ISSN 1027-5495. Functional Materials, 29, No.4 (2022), p. 611-620.
doi:https://doi.org/10.15407/fm29.04.611

Stress testing of 2,5-dihydro-1H-pyrrol-3-yl substituted acetic acid

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Received September 15, 2022

Stress testing was performed on 2-(2-(4-bromophenyl)-4-hydroxy-1-(5-methylisoxazol-3-yl)-5-oxo-2,5-dihydro-1H-pyrrol-3-yl) acetic acid, Bropa, to determine the weaknesses of pyrrolone-type heterocycles to various stressors, establish the degradation pathways and found the possible approaches to overcome potential stability problems. Since other pyrrolone derivatives had previously shown high activity against influenza A/H1N1 virus and low cytotoxicity, this class was considered a promising source of the new active substances. The influence of temperature, alkaline and acidic media, daylight, UV irradiation and hydrogen peroxide on the stability of Bropa was studied. Several analytical procedures using HPLC, FTIR, and UV-vis spectroscopy were developed for the qualitative and quantitative analysis of Bropa and the impurities in the test solutions. Bropa was found to be resistant to daylight, temperature, alkaline and acidic media, with impurity levels increasing by only 0.3–0.7 %. However, the compound proved unstable in UV tests and tests with hydrogen peroxide (30 % and 3 %), as the content of the Bropa decreased to 11.7 %, 8.3 %, and 41.2 %, respectively, with many new impurities formed, showing the susceptibility of the 5-oxo-2,5-dihydro-1H-pyrrol-3-yl fragment to oxidation and photoinduced chemical reactions. The results indicated that the photochemical and oxidative stability properties of pyrrolone-type heterocycles need to be improved to significantly reduce the number of degradation products.

Keywords: pyrrolone derivatives, multicomponent reaction, stress testing, stability, photoinduced chimical reactions, HPLC, FT-IR.

Стрес-тестування 2,5-дигідро-1Н-пірол-3-ілзаміщеної оцтової кислоти. *Я.І.Сахно, І.О.Зінченко, І.Б.Щербаков, Г.Л.Іващенко, Ю.М.Столпер, А.М.Ляпунова, К.М.Бєліков, С.М.Десенко, О.П.Безугла, В.А.Чебанов*

Проведено стрес-тестування 2-(2-(4-бромфеніл)-4-гідрокси-1-(5-метилізоксазол-3- іл)-5-оксо-2,5-дигідро-1Н-пірол-3-іл) оцтової кислоти, Bropa, з метою визначення вразливості гетероциклів піролонового типу до різних стресових факторів, зокрема, встановлення шляхів деградації та пошуку можливих підходів до подолання потенційних проблем стабільності. Оскільки інші похідні піролону раніше показали високу активність проти вірусу грипу A/H1N1 та низьку цитотоксичність, цей клас розглядався як перспективне джерело нових активних сполук. Вивчено вплив температури, лужного та кислого середовищ, денного світла, УФ-опромінення та пероксиду водню на стабільність Bropa. Розроблено низку аналітичних методик з використанням ВЕРХ, ІЧ-та УФ-спектроскопії для якісного та кількісного аналізу Bropa та домішок у досліджуваних розчинах. Встановлено, що сполука Bropa є стійкою до дії денного світла,

температури, лужних та кислотних середовищ адже при цьому вміст домішок збільшується лише на 0,3–0,7 %. Однак сполука виявилася нестабільною під дією УФ-опромінення і перекису водню (30 % і 3 % розчини) оскільки вміст основної сполуки Bropa падав до 11,7 %, 8,3 % і 41,2 % від вихідного відповідно з утворенням великої кількості нових домішок, що свідчить про чутливість 5-оксо-2,5-дигідро-1Н-пірол-3-іл фрагменту до окислення та фотоіндукованих хімічних реакцій. Результати дослідження показали, що властивості фотохімічної та окиснювальної стабільності гетероциклів піролонового типу потребують покращення для суттєвого зменшення кількості продуктів деструкції.

1. Introduction

The study of the influence of stress factors on the qualitative and quantitative composition of the new active substances is an important stage in the way of the newly synthesized compound to the final drug, because regulatory authorities require complete data on the composition, structure, reactivity, and reaction products of a potential drug [1]. However, regulatory guidelines for stability testing of drugs in the early stages of development lack specificity [2], so drug development often involves more stability studies than necessary just to avoid regulatory questions, or inadequate stability testing, leading to delays in drug development. The most common tests for drug degradation include effect of temperature, oxidation, the influence of alkalis and acids, various types of irradiation (microwaves, IR, UV, X-rays, etc.), and mechanochemical degradation. The choice of exposure factors for testing often depends on the intended purpose of the new active substance.

In the current work as the test substance was used 2-(2-(4-bromophenyl)-4-hydroxy-1-(5-methylisoxazol-3-yl)-5-oxo-2,5-dihydro- 1H-pyrrol-3-yl) acetic acid, Bropa (4, Scheme 1). The pyrrolone derivative is the privileged class of heterocycles due to their biological activities namely antiviral [3–5], antibacterial [6, 7], antimicrobial [8, 9], analgesic [10], antitumor [11, 12]. Also, pyrrol-2-ones containing γ-lactam fragment is presented in bioactive ingredients such as antibiotic pyrrocides [13] and the endothelin receptor antagonist oteromicyn [14]. In our previous studies [15], a representative of the pyrrolones, 3-hydroxy-5-(2-methoxyphenyl)-4-phenyl-1-(1H-1,2,4-triazol-5-yl)-1H-pyrrole-2(5H)-one, that was obtained by multicomponent reaction of 5-amino-1,2,4-triazole with 2-methoxybenzaldehyde and pyruvic acid, showed high activity against influenza A/H1N1 virus (EC50 = 0.57 μM) and low cytotoxicity (CC50 100 μM).

2. Materials and methods

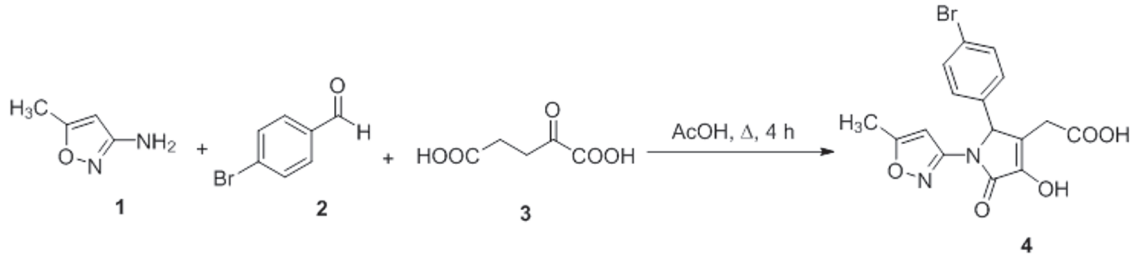
All reagents, volumetric solutions, and indicators used in the present work were supplied by commercial vendors and met the requirements of the State Pharmacopoeia of Ukraine [16].

Bropa synthesis and confirmation of the structure.

The Bropa was synthesized by known three-component reaction [15] involving 3-amino-5-methylisoxazole (1), 4-bromobenzaldehyde (2), and α-ketoglutaric acid (3) in boiling acetic acid for 4 h (Scheme 1).

Pyrrolone 4 has already been described in literature [15] and its melting point, 1H, ^{13}C NMR and mass spectra 4 are in good correlation with published data. 1H and ^{13}C NMR spectra were acquired on a Varian MR-400 instrument at 400 and 100 MHz, respectively, in DMSO-d_6. Mass spectra were registered on a GC-MS Varian 1200L (ionizing voltage 70 eV). Melting points were determined with a Stuart SMP10 apparatus.

Scheme 1



Identification tests.

The identification of Bropa was carried out by:

— infrared absorption spectrophotometry, [16, 2.2.24] within the region of 4000-400 cm^{-1} using Shimadzu 8400S spectrometer (Shimadzu, Japan);

— ultraviolet and visible absorption spectrophotometry [16, 2.2.25] within range from 220 nm to 320 nm using Shimadzu UV-1700 PharmaSpec spectrometer (Shimadzu, Japan);

— HPLC [16, 2.2.29] simultaneously with its quantitative determination using Shimadzu LC-2030C 3D chromatograph (Shimadzu, Japan).

Quantitative analysis of Bropa and the impurities.

For assay of Bropa the analytical procedure by reverse potentiometric titration [16,2.2.20] using 848 Titrino Plus (Metrohm AG, Switzerland) titrator was developed, viz.:

Dissolve 0.2 g of the substance in 15 ml of 0.1 M sodium hylroxide, in 20 min add 20 ml of water and mix. Titrate the solution with 0.1 M hydrochloric acid.

1 ml of 0.1 M hydrochloric acid is equivalent to 39.32 mg Bropa ($C_{16}H_{13}BrN_2O_5$).

In addition, the analytical procedure by HPLC [16, 2.2.29] was developed for quantitative determination of both Bropa and its impurities as it is provided below:

Buffer solution pH 2.0. Solution of sodium perchlorate (6.0 ml) in water for chromatography (1000 ml) with trimethylamine (2 ml) added and adjusted to pH (2.0±0.05) with phosphoric acid.

Dissolution mixture. Acetonitrile for chromatography — water (50:50).

Test solution. Dissolve 100.0 mg of Bropa in the 70 ml of dissolution mixture, dilute to 100 ml with the same solvent and mix. Dilute 10.0 ml of the solution to 100 ml with the dissolution mixture and mix. Filter this solution using 25 mm fluoroplastic membrane filter (pore size of 0.45 μm) discarding the first 2 ml of filtrate (100 μg/ml of Bropa).

Reference solution (a) (for the quantitative determination of Bropa). Dissolve 25.0 mg of Bropa RS* in 15 ml of dissolution mixture, dilute to 25 ml with the same solvent and mix. Dilute 5.0 ml of the solution to 50 ml with the dissolution mixture and mix (100 μg/ml Bropa).

*The substance with content of $C_{16}H_{13}BrN_2O_5$ 99.8 % was used as reference standard.

Reference solution (b) (for quantitative determination of the impurities). Dilute 1.0 ml of reference solution (a) to 100 ml with the dissolution mixture and mix (1 μg/ml of Bropa).

Reference solution (c) (for system suitability). Dilute 5.0 ml of reference solution (b) to 25 ml with the dissolution mixture and mixe (0.2 μg/ml of Bropa).

Chromatographic conditions:

— column: stainless-steel chromatographic column, 150×3.9 mm, packed with octadecylsilyl silica gel for chromatography (5 μm) (Symmetry® C18, "Waters");

Time, min	Mobile phase A, % v/v	Mobile phase B, % v/v
0→15	100→0	0→100
15–20	0	100
Column regeneration		
20–25	100	0

— mobile phase A: buffer solution pH 2.0;

— mobile phase B: R buffer solution 2.0 — acetonitrile for chromatography (25:75);

— flow rate: 1.0 ml/min;

— detection: spectrophotometer at 230 nm;

— injection: 20 μl;

— temperature: 30°C;

— disregard limit (in the case of quantification of impurities): peak which area was less than the area of the principal peak on the chromatogram obtained with *reference solution* (c) (0.2 %).

System suitability:

— column performance calculated by the peak due to Bropa in the chromatogram obtained with the reference solution (a) or (c) should be not less than 25,000 theoretical plates;

— symmetry factor should be within range 0.8–1.5;

— relative standard deviation should meet the requirements of [16, 2.2.46];

— signal-to-noise ratio should be not less than 10.

All the developed analytical procedures were appropriately validated in accordance with the requrements of [16] harmonized with the provisions of ICH guideline Q 2 [17].

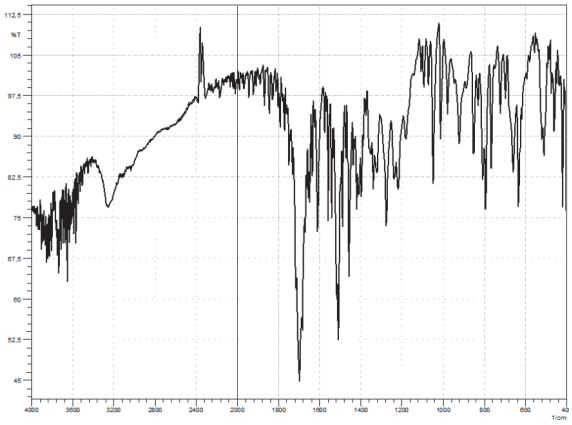


Fig. 1. FTIR spectrum of Bropa.

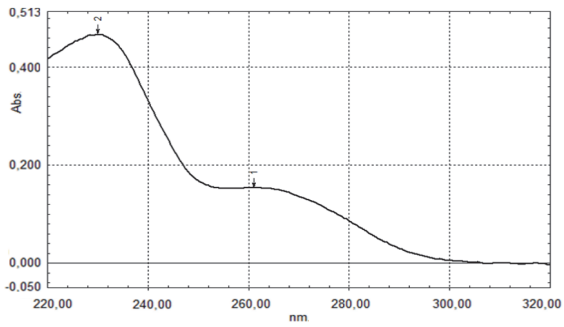


Fig. 2. UV absorption spectrum of Bropa in methanol.

The preparation of the solutions for Bropa stress testing.

The following solutions were used: the solvent (acetonitrile — water for chromatography 1:1); Bropa solution (100 μg/ml in solvent); the control solution (Reference solution (a) — water 1:1); Bropa synthesis components (containing 86.0 μg/ml of Bropa, 720.8 μg/ml of α-ketoglutaric acid, 120.0 μg/ml of 3-amino-5-methylisoxazole and 133,6 μg/ml of 4-bromobenzaldehyde) in the solvent.

HCl test. Mixture of 5.0 ml of Bropa solution and 0.4 ml of a 1.0 M hydrochloric acid was kept at 40°C for 60 minutes and cooled to room temperature. Then the mixture was neutralized with 1.0 M sodium hydroxide, quantitatively transferred into a 10 ml measuring flask and diluted to 10 ml with the solvent.

NaOH test. Mixture of 5.0 ml of Bropa solution and 0.4 ml of 1.0 M sodium hydroxide was kept at 40°C for 60 minutes and cooled to room temperature. Then the mixture was neutralized with a 1.0 M solution of hydrochloric acid, quantitatively transferred into a 10 ml measuring flask and diluted to 10 ml with the solvent.

Hydrogen peroxide tests. Mixture of 0.5 ml of Bropa solution and 0.5 ml of 30 % hydrogen peroxide solution or 0.5 ml of 3 % hydrogen peroxide solution were placed into chromatographic vials and mixed. They were kept at a temperature of 40°C for 60 minutes and cooled to room temperature.

UV test. Bropa solution was placed in a quartz closed cuvette and irradiated with UV light at 254 nm for 30 minutes, then 0.5 ml of solution was placed into the chromatographic vial with 0.5 ml of solvent added and mixed.

Temperature test. 0.5 ml of Bropa solution and 0.5 ml of the solvent were placed into a chromatographic vial and mixed, then heated in an oven at 60°C for 1 hour.

Daylight test. 0.5 ml of the Bropa solution and 0.5 ml of the solvent were placed into a chromatographic vial, mixed and exposed to daylight at 25°C for 1 hour.

Control solution. 0.5 ml of Bropa solution and 0.5 ml of the solvent were placed in a chromatographic vial, mixed and immediately chromatographed.

3. Results and discussion

FTIR spectrum of Bropa measured in QATR mode showed all necessary characteristic peaks (Fig. 1). The UV-absorption spectrum of 8 μg/ml Bropa solution in methanol in the range from 220 to 320 nm contains the maximum at 230 nm and a shoulder peak at 260 nm (Fig. 2), the specific absorbance measured at 230 nm was within 0.45–0.48 abs.

Chromatogram obtained with Bropa as the control solution (Fig. 3) shows the substance contained two unidentified impurities with *Rt* ~ 12.8 min and Rt ~ 14.9 min hereinafter referred as Impurity A and Impurity B with the content about 2.1 % and 0.2 %, respectively.

Chromatogram obtained with test sample after the daylight exposure contains no additional peaks of impurities other than the impurities A and Impurity B observed in the original substance (Fig. 4).

The HPLC data after the temperature test (Fig. 5) show only one additional peak of the new impurity with *Rt* ~ 11.8 (about 0.6 %); the content of both Impurity A and Impurity B originally present in the substance did not change significantly.

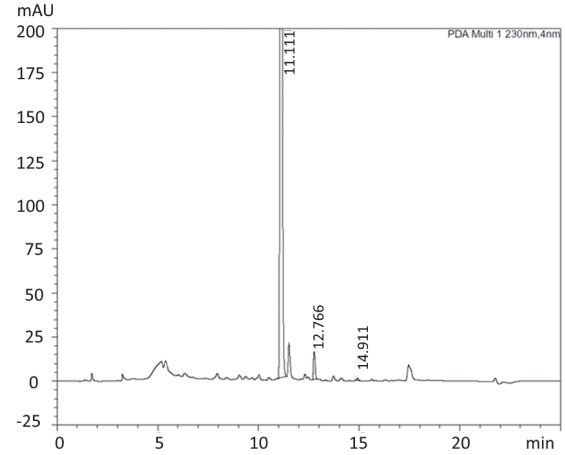


Fig. 3. Chromatogram obtained with the control solution.

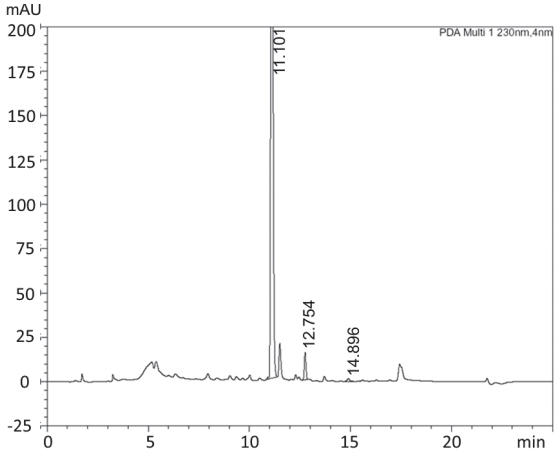


Fig. 4. Chromatogram obtained with test sample after the daylight exposure.

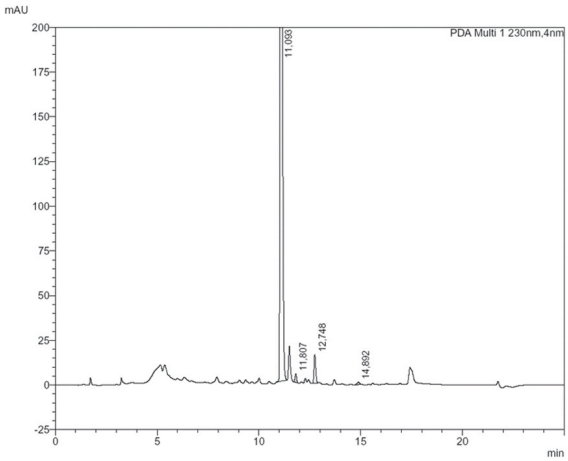


Fig. 5. Chromatogram obtained with test sample after the temperature degradation test.

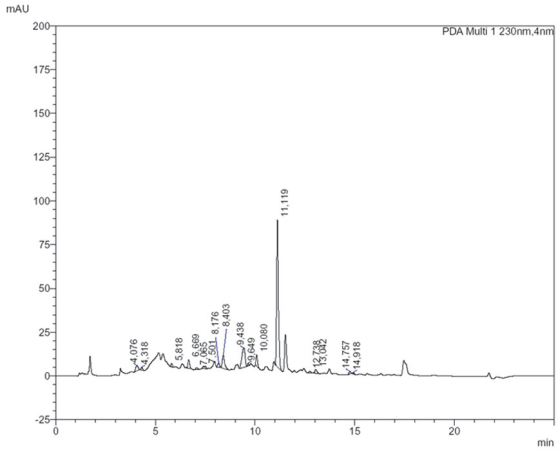


Fig. 6. Chromatogram obtained with test sample after the UV exposure.

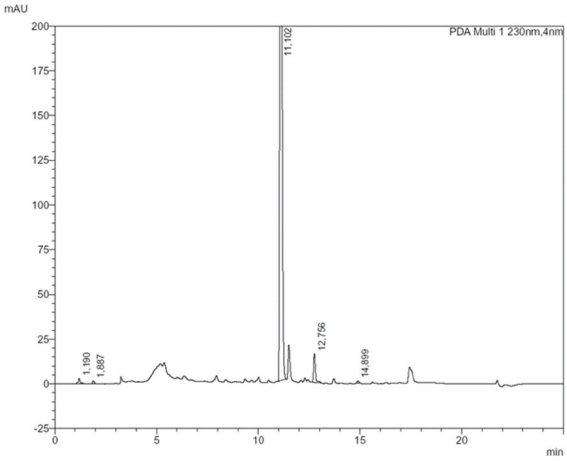


Fig. 7. Chromatogram obtained with test sample after the HCl degradation test.

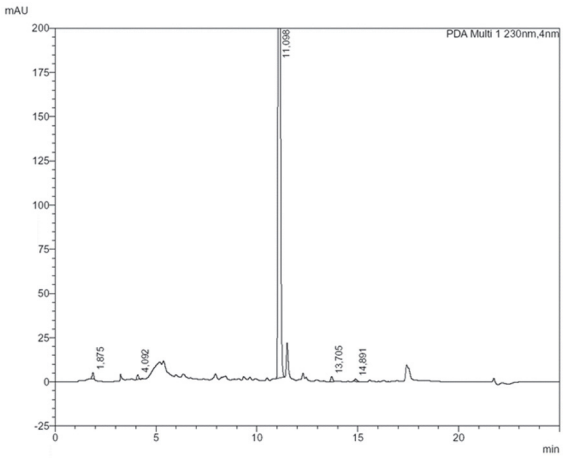


Fig. 8. Chromatogram obtained with test sample after the NaOH degradation test.

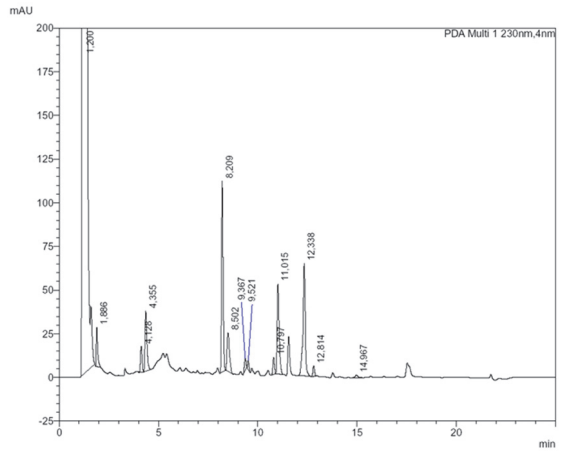


Fig. 9. Chromatogram obtained with test sample after the 30 % H_2O_2 degradation test.

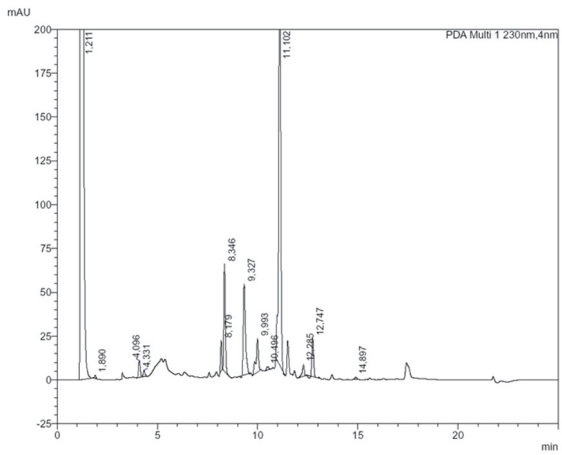


Fig. 10. Chromatogram obtained with test sample after the 3 % H_2O_2 degradation test.

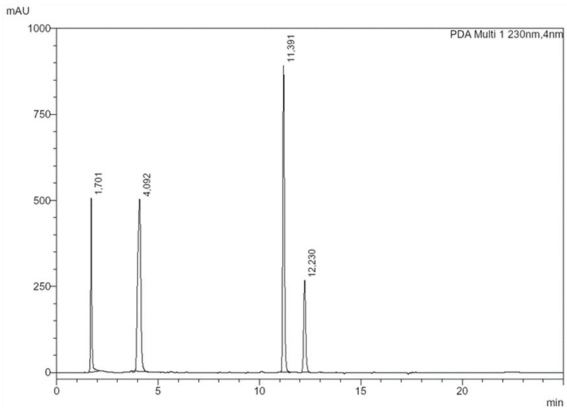


Fig. 11. Chromatogram obtained with the reaction components of the Bropa synthesis.

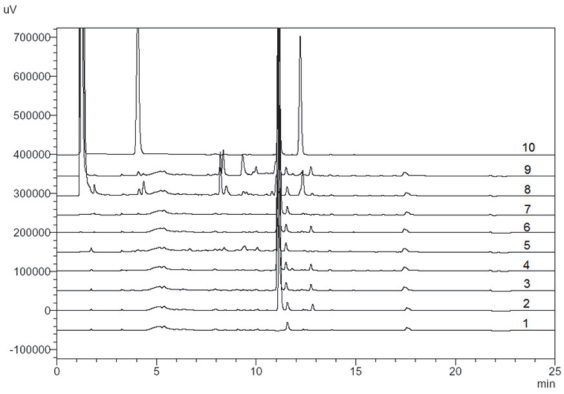


Fig. 12. Summary HPLC data: *1* — solvent; *2* — control solution; *3* — test sample after the daylight test; *4* — test sample after the temperature test; *5* — test sample after the UV test; *6* — test sample after the HCl test; *7* — test sample after the NaOH test; *8* — test sample after the 30 % H_2O_2 test; *9* — test sample after the 3 % H_2O_2 test; *10* — solution of Bropa and the synthesis components.

The content of Bropa in the substance after the UV test (Fig. 6) decreased to 11.7 % of the original content and 14 new impurities were detected by HPLC.

The HPLC data after the HCl test (Fig. 7) show the presence of both Impurity A and Impurity B with *Rt* ~ 12.8 and *Rt* ~ 14.9 min with two other unidentified impurities with *Rt* ~ 1.2 and *Rt* ~ 1.9 min and an estimated content of about 0.5 and 0.2 %, respectively.

The NaOH test (Fig. 8) gives the new four peaks of the impurities, assigning the impurity at *Rt* ~ 14.9 min to Impurity B without significant changes in content, and the unidentified impurities at *Rt* ~ 1.9 and *Rt* ~ 13.7 min and estimated content of about 0.2 and 0.4 %, respectively, and 3-amino-5-methylisoxazole 1 (Scheme 1) at *Rt* ~ 4.1 min.

The interaction with hydrogen peroxide leads to the destruction of Bropa with the formation of a considerable amount of impurities (Figs. 9, 10), while the content of the main substance in the solutions exposed to 30 % and 3 % hydrogen peroxide solutions decreased to 8.3 % and 41.2 %, respectively, compared to the initial amount.

The chromatogram obtained with the reaction components of the Bropa synthesis is shown in Fig. 11. The peaks with retention times of 1.719, 4.072, 11.120, and 12.213 correspond to α-ketoglutaric acid 3, 3-amino-5-methylisoxazole 1, Bropa 4, and 4-bromobenzaldehyde 2, respectively (Scheme 1), while the peak with *Rt* = 14.920 min was assigned to the Impurity B.

The summarized HPLC data after all stress tests are shown in Fig. 12.

Summary data of all registered HPLC peaks, ordered by their retention time, from all samples are shown in Fig. 12 and

Table 1. Summary of HPLC data obtained for the studied solutions with the peaks characteristics

Component assignment	Test	Peak Label	R_t, min	Area, a.u.	Tailing Factor (10 %)	Resolution (USP)
Unidentified01	HCl	Imp1	1.19	20527	1.2	–
Hydrogen peroxide	H_2O_2, 30 %	Imp1	1.2	42009914	1.876	–
	H_2O_2, 3 %	Imp1	1.211	25154919	1.111	–
α-ketoglutaric acid	Synthetic mixture	Sub3	1.701	1977032	1.283	–
Unidentified02	NaOH	Imp1	1.875	11006	1.418	–
	H_2O_2, 30 %	Imp2	1.886	109211	1.911	3.701
	HCl	Imp2	1.887	9152	1.384	4.626
	H_2O_2, 3 %	Imp2	1.89	8178	1.42	4.457
3-amino-5-methylisoxazole	UV-light	Imp1	4.076	26859	1.014	–
	H_2O_2, 3 %	Imp3	4.086	57036	1.115	14.17
	NaOH	Imp2	4.092	13661	1.033	15.328
	Synthetic mixture	Sub1	4.092	5239040	0.913	12.14
	H_2O_2, 30 %	Imp3	4.128	80641	1.148	14.531
Unidentified03	UV-light	Imp2	4.318	8396	1.116	1.19
	H_2O_2, 3 %	Imp4	4.331	21177	1.325	1.333
Unidentified04	H_2O_2, 30 %	Imp4	5.355	234973	1.424	1.245
Unidentified05	UV-light	Imp3	5.818	9231	1.083	9.581
Unidentified06	UV-light	Imp4	6.669	29720	1.022	5.175
Unidentified07	UV-light	Imp5	7.065	7060	1.015	2.055
Unidentified08	UV-light	Imp6	7.501	13708	0.683	2.17
Unidentified09	UV-light	Imp7	8.176	1538	0.919	3.479
	H_2O_2, 3 %	Imp5	8.179	76215	0.893	25.367
	H_2O_2, 30 %	Imp5	8.209	574152	1.183	22.071
Unidentified10	H_2O_2, 3%	Imp6	8.346	314494	1.204	1.186
	UV-light	Imp8	8.403	60650	1.415	1.102
	H_2O_2, 30 %	Imp6	8.502	185570	1.325	1.487
Unidentified11	H_2O_2, 3 %	Imp7	9.327	394287	1.508	5.378
	H_2O_2, 30 %	Imp7	9.367	40092	0.964	4.302
	UV-light	Imp9	9.438	120801	0.791	4.071
Unidentified12	H_2O_2, 30 %	Imp8	9.521	22422	1.136	1.025
	UV-light	Imp10	9.649	5789	1.107	1.048
	H_2O_2, 3 %	Imp8	9.993	165808	0.738	3.278
Unidentified13	UV-light	Imp11	10.08	30682	1.235	3.275
Unidentified14	H_2O_2, 3 %	Imp9	10.496	15855	1.026	2.441
Unidentified15	H_2O_2, 30 %	Imp9	10.797	54557	1.05	8.363
Bropa	H_2O_2, 30 %	Bropa	11.015	362480	1.388	1.183
	temperature	Bropa	11.093	4419651	1.156	–
	NaOH	Bropa	11.098	4328757	1.157	40.966
	daylight	Bropa	11.101	4304779	1.147	–
	HCl	Bropa	11.102	4378235	1.159	57.332
	H_2O_2, 3 %	Bropa	11.102	1790970	1.022	3.052
	control	Bropa	11.111	4345324	1.159	–
	UV-light	Bropa	11.119	508612	1.233	6.361
	synthetic mixture	Bropa	11.391	4978679	1.211	31.249

Unidentified16	temperature	Imp1	11.807	25609	1.13	4.191
4-bromobenzalde-hyde	Synthetic mixture	Sub2	12.23	1805369	1.149	5.716
Unidentified17	H_2O_2, 3 %	Imp10	12.285	46342	0.75	6.425
Unidentified18	H_2O_2, 30 %	Imp10	12.338	513289	0.823	6.526
Impurity A	UV-light	Imp12	12.738	5902	1.248	8.248
	H_2O_2, 3 %	Imp11	12.747	123153	1.074	2.519
	temperature	Imp2	12.748	93306	1.102	5.552
	daylight	Imp1	12.754	89789	1.086	9.445
	HCl	Imp3	12.756	100232	1.128	9.435
	control	Impurity A	12.766	91477	1.102	9.525
	H_2O_2, 30 %	Imp11	12.814	34621	1.075	2.534
Unidentified19	UV-light	Imp13	13.042	16998	1.161	1.399
Unidentified20	NaOH	Imp3	13.705	18959	1.145	213.944
Unidentified21	UV-light	Imp14	14.757	9816	0.908	8.765
Impurity B	NaOH	Imp4	14.891	10819	1.074	5.65
	temperature	Imp3	14.892	11699	0.906	10.65
	daylight	Imp2	14.896	14265	1.002	10.241
	H_2O_2, 3 %	Imp12	14.897	10891	0.983	10.695
	HCl	Imp4	14.899	8431	1.036	11.438
	control	Impurity B	14.911	8133	1.18	11.585
	UV-light	Imp15	14.918	4481	1.409	0.943
	H_2O_2, 30 %	Imp12	14.967	15.317	1.008	10.267

Table 1. A total of 23 unidentified impurities were detected, two of which were designated Impurity A and Impurity B, which appear to be byproducts of the Bropa synthesis.

The Bropa content after influence of stress factors compared to that of the control solution can be found in Fig. 13. It can be seen that daylight, temperature, NaOH and HCl have no effect on the compound, while UV and hydrogen peroxide sufficiently reduce the Bropa content.

To confirm the assignment of the peaks in Table 1, the Rt_{min} and Rt_{max} values were estimated to meet the stated requirements for the identification of Bropa in the substance by HPLC (Table 2), and it can be seen that all the *Rt* values found were within the min-max ranges, so the assignments in Table 1 were correct.

The situation with both Impurity A and Impurity B was interesting and provided information about their chemical properties. Impurity A was not found after the NaOH test, indicating its possible high reactivity in alkaline medium, but its content increased slightly to 2.8 % after the 3 % H_2O_2 test, while UV light and the 30 % H_2O_2 test decreased its content to 0.14 % and 0.8 %, respectively.

In contrast, Impurity B was found after all the tests and only HCl had no effect on its content while UV, daylight, and both hydrogen tests showed up to a twofold increase in its original content. It can therefore be assumed that the formation of impurity B is probably caused by various photochemical processes.

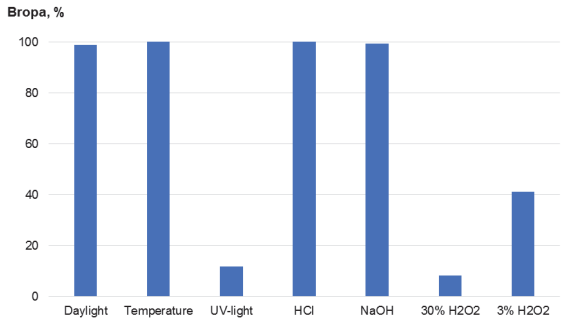


Fig. 13. Bropa content after the stress tests compared to the control solution.

It should be noted the presence of 3-amino-5-methylisoxazole after the UV test (0.6 % found), the NaOH test (0.3 % found), and both the hydrogen peroxide tests (1.9 % and 1.3 % for concentrated and diluted H_2O_2, respectively), while the HCl and daylight tests did not elicit its formation. This suggests some susceptibility of the 5-oxo-2,5-dihydro-1H-pyrrol-3-yl fragment of Bropa to oxidation and photoinduced chemical reactions.

Table 2. Retention time data check to prove the component assignment of peaks

Component assignment	Samples found in	Rt_{avg}	RSD Rt	Rt_{min}	Rt_{max}
Bropa	9	11.126	0.098	10.903	11.348
Impurity B	8	14.909	0.024	14.611	15.207
Impurity A	7	12.760	0.023	12.505	13.016
3-amino-5-methylisoxazole	5	4.095	0.018	4.013	4.177
Unidentified02	4	1.885	0.006	1.847	1.922
Unidentified12	3	9.811	0.199	9.615	10.007
Unidentified09	3	8.188	0.015	8.024	8.352
Unidentified10	3	8.417	0.064	8.249	8.585
Unidentified11	3	9.377	0.046	9.190	9.565
Unidentified03	2	4.325	0.007	4.238	4.411

Four unidentified impurities with indices 09-12 (Table 1) were found only after the UV and the two hydrogen peroxide tests and can therefore be assumed to be products of direct oxidation and photochemical degradation of Bropa.

While the impurity Unidentified02 was found after all tests except the UV and daylight tests, the impurity Unidentified03 was found after the UV and 30 % H_2O_2 tests, so no clear assumptions can be made about its possible formation.

The unidentified impurities with other indices are detected once in the corresponding tests (Table 1), while the UV test showed the largest number of impurities formed, probably due to the multiple possibilities of photochemical degradation of Bropa.

4. Conclusions

In the present work, the stability of the 2-(2-(4-bromophenyl)-4-hydroxy-1-(5-methylisoxazol-3-yl)-5-oxo-2,5-dihydro-1H -pyrrol-3-yl) acetic acid (Bropa), which is the structural analog of the compound with high antiviral activity, was studied providing complete qualitative and quantitative data for the degradation products.

Since two unidentified impurities labelled as Impurity A and Impurity B with retention times of ~ 12.8 min and ~ 14.9 min, respectively, were detected in the Bropa with content 2.1 % and 0.2 %, respectively, there is a need for further study of the reaction products of the Bropa synthesis.

The Bropa proved to be resistant to daylight, temperature, NaOH, and HCl, as an increase in impurity content of only 0.3–0.7 % was observed.

However, the Bropa was found to be unstable in UV and both hydrogen peroxide tests, as the content of the $C_{16}H_{13}BrN_2O_5$ decreased to 11.7 %, 8.3 % and 41.2 %, respectively, and 11-14 of the new impurities were detected by HPLC.

To ensure the safety of the new compound in preclinical and clinical development, it is important that the degradation products are quantified and structurally identified if they content exceeds the identification threshold established in ICH Q3A. And the large number of degradation products of a new compound makes this process lengthy, resource and labor intensive, and ultimately expensive. Thus, to be successfully selected as a promising drug substance, Bropa needs to be improved in regard to its photochemical and oxidative stability in order to significantly reduce the number of the degradation products if the long-term and accelerated stability testing proves that such its properties could pose a problem.

Acknowledgements. Authors thank to the National Academy of Sciences of Ukraine for financial support within the project 0122U001857, the Ministry of Education and Science of Ukraine for financial support within the project 0121U112886, and all brave defenders of Ukraine for allowing us to continue our scientific work and complete this research in besieged Kharkiv.

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