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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PIPERIDINIUM 2-((5-(FURAN-2-YL)-4-PHENYL-4*H*-1,2,4-TRIAZOL)-3-YL) ACETATE FORCED DEGRADATION STUDY

PİPERİDİNYUM 2-((5-(FURAN-2-İL)-4-FENİL-4H-1,2,4-TRİAZOL)-3-İL) ASETATIN ZORLANMIŞ BOZUNMA ÇALIŞMASI

Borys VARYNSKYI[1,*]

[1]Zaporozhye State Medical University, Pharmaceutical Faculty, Physical and Colloidal Chemistry Department, 69035 Zaporozhye, Ukraine

ABSTRACT

Objective: *Aim of the research to make forced degradation study of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol)-3-yl) acetate in active pharmaceutical ingredient (API), 0.1% solution and 1% solution for injection. Influence of the sodium hydroxide, hydrochloride acid, 3% H_2O_2, temperature, UV radiation on piperidinium 2-((5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol)-3-yl) acetate substance, 0.1% solution and 1% solution for injections was studied.*

Material and Method: *Agilent 1260 Infinity HPLC System. Agilent single-quadrupole mass spectrometer 6120.*

Result and Discussion: *Dependence of the quantitative content of the piperidinium 2-((5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol)-3-yl) acetate from exposition time was determined. The composition of degradation products formed under the action of an oxidizer (3%H_2O_2) was established. The composition of degradation products which were formed under the influence of UV radiation was proposed.*

Keywords: *ESI mass spectrometry; forced degradation study; high pressure liquid chromatography; triazoles*

ÖZ

Amaç: *Bu araştırmanın amacı; aktif farmasötik içerikteki (API) 2-((5-(furan-2-İl)-4-fenİl-4H-1,2,4-trİazol)-3-İl) asetat maddesinin % 0.1 çözeltisinin ve enjeksiyonluk % 1 çözeltisinin zorlanmış bozunmasının incelenmesidir. Çalışma, sodyum hidroksit, hidroklorik asit,% 3 H_2O_2, sıcaklık ve UV radyasyonunda bulunan piperidinyum 2–((5-(furan-2-il)-4-fenil-4H-1,2,4-triazol)-3-il) asetatın % 0.1 çözelti ve enjeksiyonluk % 1'lik çözeltisinde gerçekleştirilmiştir.*

Gereç ve Yöntem: *Agilent 1260 Infinity HPLC Sistemi. Agilent dört kutuplu kütle spektrometresi 6120.*

Sonuç ve Tartışma: *Piperidinyum 2-((5-(furan-2-il)-4-fenil-4H-1,2,4-triazol)-3-il) asetatın kantitatif içeriğinin, maruz kalma süresine bağlı olduğu belirlenmiştir. Oksitleyicinin (% 3 H_2O_2) etkisi altında oluşan*

* **Corresponding Author / Sorumlu Yazar:** Borys Varynskyi
e-mail: varinsky@zsmu.zp.ua

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bozunma ürünlerinin bileşimi gösterilmiştir. UV radyasyonunun etkisi altında oluşan bozunma ürünlerinin bileşimi önerilmiştir.

Anahtar Kelimeler: *ESI kütle spektrometresi; triazoller; yüksek basınçlı sıvı kromatografisi; zorlanmış bozunma çalışması*

INTRODUCTION

Piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate is the active pharmaceutical ingredient of drug "Tryfuzol" (API). It is used in veterinary as an immunomodulatory agent. It increases the resistance of organisms to viral diseases. Forced degradation conditions create the model influence of various environmental factors on the active substance. In these conditions, various impurities may be formed in the decomposition, which may alter or weaken the biological activity of the active compound, as well as increase toxicity. Thus, it is possible to predict which impurities may be generated during the storage or transportation of drugs containing the investigated API. It will also help to offer conditions for the protection of this substance from the influence of harmful factors. Therefore, this study has a significant relevance.

Methods for investigating force degradation effects have been described in a number of publications [1-6]. Regulatory aspects in Development of Stability-Indicating Methods were presented in the review of Renu Sehrawat *et al.* [1]. The condition for stress degradation which usually studied are: acid hydrolysis, base hydrolysis, thermal hydrolysis, oxidation, thermal degradation, photodegradation.

Authors [7] proposed potentiometric titration method for quantitative determination of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate in the 1% and 2.5% solutions. Method is not selective and it is not applicable for determination of impurities. Method based on adsorbtion of this API in the ultraviolet region of the spectrum was elaborated [8]. Low selectivity and sensitivity of the method are not permit to measure of impurities.

Our HPLC-DAD method of determination of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate in 1 % solution shown satisfied quality of separation of API from impurities [9]. This work was not contained forced degradation study.

Aim of the research to make forced degradation study of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate in active pharmaceutical ingredient, 0.1% solution and 1% solution for injection.

MATERIAL AND METHOD

Chemicals and reagents

Piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl)acetate was obtained from Toxicological and Inorganic Chemistry Department. Substance was synthesized and its structure was confirmed by the Parchenko V.V. [10,11]. 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 HPLC System (degasser, binary pump, autosampler, thermostat column compartment, DAD). Agilent single-quadrupole mass spectrometer 6120 with electrospray ion source (ESI); OpenLAB Software CDS.

Chromatography conditions

The chromatography study was carried out by elution with a water-acetonitrile mixture (70:30) with the addition of 0.1% methanoic acid. Column Zorbax SB-C18, 30 mm x 4.6 mm, 1.8 um. Column Temp. 40 ^{o}C. Flow rate was 0.400 ml/min.

Mass spectrometry conditions

Temperature of drying gas was 100 ^{o}C. Drying gas (nitrogen) flow rate was 10 l/min. Nebulizing gas (N2) pressure was 53 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 HPLC system. Volume of injection for 0.1% solution was 5 uL, for 1% solution was 0.5 uL. Content (%) was taken from the report of OpenLab CDS Software from Signal of the DAD detector at 276 nm.

Laboratory conditions degradation

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

Thermal degradation

Influence of temperature was studied in the thermostat at the 66 ^{o}C for the 0.1%, 1% solutions and substance. The samples were kept at 66 ^{o}C during 5 days.

Oxidative degradation

Hydrogen peroxide (3%) was used for 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 by the luminescent UV lamp, YF UV-9W 365 nm, which radiates in the range of long-wavelength ultraviolet with a maximum radiation of 365 nm. The illumination was measured with a luxmeter and was approximately 2000 lux. Solid substance and solutions with concentrations 0.1%, 1% were studied. Maximal period of exposure was 4 days.

Acid hydrolysis

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

Alkaline hydrolysis

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

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

Solution with concentration 0.1% was prepared by dissolution of 0.001 g of API in 1 mL of water. Solution with concentration 1% was prepared according to pharmaceutical preparation "1% solution for injections", viz. 0.01 g of API was dissolved in the 1 mL of water, 0.0059 of sodium chloride was added.

When the solid substance was studied, 0.001 g was dissolved in 1 mL water and 5 uL of solution was injected to the HPLC.

RESULT AND DISCUSSION

Optimized chromatography conditions

2-((5-(Furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid was formed in the stream of solvent from the API (salt). Therefore, the detector identified the acid. Thus, API was determined in form of the acid.

Results of the study of the substance decomposition are shown in Table. 1. Mass balance, % (content of the main substance, % plus content of degradation products and impurities, %) in all cases was equaled 100%.

Table 1. Quantitative content of the piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate.

Days / Terms of decomposition	0	1	2	3	4	5	6
Laboratory conditions, 0.1% solution	99.64	99.54	99.53	99.47	99.47	99.46	99.48
Laboratory conditions, 1% solution	99.97	99.97	99.97	99.94	99.94	99.93	
Alkaline hydrolysis. 0.1 M solution of NaOH	99.64	99.58	99.66	99.64	99.64	99.61	99.66
3% H_2O_2	99.63	79.10	73.20	69.54	65.44	61.57	55.49
Thermal effect 66 °C, 0.1% solution	99.64	99.53	99.35	99.25	99.25	99.24	
Thermal effect 66 °C, 1%solution	99.97	99.94	99.92	99.87	99.85	99.85	
Thermal effect 66 °C, substance	99.64	99.80	99.72	99.90	99.80	99.81	
UV light irradiation, solution 0.1%	99.64	97.41	89.31	77.61	56.25		
UV light irradiation, solution 1%	99.97	97.29	93.36	89.68	80.03		
UV light irradiation, substance	99.64	99.80	99.76	99.23	99.76		

Laboratory conditions degradation

During the storage of the 0.1% reference API solution in the laboratory conditions, percentage of the substance was decreased about 0.1% for 6 days. The level of the substance in 1% solution under these conditions was not changed for 5 days.

Thermal degradation

Thermal effect (66 °C) on the 0.1% solution of API leads to its decomposition by approximately 0.4% over 5 days (Fig.1). Substantial degradation products, however, was not identified.

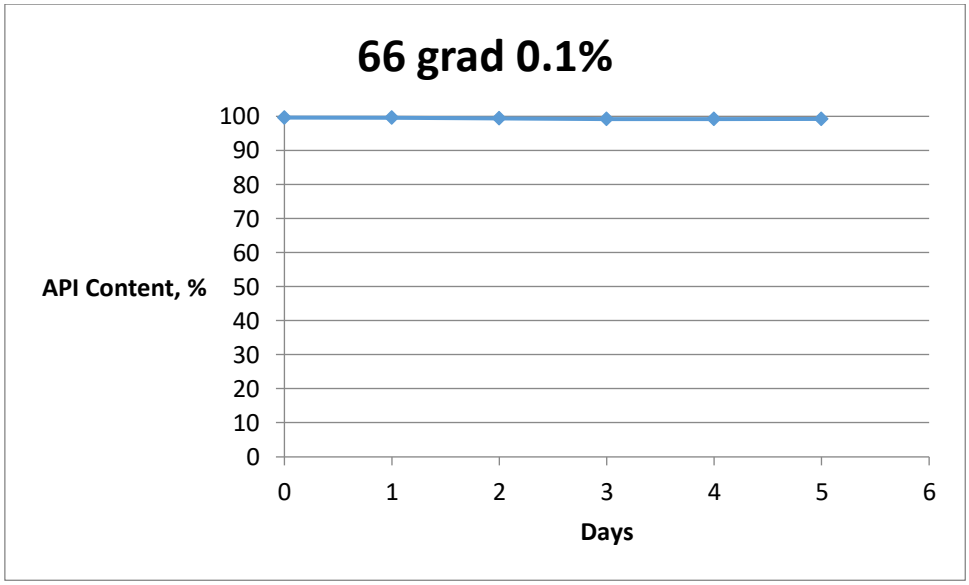

Figure1. The API degradation curve in the 0.1% solution at a temperature 66 °C

At the same time, under the influence of the temperature (66 °C) on the 1% solution decomposition occurs only about 0.1% (Fig. 2). During the study of the thermal effect (66 °C) on the solid substance (API) the content of API in a substance was not changed.

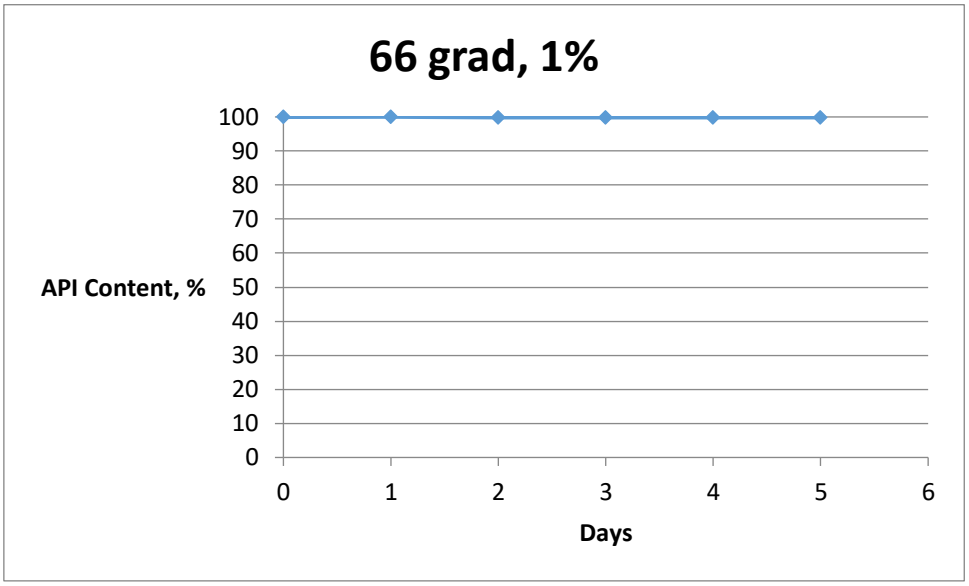

Figure 2. The API degradation curve in the 1% model solution for injection at a temperature 66 °C

Oxidative degradation

The effect of 3% hydrogen peroxide over 6 days results in a decrease in the concentration of API about 2 times (Fig. 3).

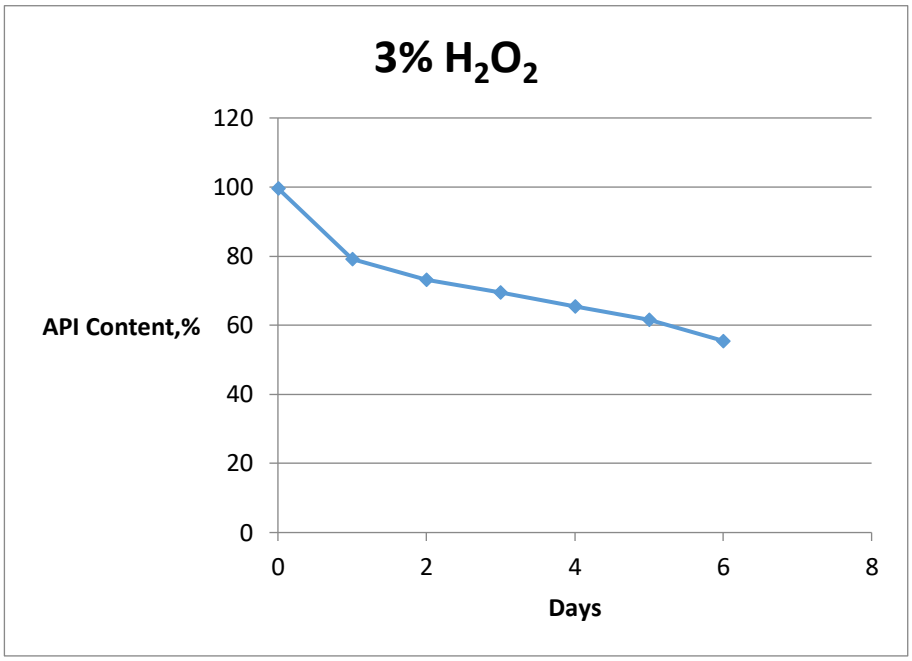

Figure3. The API degradation curve under action 3% H_2O_2

Ultraviolet (UV) degradation

UV light irradiation causes the decomposition of the 0.1% solution during four days at more than 40% (Fig. 4).

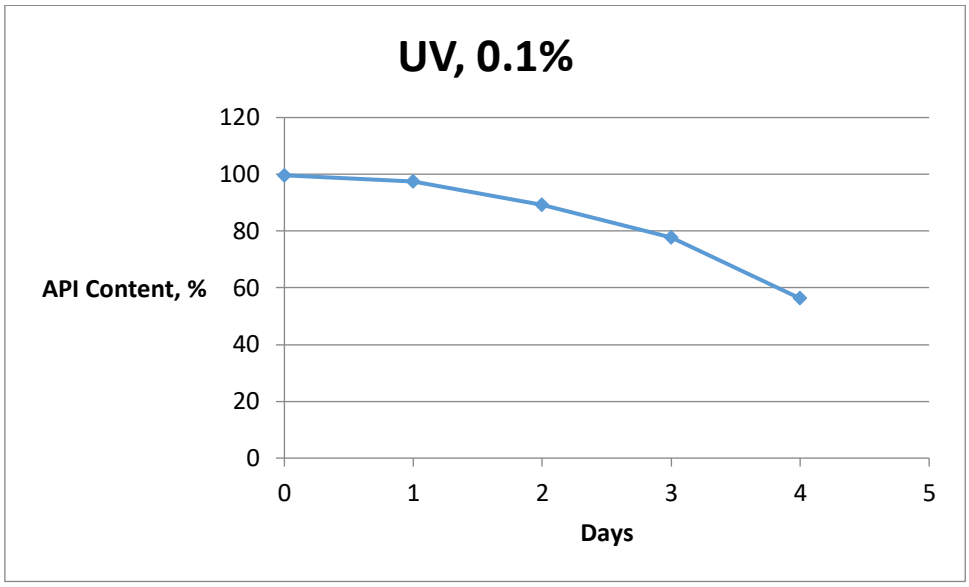

Figure 4. The API degradation curve in 0.1% solution

At the same time, for 1% solution the concentration was decreased about 20% (Fig. 5). The API content was not changed during irradiation of dry substance for 4 days.

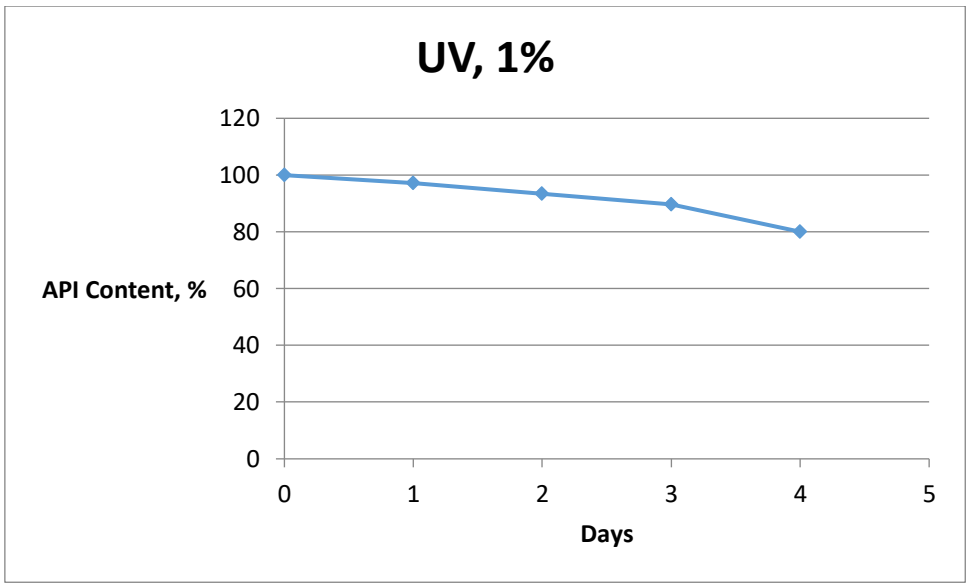

Figure 5. The API decomposition curve in the 1% solution for injection.

Acid hydrolysis

Under the action of 0.1 M solution of chloride acid API immediately decomposes with formation 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid, which is insoluble in water. So the study of exposure of 0.1 M chloride acid was finished at this step.

Alkaline hydrolysis

Under the action of 0.1 M solution of sodium hydroxide, the content of the API was not changed for 6 days.

Determination of the structure of degradation products

Possible structures of compounds formed as a result of API degradation under stress conditions was proposed after study of the mass spectra of the corresponding chromatography peaks.

The structure determination of API degradation products formed by the action of 3% hydrogen peroxide.

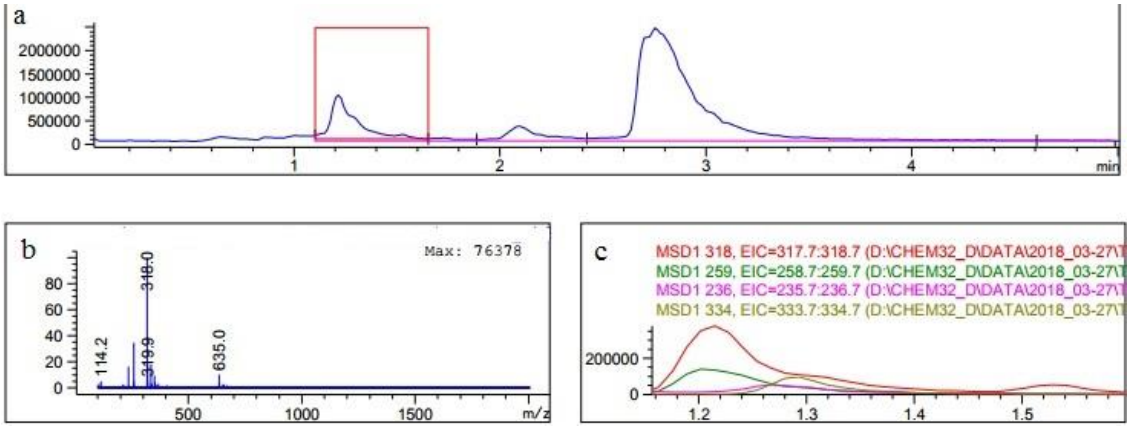

Figure 6. The TIC chromatogram of API degradation products formed by action of 3%H_2O_2 at 150V (a). Mass spectrum of peak at 1.219 min (b). EIC chromatogram (c).

Chromatography of the degradation products appeared after action of 3% H_2O_2 shown two peaks (Fig. 6). First peak (at 1.219 min) was not pure. The most intensive peak in extracted ion chromatogram (EIC) had m/z=318. It corresponded to the sulfoxide (Fig. 7). It is known reaction of sulfoxide formation from organic compounds of sulfur with valence two by the influence of the H_2O_2 solution [12].

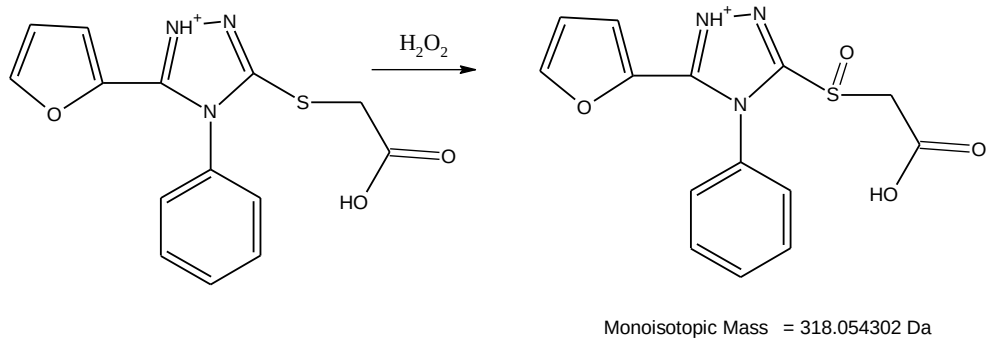

Figure 7. Formaton of 3-[(carboxymethyl)sulfinyl]-5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-1-ium cation (m/z=318).

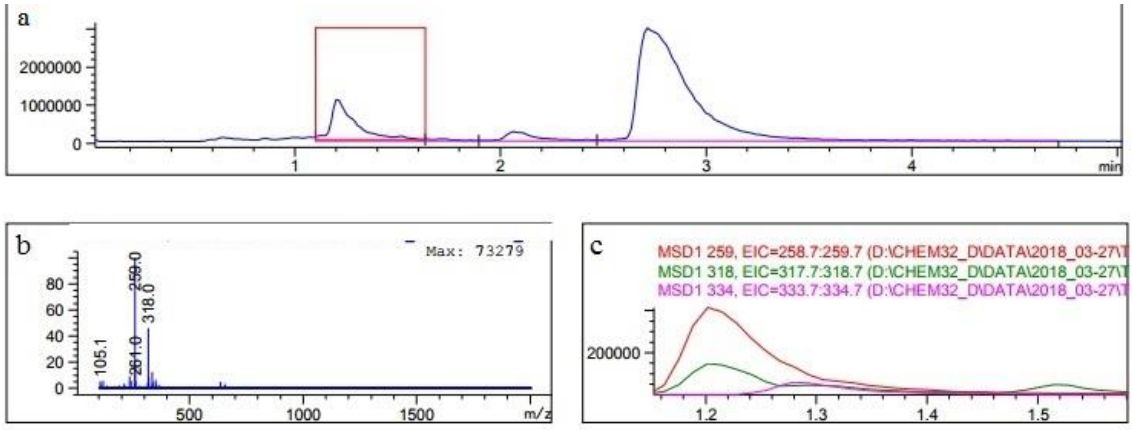

Figure 8. The TIC chromatogram of API degradation products formed by action of 3% H_2O_2 at 200V (a). Mass spectrum of peak at 1.217 min (b). EIC chromatogram (c).

When fragmentation voltage was increased till 200 V the ion with the m/z 259 in the mass spectra of first peak was appeared (Fig.8). The possible structure of this ion is presented at Fig. 9.

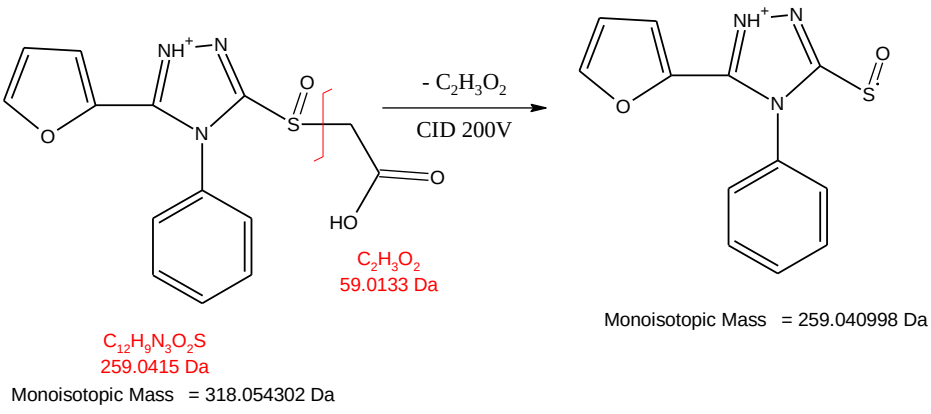

Figure 9. Transformation of cation with m/z 318 during fragmentation in CID at 200V

Second peak of the degradation product was at 2.140 min (Fig. 10).

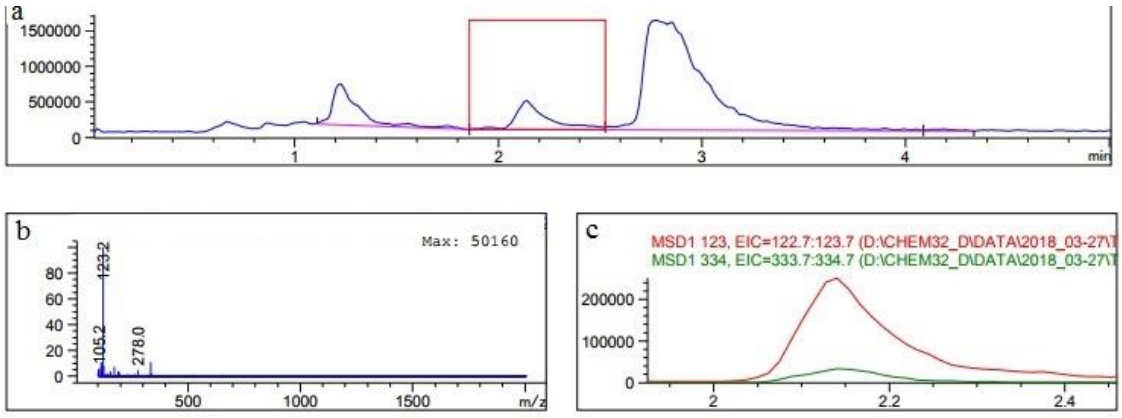

Figure 10. The TIC chromatogram of API degradation products formed by action of 3% H_2O_2 at 100V (a). Mass spectrum of peak at 2.140 min (b).EIC chromatogram (c).

Quazimolecular ion with m/z = 334 correspond to the sulfone which was formed at the second step oxidation by the H_2O_2 (Fig. 11). It is well-known reaction [12].

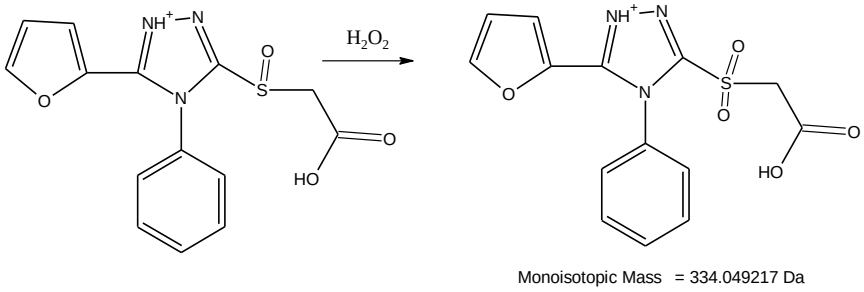

Figure 11. Formation of 3-[(carboxymethyl)sulfonyl]-5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-1-ium cation (m/z=334).

There are two fragment ions present in mass spectra of second peak at 100 V (m/z =278.0 and m/z=123.2). Possible structure of first ion present at Fig. 12.

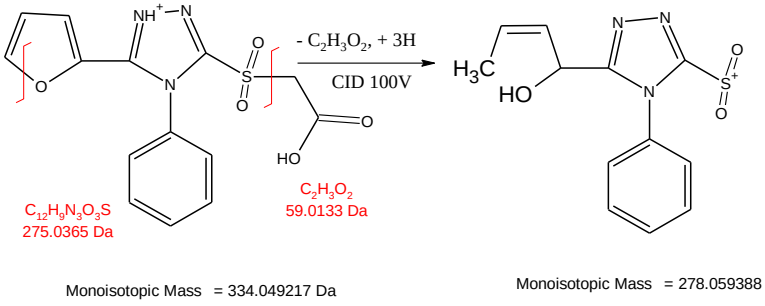

Figure 12. Converting of cation with m/z 344 during fragmentation in CID at 100V to product the cation with m/z 278.

Reaction formation of the ion with m/z 123 present at Fig.13.

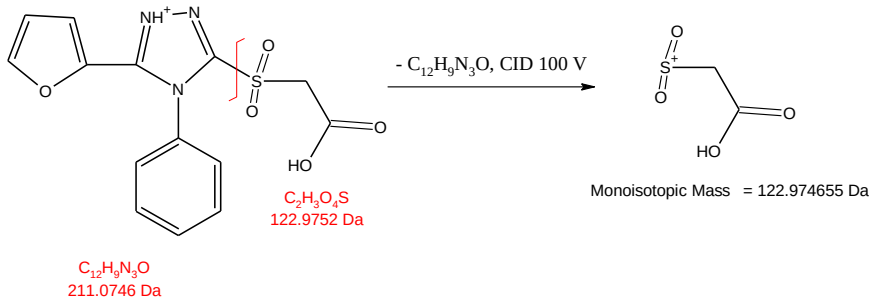

Figure 13. Transformation of cation with m/z 344 during fragmentation in CID at 100V to product the cation with m/z 123.

The structure determination of API degradation products formed by the influence of UV radiation on 0.1% solution.

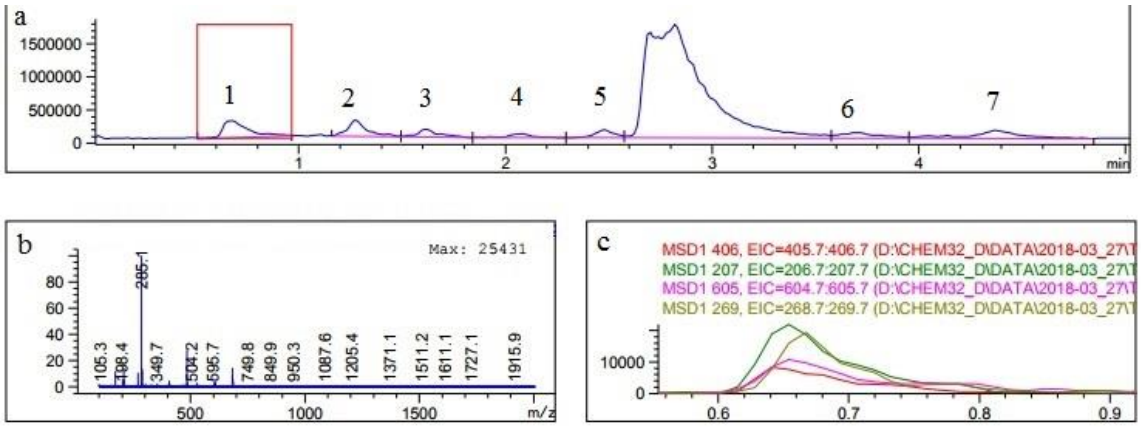

Figure 14. The TIC chromatogram of API degradation products formed by UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (1) at 0.675 min (b). EIC chromatogram (c).

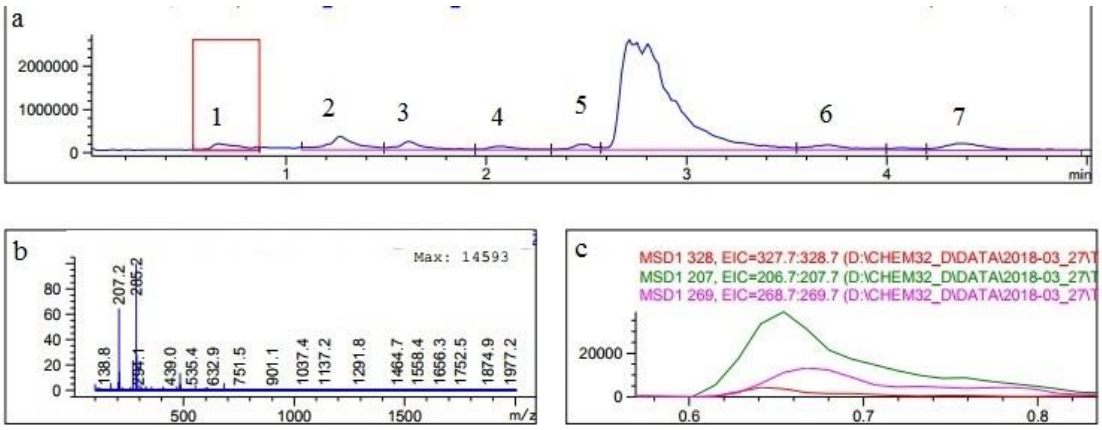

Figure 15. The TIC chromatogram of API degradation products formed by UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (1) at 0.670 min (b). EIC chromatogram (c).

The first peak was not identified (Fig. 15). The monoisotope mass m/z = 285.2 and m/z = 207.2 in the mass spectrum of the unidentified peak (1) was observed (Fig.14, 15).

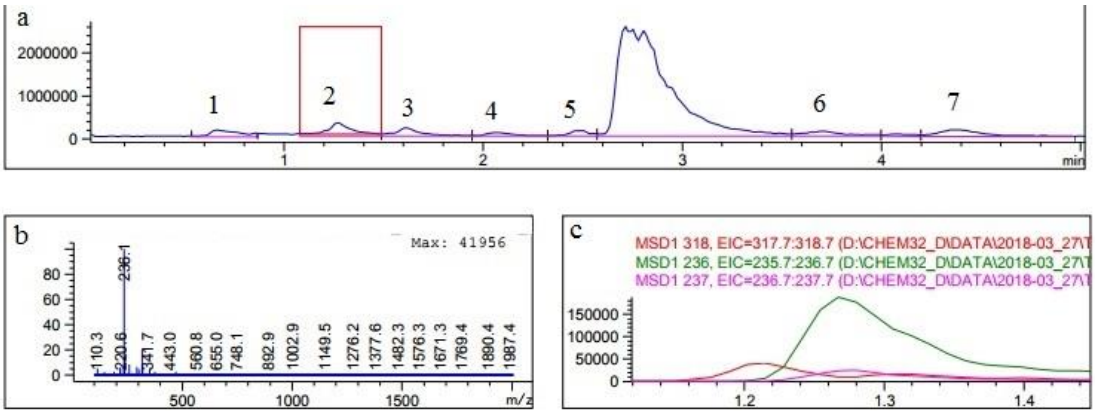

Figure 16. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (2) at 1.275 min (b). EIC chromatogram (c).

Sulfoxide was also observed at 150V in API degradation products formed by the action of UV radiation. The retention time was close to 1.2 (peak 2), m/z 318 (Fig. 16).

There was an impurity that is associated with the cleavage of the furan cycle to form the corresponding structure with m/z 236.1 (Fig.17). On the second day of irradiation there was a peak of dimer ion with m/z 471, which confirms that the quasimolecular ion has a mass 236.

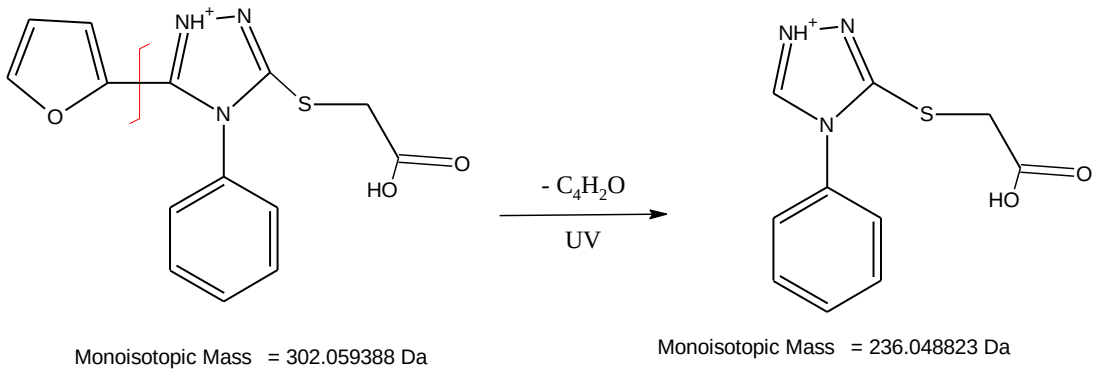

Figure 17. Possible way of degradation of the API at UV radiation influence with cleavage of the furan ring.

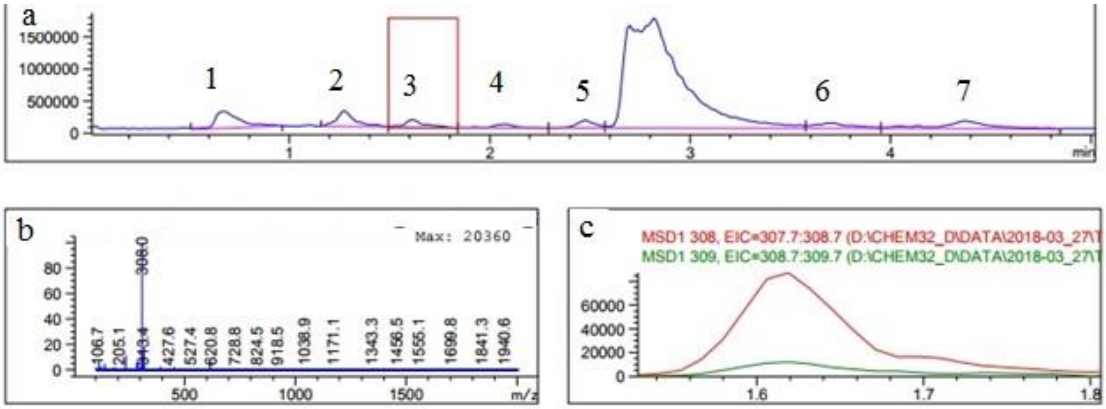

Figure 18. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (3) at 1.616 min (b).EIC chromatogram (c).

Possible structures of the ion with m/z 308 (Fig. 18) ($[M+H]^+$) proposed at Fig. 19. They are formed as a result of the reduction and opening of furan cycle. The dimeric ion with m/z 615 ($[2M+H]^+$) was detected in the mass spectrum on the second day of irradiation. The presence of the corresponding dimer ion confirms that the ion with m/z 308 is a quasimolecular ion.

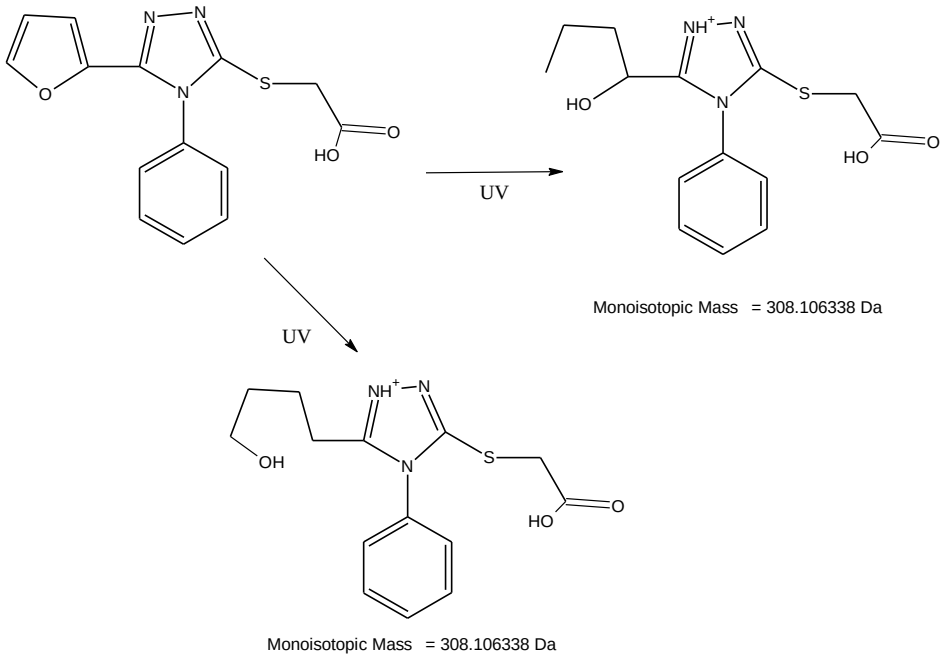

Figure 19. Possible API photodegradation pathway with formation of the product with molecular mass 308.1

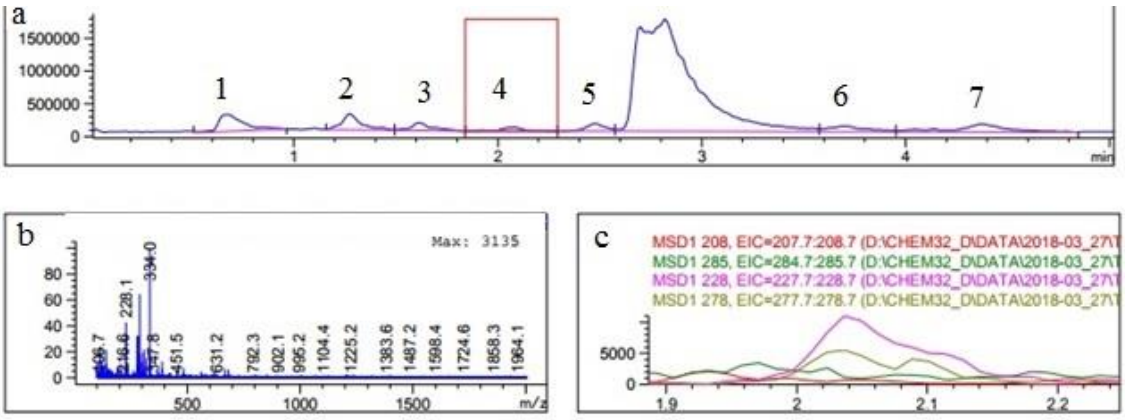

Figure 20. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (4) at 2.070 min (b).EIC chromatogram (c).

There is also sulfone in products of the photodegradation, the retention time is approximately 2.1, m/z 334 (Fig.20).

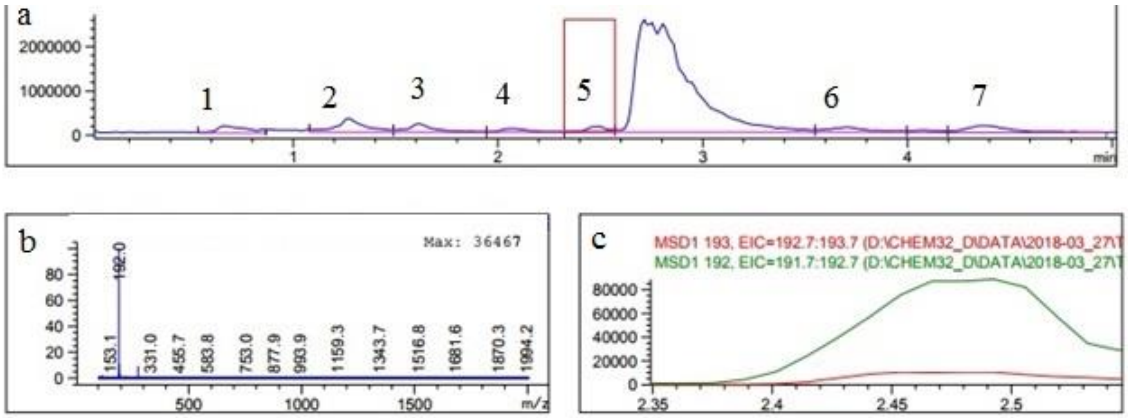

Figure 21. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (5) at 2.478 min (b).EIC chromatogram (c).

An impurity with a retention time approximately 2.5 min and m/z 192 was observed (Fig. 21). It is the product of the breakaway of the furan cycle, as well as carbon dioxide (decarboxylation) from protonated 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate acid (Fig. 22).

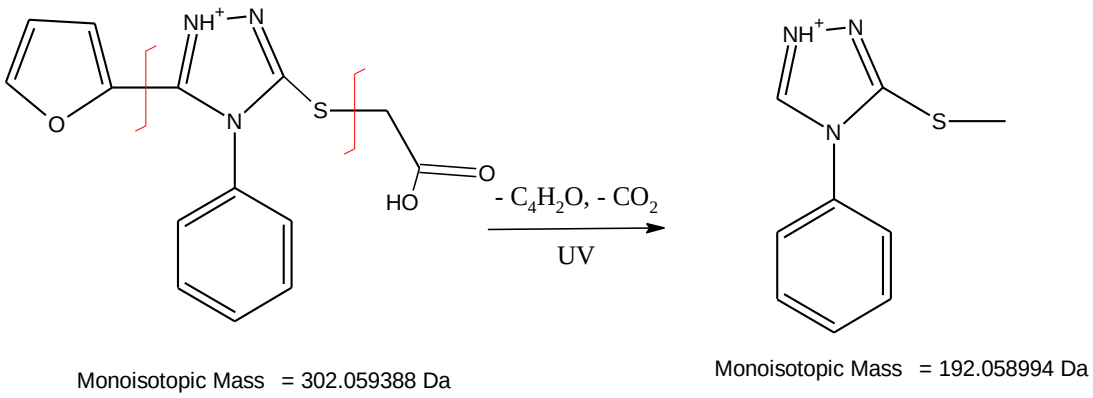

Figure 22. Cleavage of furan cycle and decarboxilation of API

The formation of the thione under the influence of UV radiation was observed. The 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione is precursor in the synthesis of API [10, 11]. It was confirmed by the retention time and m/z. The retention time corresponds to the retention time from chromatography of the standard solution of the corresponding thione (3.7 min), m/z of quasimolecular ion equals 244, which corresponds to the molecular weight of the protonated compound (Fig.23, 24).

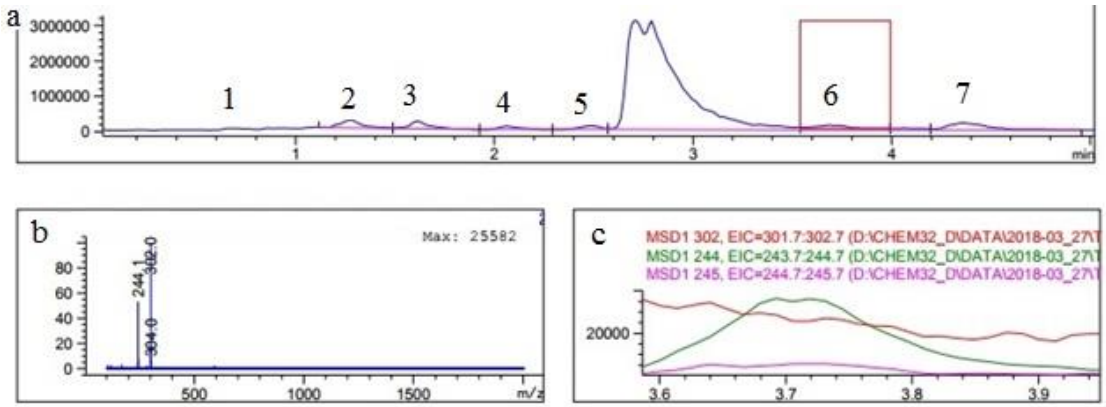

Figure 23. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 200V) (a). Mass spectrum of peak (6) at 3.695 min (b).EIC chromatogram (c).

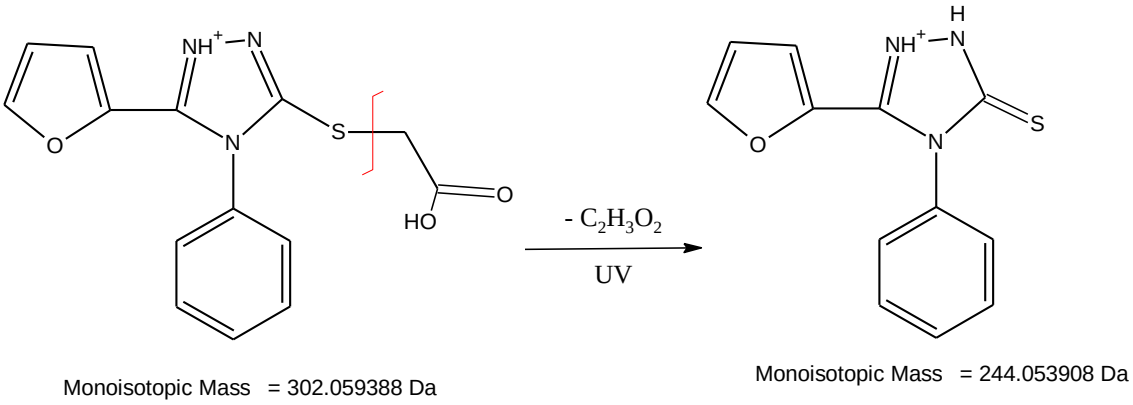

Figure 24. The 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione formation.

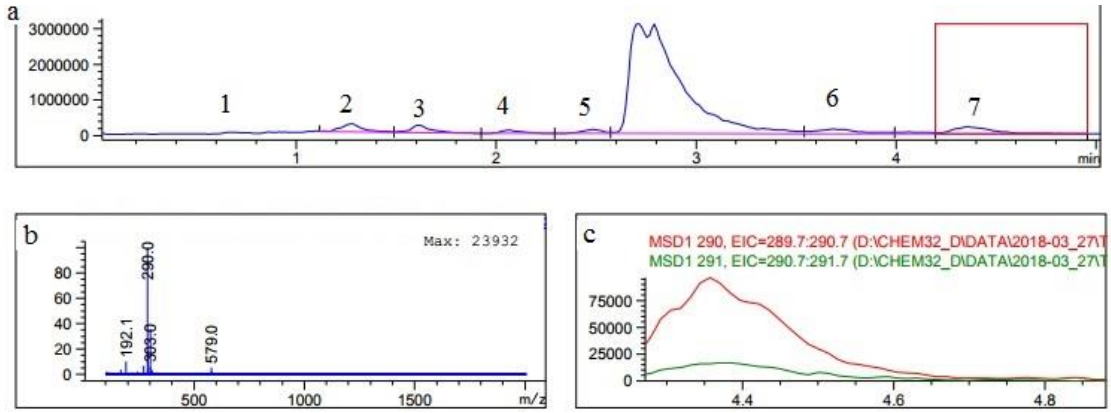

Figure 25. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 200V) (a). Mass spectrum of peak (7) at 4.363 min (b). EIC chromatogram (c).

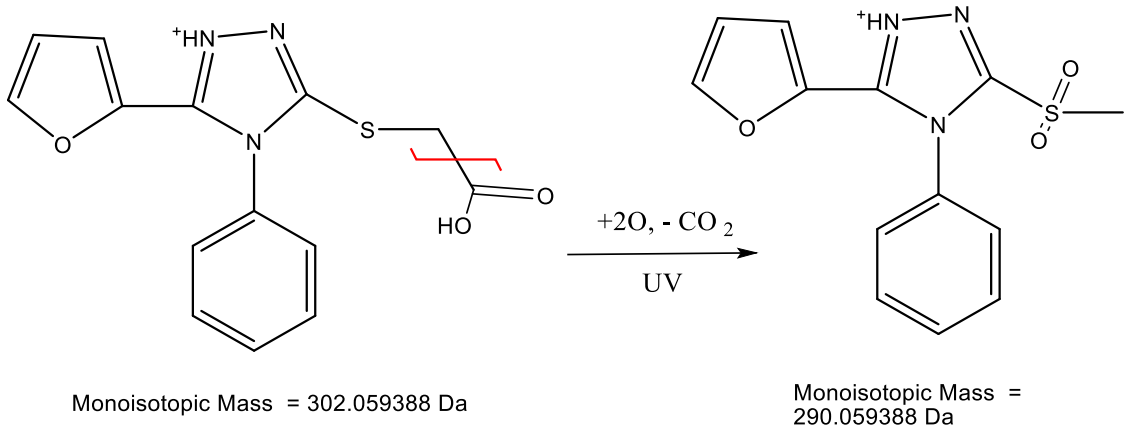

Figure 26. Formation of decarboxilated sulfone.

Anion with m/z = 290.0 was formed in the ion source ($[M+H]^+$), as well as dimer ion with m/z 579.0 ($[2M+H]^+$), which confirms that the ion with m/z 290 is a quasimolecular ion (Fig. 25). The carbon dioxide was eliminated and the sulfur atom was oxidized under the action of UV light to form the methylsulfone with the monoisotope mass 289.0 (Fig. 26).

Table 2. Impurities were formed in stressful conditions.

#	Compound	3% H_2O_2	UV	Retention time	m/z quasimolecular ion	Monoisotope molecular weight
0	API			2.8	302	301
1	2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfinyl)acetic acid	+*	+	1.2	318	317
2	2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfonyl)acetic acid	+	+	2.1	334	333
4	2-((4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)aceticacid	-**	+	1.3	236	235
5	2-((5-(1-hydroxybutyl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid, 2-((5-(4-hydroxybutyl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid	-	+	1.6	308	307
6	3-(methylthio)-4-phenyl-4*H*-1,2,4-triazole	-	+	2.5	192	191
7	5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione	-	+	3.7	244	243
8	3-(furan-2-yl)-5-(methylsulfonyl)-4-phenyl-4*H*-1,2,4-triazole	-	+	4.4	290	289

*Substance was found in degradation products

**Substance was absent in degradation products

Influence of the sodium hydroxide, hydrochloride acid, 3% H_2O_2, temperature, UV radiation on piperidine 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate substance, 0.1% solution and 1% solution for injections were studied. Dependence of the quantitative content of the piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate from exposition time was determined.The composition of degradation products formed under the actionof anoxidizer was established (3%H_2O_2). This is sulfoxide and sulfone corresponding to the API. The composition of degradation products which were formed under the influence of UV radiation was proposed.

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There are no conflicts of interest have been declared.

REFERENCES

1. Sehrawat, R., Maithani, M., Singh,R. (2010). Regulatory Aspects in Development of Stability-Indicating Methods: A Review. Chromatographia, 72 (1/2), 1–6.

2. Blessy, M, Ruchi, D.P., Prajesh, N.Pr., Agrawal, Y.K. (2014). Development of forced degradation and stability indicating studies of drugs-A review. Journal of Pharmaceutical Analysis; 4(3), 159–165.

3. Klick, S., Muijselaar, P.G., Waterval, J., Eichinger, Th., Korn, C., Gerding, Th.K., Debets, Al.J., Van de Griend, C.S., Van den Beld, C., Somsen, G.W. and De Jong, G.J. (2005). Stress Testing of Drug Substances and Drug Products. Pharmaceutical Technology, FEBRUARY; 48–66.

4. Chakravarthy, V.A., Sailaja, B.B.V., Kumar, A.P. (2015). Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Enrofloxacin and Its Degradation Products in Tablet Dosage Forms. Journal of Analytical Methods in Chemistry, 2015, 1 – 11.

5. Ahirrao, V.K., Patil, C.S., Bembalkar, S.R., Katariya, M.V., Sonnekar, V.S., Marathe, R.P., Nawale,R. B.,Pawar, R. P.(2012). Stress degradation studies of dronedarone in pharmaceutical dosage form by a validated stability-indicating LC method. Journal of the Chilean Chemical Society, 57(3); 1272 – 1276.

6. Patel, J. K., Patel, N. K.(2014). Stability-Indicating RP-HPLC Method for the Determination of Ambrisentan and Tadalafil in Pharmaceutical Dosage Form. Scientia Pharmaceutica, 82, 749–763.

7. Parchenko, V. V., Panasenko, O. I., Knysh, E. G., Vasyuk S. O., Tarkhanova, O. O. (2009). Working out of methods quantitative determination piperidiniy 2-[5-(furan-2-il)-4-phenil-1,2,4-triazol-3-ilthio]acetate in the substance. Ukrainskyi biofarmatsevtychnyi zhurnal, 4, 44–45.

8. Parchenko, V.V., Panasenko, O.I., Knysh, E.G., Vasiuk, S.O., Tarkhanova, O. O. (2009). Qualitative and quantitative determination of the Piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate 1% and 2,5% solutions. Zaporozhye medical journal, 3, 111–112.

9. Varynskyi, B. O., Parchenko, V.V., Kaplaushenko, A. G. (2017) Development and validation of HPLC-DAD method of determination piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate in 1 % solution, Zaporozhye medical journal, 19 (6), 827-832.

10. Parchenko, V. (2006). Synthesis, physicochemical and biological properties of derivatives of 1,2,4-triazoles-3-thione containing core furan. Candidate of Pharm Scithesis, Zaporozhye State Medical University, Zaporozhye, 208 p.

11. Parchenko, V. (2014). Synthesis, transformation, physico-chemical and biological properties in a number of 5-furyl-substituted 1,2,4-triazole-3-thiones. Dr. Pharmacist Sciences thesis, Zaporozhye State Medical University, Zaporozhye, 460 p.

12. Tietze, L.F., Eicher, Th. (1991). Reaktionen und Synthesen im organisch-chemischen Praktikum und Forschungs laboratorium. 2. Aufl., Georg Thieme Verlag, Stuttgart – NewYork. DM 68, 672 S.