume of injection for 0.1% solution was 2uL, for 1% solution was 0.5uL. Content (%) was taken from the report of OpenLab CDS Software. The signal of the DAD detector was acquired at 272nm.

Laboratory conditions degradation

Substance and solutions (0.1%, 1%) were stored at room temperature in laboratory conditions.

Thermal degradation

The temperature influence was studied in the thermostat at the 60°C for the 0.1%, 1% solutions, and substance.

Oxidative degradation

Hydrogen peroxide (3%) was used for a study of the influence of oxidizing agent. About 0,001 g of API was dissolved in the 1 mL of 3% hydrogen peroxide.

Ultraviolet (UV) degradation

The irradiation was carried out with the help of a luminescent UV lamp, YF UV-9W 365 nm, which radiates in the range of long wavelength ultraviolet with maximum radiation of 365 nm. The illumination was measured with a luxmeter and was approximately 2000 lux. Bulk drug and solutions with concentrations 0.1%, 1% were studied.

Acid hydrolysis

Influence of acid was studied. About 0.001 g of API was mixed with the 1mL of the 0.1mole/L of HCl.

Table I. APII content change during force degradation study

Terms of decomposition	Days				
	0	1	2	3	4
Laboratory conditions, 0.1% solution of APII	99.33	97.92	98.33	98.05	98.32
Laboratory conditions, 1% solution	98.46	98.46	98.59	98.55	98.69
HCl, 0.1M solution	99.33	99.30	99.23	99.23	98.92
NaOH, 0.1M solution	99.33	100	99.36	100	100
3% H_2O_2	99.33	71.68	66.98	62.48	56.66
Thermal effect, 60°C, solution 0.1%	99.33	99.20	99.23	99.22	99.21
Thermal effect, 60°C, solution 1%	98.21	98.24	98.18	98.18	98.13
Thermal effect, 60°C, substance	99.33	98.98	99.30	98.39	99.38
UV light irradiation, solution 0.1%	99.33	96.66	92.82	89.57	76.14
UV light irradiation, solution 1%	98.21	94.30	82.45	76.59	63.48
UV light irradiation, substance	99.33	100.00	100	100	100

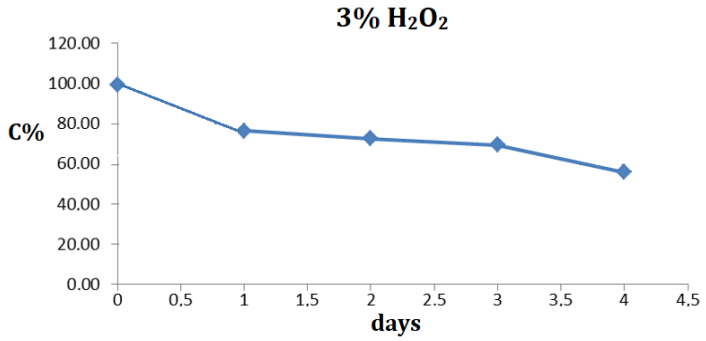


Figure 1. Graph of APII content (C%) dependence from number of days for oxidation by H_2O_2.

Alkaline hydrolysis

About 0.001 g of APII was mixed with the 0.1mole/L sodium hydroxide solution.

Preparation of solutions for laboratory conditions degradation study, thermal decomposition study, UV degradation study.

A solution with concentration 0.1% was prepared by dissolution of 0.001 g of APII in 1mL of water. A solution with concentration 1% was prepared according to pharmaceutical preparation "1% solution for injections", viz. 0.01g of APII was dissolved in the 1mL of water, 0.0069g of sodium chloride was added for isotonicity.

If the solid substance was studied, 0.001g was dissolved in 1 mL water and 2uL of the solution was injected into the HPLC. The period of exposition of stress factors was four days.

RESULTS AND DISCUSSION

Elution of the APII by the water-acetonitrile mixture (84:16) with the addition of 0.1% methanoic acid leads to formation of the 2-((4-(2-methoxyphenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl) thio) acetic acid. Therefore, the detecting of the acid takes place directly on the detector. The APII was determined in form of the acid.

The results of the study of forced degradation (Table I). Mass balance,% (content of the main substance,% plus content of products of degradation and impurities,%) in all cases was equaled 100%. When storing 0.1% of the reference APII solution in the laboratory conditions the content of the main component was decreased at about 0.1% for four days. The content of the APII in 1% solution under these conditions was not changed for four days.

The APII content was not changed for four days under the action of the 0.1M solution of chloride acid. The content of the main component was not changed during the action of the 0.1M sodium hydroxide solution for four days. The effect of 3% hydrogen peroxide over four days results in a decrease the APII concentration by more than 40% (Figure1).

Thermal effect (60°C) on 0.1% and 1% API solution leads to its degradation about 0.1% within four days. The APII content during the thermal influence on the bulk drug was not changed.

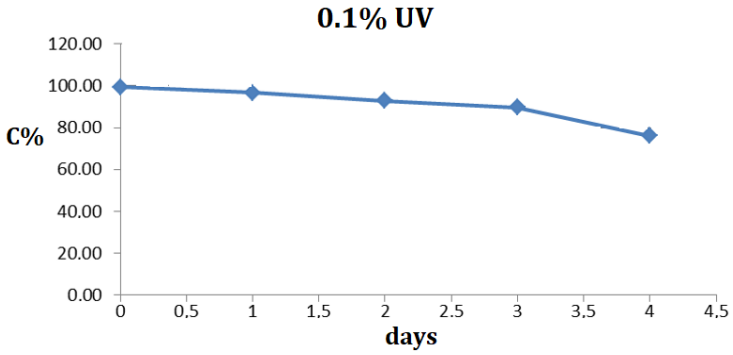


Figure 2. Graph of APII content (C%) dependence from number of days for ultraviolet influence on the 0.1% solution.

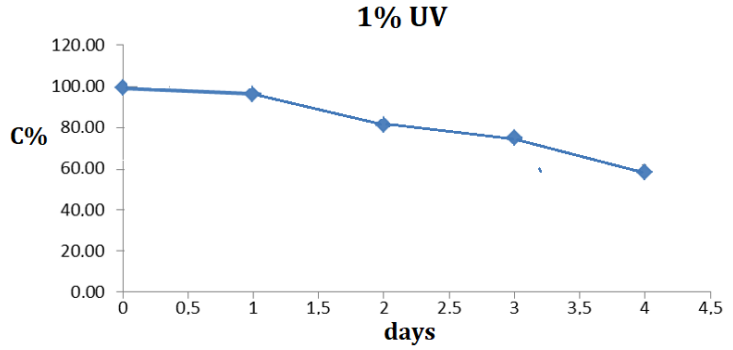


Figure 3. Graph of APII content (C%) dependence from number of days for ultraviolet influence on the 1% solution.

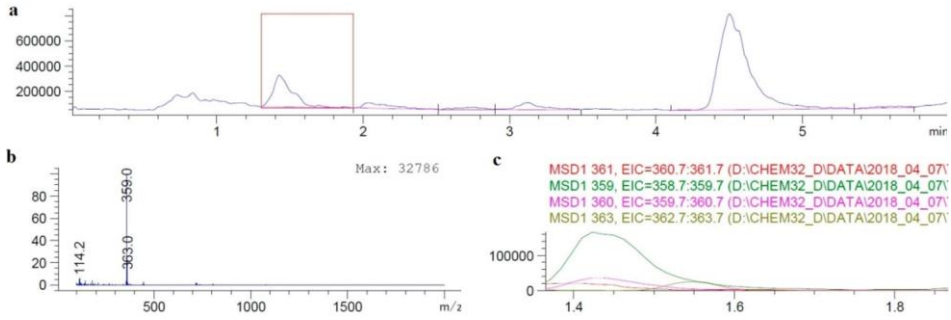


Figure 4. TIC chromatogram after four days influence of 3% H_2O_2 at fragmentation 100 V, (a). Mass spectrum for peak at 1.435 min (b). EIC chromatograms: main peak at m/z 359 (c).

UV light exposure causes degradation of 0.1% solution over four days to more than 20% (Figure2). At the same time, for 1% solution, the concentration decreases by about 40% (Figure3). The API content was not changed during irradiation of bulk drug for four days.

Oxidation Influence of 3% H_2O_2.

Peaks with retention time 1.4min and 2.0min were contained quasi-molecular ion with m/z 359. They are corresponding to isomeric compounds with molecular mass 358. Probable structure of one isomer is proposed (Figure 6).

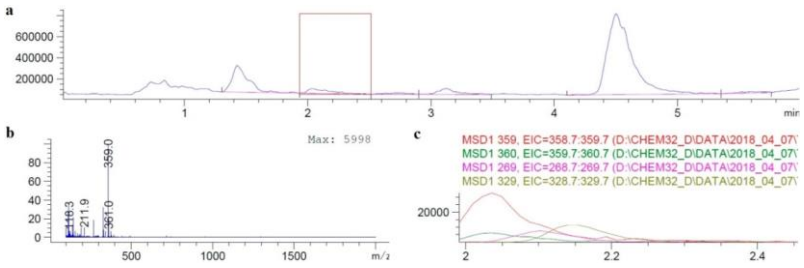


Figure 5. TIC chromatogram after four days influence of 3% H_2O_2 at fragmentation 100 V(a). Mass spectrum for peak at 2.044 min (b). EIC chromatograms: main peak at m/z 359 (c).

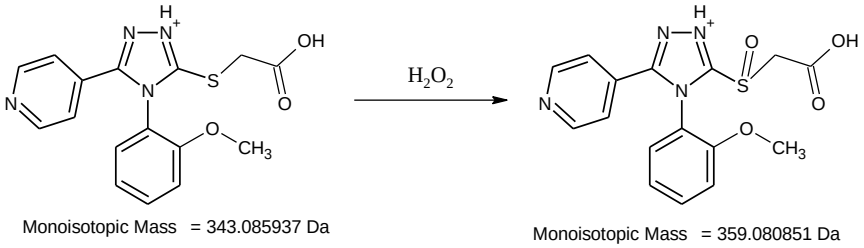


Figure 6. Oxidation of the APII by the 3% H_2O_2 with formation of the sulfoxide

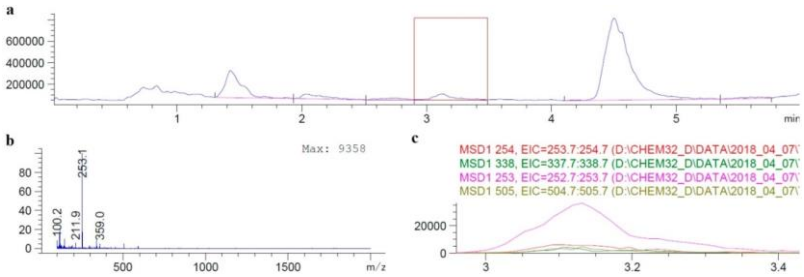


Figure 7. TIC chromatogram after four days influence of 3% H_2O_2 at fragmentation 100 V(a). Mass spectrum for peak at 3.122 min (b). EIC chromatograms: main peak at m/z 253 (c).

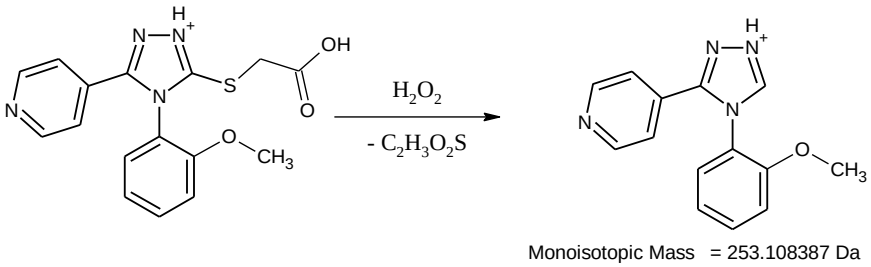


Figure 8. Possible reaction of elimination of the thioacetate fragment from the APII by the action of 3% H_2O_2

It is well-known that organic sulfides react with H_2O_2 to form sulfoxides (Tietze *et al.*, 1991).

The compound which corresponds to the quasi-molecular ion with m/z=253 (retention time 3.1min) was formed during cleavage of the chemical bond between sulfur and triazole cycle.

The trace component with m/z 375 was detected in products with retention time about 2min. It corresponds to the sulfone. So the reaction of formation of sulfone proceeds insignificantly (Figure 9). The traces of APII decarboxylation result were found in products of oxidation.

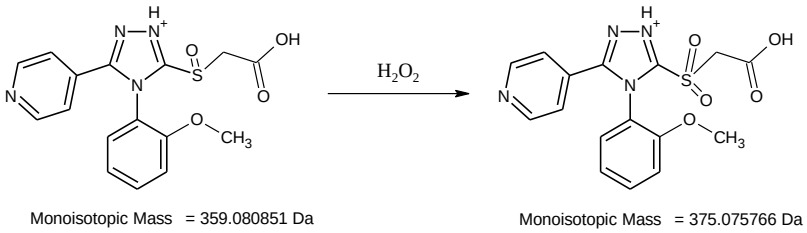
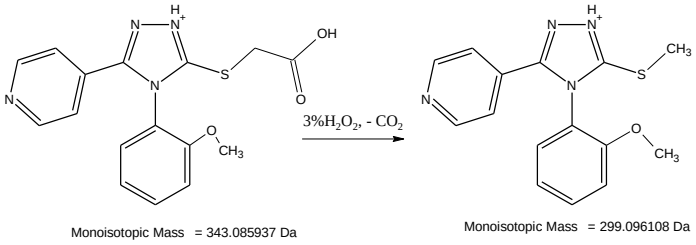
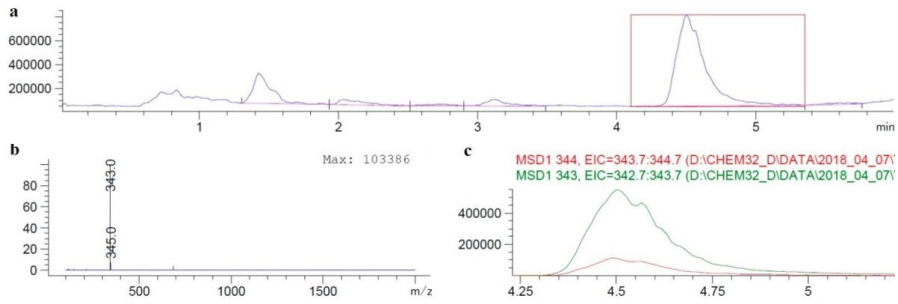


Figure 9. Formation of sulfone in the reaction of oxidation of APII by the 3%H_2O_2



Figue 10. Decarboxylation of the APII by the 3%H_2O_2, four days of influence.



Figue 11. Peak purity of APII peak after four days influence of 3%H_2O_2.

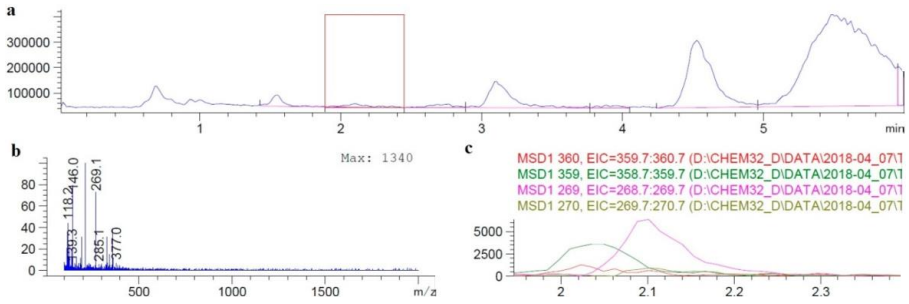


Figure 12. TIC chromatogram after four days UV influence at fragmentation 100 V(a). Mass spectrum for the a peak at 2.101 min (b). EIC chromatogram: main peaks are m/z 359 and m/z 269(c).

It detected as an ion with m/z 299 and retention time about 5.5min (Figure 10). Peak purity (Figure 11) was shown that the peak of APII was a pure peak after four days of irradiation. This was confirming that products of oxidation were not interfering with the determination of APII. So the method of APII determination presented earlier is selective (Varynskyi *et al.,* 2017, 2018). They confirmed that the molecular weight of this degradation product was 298.1.

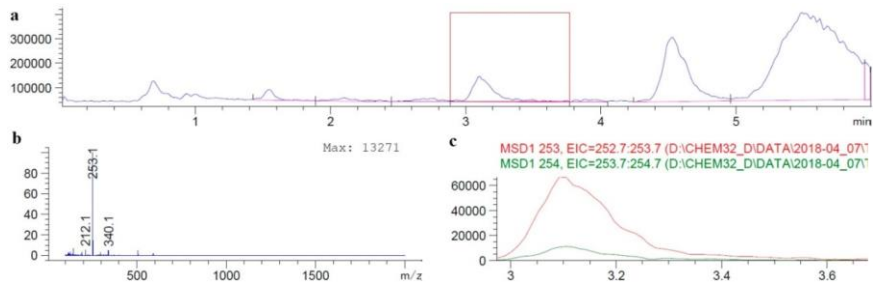


Figure 13. TIC chromatogram after four days UV influence at fragmentation 100V, (a). Mass spectrum for a peak at 3.100min (b). EIC chromatogram: a main peak at m/z 253, a peak of the dimer ion m/z 505.1 (c).

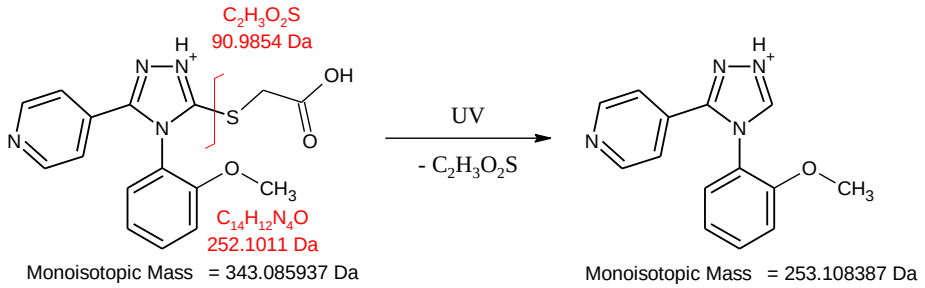


Figure 14. Thioacetate cleavage from API during the UV influence

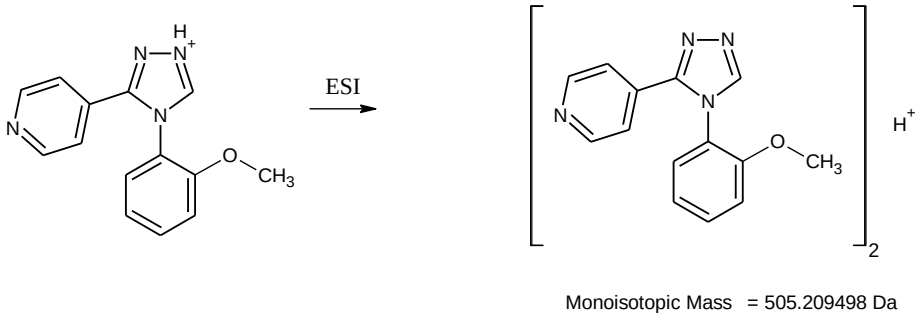


Figure 15. Dimerization in ESI of the ion with m/z = 253.1

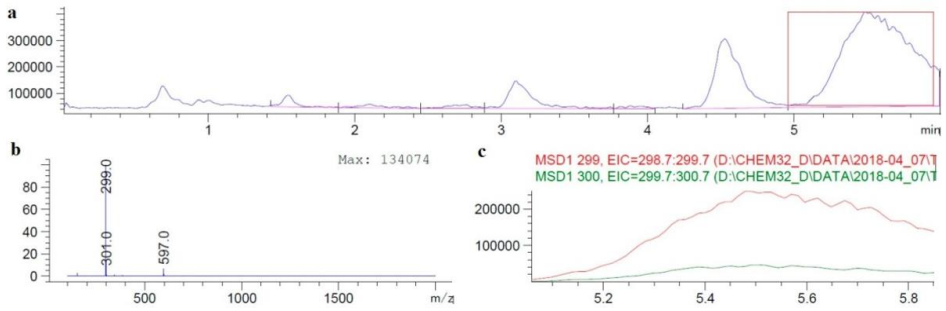


Figure 16. TIC chromatogram after four days UV influence at fragmentation 100V, (a). Mass spectrum for peak at 5.504 min (b). EIC chromatogram: main peak at m/z 299(c).

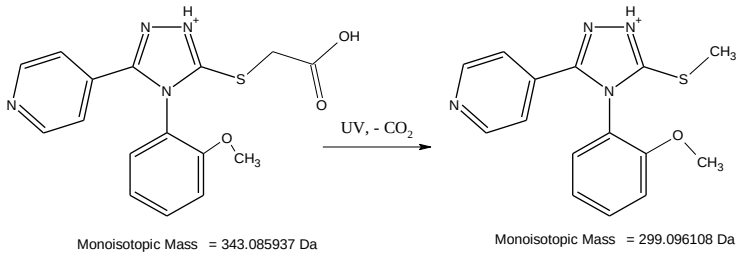


Figure 17. The product of UV decarboxylation of the APII correspondent to the peak at 5.504 min, four days of UV irradiation.

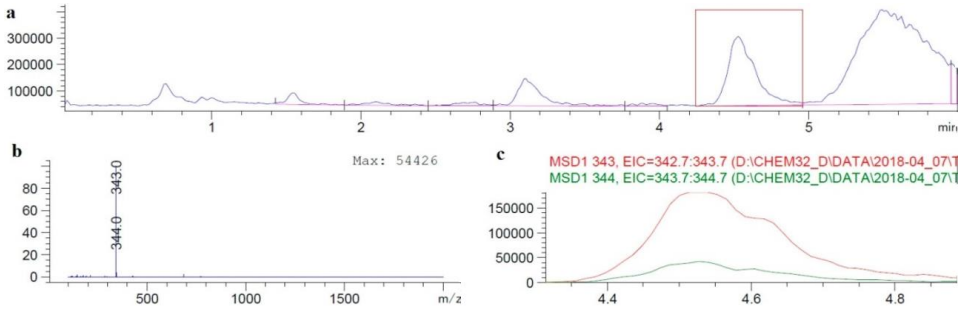


Figure 18. Peak purity of APII peak after four days of UV irradiation

Table II. Impurities were formed in stress conditions.

#	Compound	3% H_2O_2	UV	Retention time, min	m/z quasimolecular ion	Monoisotope molecular weight
0	API			4.5	343	342
1	5-[(carboxymethyl) sulfinyl)-4-(2-methoxyphenyl) -3-(pyridin-4-yl]-4*H*-1,2,4-triazol-1-ium	+*	+	1.4	359	358
2	4-(2-methoxyphenyl)-3-(pyridin-4-yl)-4*H*-1,2,4-triazol-1-ium	+	+	3.1	253	252
3	5-[(carboxymethyl) sulfonyl)-4-(2-methoxyphenyl)-3-(pyridin-4-yl]-4*H*-1,2,4-triazol-1-ium	traces	-**	~2	375	374
4	4-(2-methoxyphenyl)-5-(methylthio)-3-(pyridin-4-yl)-4*H*-1,2,4-triazol-1-ium	traces	+	5.5	299	298

*Substance was found in degradation products; **Substance was absent in degradation products

Influence of the UV irradiation.

The peaks of degradation products much strictly recognized after four days of irradiation. The peak with m/z 359 and retention time is about 2min (Figure 12) is same as peak which presents in degradation products appeared by the action of 3% H_2O_2 (Figure 5, 6). In this case, a possible product of degradation of APII is sulfoxide. A substance (m/z = 253.1) which was eluted at 3.1min was identified (Figure 13). It has the same structure as the product of oxidation by H_2O_2 (Figure 7, 8). Formation of the dimer ion in the ESI source with m/z 505.1 is additional confirmation (Figure14, 15).

UV irradiation was a cause of the decarboxylation of the APII to product the methylthiocompound with retention time about 5.5 (Figure16, 17). In spectra of this compound were presented quasi-molecular ion ($[M+H]^+$) with m/z=299.1 and dimer ion ($[2M+H^+]$) with m/z 597.

During UV irradiation also were formed different products of degradation. That is why was proposed to keep solutions in dark place. Peak purity after four days of the UV irradiation was presented at Figure 18. It was shown that is a peak of API was pure. Products of UV degradation were not interfering with API determination. It is additional proof of method selectivity which presented earlier (Varynskyi *et al*., 2017; Varynskyi *et al.,* 2018).

CONCLUSIONS

Influence of the such factors as acid, alkali, H_2O_2, temperature, UV radiation on the morpholinium 2 -((4-(2- methoxyphenyl) -5-(pyridin-4-yl) -4*H*-1,2,4-triazol-3-yl)thio)acetate in bulk drug, 0.1% solution and 1% "solution for injection" were studied. Dependence of content of APII from a number of days of factors exposition was studied. Most destructive action was during the influence of the H_2O_2 and UV radiation. APII was stable during the storage at laboratory conditions, acid, alkali and temperature (60°C) influence. Some degradation products structure was proposed. The sulfoxide and sulfone in H_2O_2 oxidation products of degradation are presented. The most abundant UV degradation product was formed due to the API decarboxylation.

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