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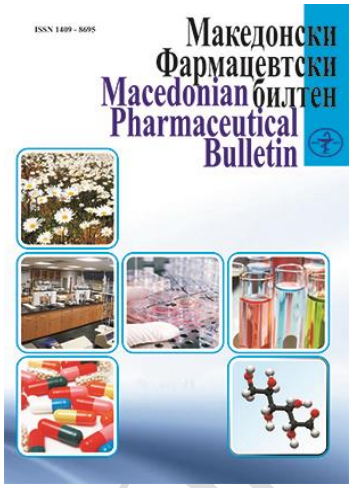

Title: A new validated RP-HPLC-UV DAD method for assay of bisoprolol fumarate and related substances in tablets

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A new validated RP-HPLC-UV DAD method for assay of bisoprolol fumarate and related substances in tablets

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Abstract

The research focus of this work was development and validation of an efficient analytical method that can be used for separation and determination of related substances of bisoprolol in finished drug product using reversed-phase HPLC with UV-DAD. In a previous systematic study of various stationary phases and elution conditions it was found that several octadecylsilane phases can be successfully employed for separation of the critical pairs of bisoprolol and its related degradation products. Namely, method development led to conclusions that satisfactory resolution and peak shapes were obtained with gradient elution with water with 0.2% perchloric acid and acetonitrile and the reversed-phase columns: Hypersil 3 BDS C18 (100 x 4 mm, 3 μm); Zorbax SB C18 (150 x 4.6 mm, 3.5 μm); Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 μm), and Xterra MS C18 (100 x 4.6 mm, 3.5 μm). Since regular quality control includes the parameter related substances, which is of great importance for the overall quality of dosage forms, this study was oriented towards widening the available validated analytical methods for determination of this parameter. In this work, the results from the validation of the method for determination of related substances of bisoprolol using Zorbax SB C18 150 x 4.6 mm, 3.5 μm, are presented. Validation parameters that were tested (linearity, accuracy, precision, selectivity) confirmed that the method is suitable for its intendance and it was used for characterization of the samples from a forced degradation study of bisoprolol tablets.

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Keywords: Bisoprolol, RP-HPLC-UV DAD, validation, related substances

Introduction

Quality control of products in pharmaceutical industry demand analytical methods that are easily implemented in the laboratory practice and are time/cost-effective, as well as valid for their purpose. It is also very important to test various stationary phases and mobile phases and have validated methods with a variety of possibilities that can be implemented in various laboratories. For this reason, in our laboratory a variety of methods have been tested for determination of bisoprolol and its related substances (Lazarevska et al., 2021), which have been found of great importance due to its extensive production and use. Bisoprolol belongs to the pharmacological group of synthetic beta-1-adrenergic blockers that are widely used in every day clinical practice for treatment of hypertension and angina pectoris (Bakheit et al., 2021).

A thorough literature survey has shown that there are many available analytical methods for determination of the content of bisoprolol fumarate as an individual active ingredient or in combination with other antihypertensive or diuretic active pharmaceutical ingredients published by Arjun et al. (2009), Joshi et al. (2010), Logoyda et al. (2019), Mahu et al. (2016), Panainte et al. (2015), Patel et al. (2006), Shaikh et al. (2008) and Witek et al. (1999). On the other side, there are not so many published methods that have been developed for separation and determination of bisoprolol and its potential related substances.

Methods that have been proposed for determination of related substances of bisoprolol fumarate are mostly transfers of the pharmacopoeial method described in British Pharmacopoeia, edition 2018 for bisoprolol fumarate as active substance as well as in the European Pharmacopoeia, 10th edition (first introduced in the 8th edition). There are only a few available methods such as the optimized HILIC methods for analysis of bisoprolol and its related substances developed by Rakic et al. (2014), and the method for monitoring bisoprolol stability under different stress conditions published by Kasagic-Vujanovic et al. (2017). Szalka et al. (2012), have developed methods using sub-2-μm adsorbents, which were of interest since they can reduce analysis run time and mobile phase consumption. Another study has been published for identification and structural

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characterization of one unidentified impurity in bisoprolol film-coated tablets by Mitrevska et al. (2017).

Considering the above-mentioned questions, it can be concluded that there is an interest in developing new efficient analytical methods for determination of related substance of bisoprolol. A systematic study aimed at development and optimization of analytical methods for determination of related substances of bisoprolol in a time-effective frame was performed in our laboratory using HPLC/DAD (Lazarevska et al., 2021). Different bonded phases (including alkyl and phenyl) with variable particle diameters and active surface areas and column dimensions, as well as variations in temperature and mobile phases with different pH and additives were tested. In the mentioned paper it was concluded that four stationary phases were selected as most successful, using the columns Hypersil 3 BDS C18 (100 x 4 mm, 3 μm); Zorbax SB C18 (150 x 4.6 mm, 3.5 μm); Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 μm), and Xterra MS C18 (100 x 4.6 mm, 3.5 μm). Full validation of the analytical method using Xterra MS C18 100 x 4.6 mm, 3.5 μm was published in the mentioned paper of Lazarevska et al. (2021).

In this work, the other suggested optimized experimental conditions using Zorbax SB C18 150 x 4.6 mm, 3.5 μm were tested in order to confirm that they can be implemented during everyday laboratory quality control as well as in studies of forced degradation of bisoprolol solid dosage forms.

Materials and methods

Chemicals and reagents

Acetonitrile and hydrogen peroxide solution 30% were from Carlo Erba; perchloric acid 70% was from Sigma Aldrich; hydrochloric acid 37% was from Merck, and sodium hydroxide was purchased from Riedel-de Haën.

Bisoprolol for system suitability (European Pharmacopoeia Certified reference standard, EPCRS) and bisoprolol for peak identification EPCRS were supplied by EDQM. Bisoprolol fumarate used for quantification was standardized versus a valid batch of bisoprolol fumarate EPCRS, supplied from EDQM.

Bisoprolol film-coated tablets 2.5 mg used in the study were from ReplekFarm, Skopje.

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Standards, test solutions and samples

Preparation of test solution: solution is prepared by dissolving a quantity of tablet mass to obtain concentration of 1 mg bisoprolol fumarate/mL solution. 20% acetonitrile in water (*v/v*) was used as a solvent.

Reference solution (a) was prepared by dilution of the test solution to obtain final concentration of 0.002 mg/mL bisoprolol fumarate solution.

Bisoprolol for peak identification was prepared by dissolving the content of the vial bisoprolol for peak identification CRS in 1 mL solvent (it contains: fumaric acid, bisoprolol impurity A, bisoprolol and bisoprolol impurity E).

Bisoprolol for system suitability was prepared by dissolving the content of the vial for bisoprolol for system suitability CRS in 1 mL solvent (it contains: fumaric acid, bisoprolol and bisoprolol impurity G).

Forced degradation studies

Forced degradation studies were divided as: hydrolytic (acid, base, oxidative and neutral), as well as thermal degradation, and exposure to moisture and light as in the ICH guidelines for Stability testing of new drug substance and product (EMA, 2003), and for Photostability testing of new active substances and medicinal products (EMA, 1998).

Hydrolytic forced degradation studies were performed by using of 1 M hydrochloric acid, 1 M sodium hydroxide and 3% (*v/v*) hydrogen peroxide, simultaneously on powdered tablet mass and placebo powder. Solutions that were prepared for testing were treated on ultrasonic bath at 60 °C for 1 hour for acid hydrolysis and 3 hours for the other tests. After the exposure, samples were cooled and neutralized, and then analysed according to the described analytical procedure.

Thermal degradation studies were performed by exposing samples of powdered tablet mass and placebo powder in open quartz dish at 105 °C for 5 hours in an oven with temperature control (ICH guidelines for Stability testing of new drug substance and product, EMA, 2003). Afterwards they were prepared and analysed according to the described analytical procedure.

Exposing the samples to UV radiation and heat in special chambers (according to ICH recommended photostability conditions), with overall illumination of not less than 1.2 million lux h along with UV energy not less than 200 W h/m^2. The powdered tablet

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mass and placebo powder were placed on an open quartz dish and kept for 10 days in the specified chamber. Then they were prepared and analysed according to the described analytical procedure.

HPLC analysis

HPLC/DAD system used for the analyses was UPLC System Shimadzu Nexera-I LC 2040C 3D Plus with LabSolution version 5.97.

The stationary phase used for the experiment was Zorbax SB C18 150 x 4.6 mm, 3.5 μm (Agilent Technologies, USA). This column packaging is characterised with pore sizes of 80 Å, active surface 180 m^2/g and carbon load of 10%. This phase is end-capped and base deactivated, has wide pH range from 0.8 - 8.0, and its separation efficiency is expressed as NTP/m (number of theoretical plate/meter) of 154186.

The mobile phase consisted of A: 0.2% (*v/v*) solution of perchloric acid in water, and B: acetonitrile. The gradient elution program was as follows: 0 - 9 min 27% B; 9.0 1-11.5 min 60%; 11.6 - 18 min 27% B. The flow rate was 1.2 mL/min, the column temperature was 20 °C, the injection volume was 4 μL.

Results and discussion

Method description and brief overview of chromatographic conditions

The main objective in this research work was developing a method that will enable satisfactory resolution of bisoprolol and its related degradation substances and its validation that will demonstrate its suitability for determination of related substances of bisoprolol in solid pharmaceutical dosage forms.

The analytical method that is here presented was developed in order to separate related substances listed in the monograph for bisoprolol fumarate in British Pharmacopoeia, 2018. It was listed as one of four effective methods for determination of related substances described by Lazarevska et al. (2021), which are listed in Table 1. The experimental data given in Table 1 briefly present the chromatographic conditions and results obtained during the systematic evaluation of a variety of stationary and mobile phases for separation of bisoprolol impurity A, G, and E from each other, and at the same time from the main peak of active substance bisoprolol. The advantages and disadvantages of all presented experiments in Table 1 are described in the paper

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mentioned before, as well as validation of the analytical method that uses Xterra MS C18 100 x 4.6 mm, 3.5 µm.

The method that employs the column Zorbax SB C18 with dimensions 150 x 4.6 mm, and particle diameter of 3.5 µm was considered as one of the suitable columns that allows satisfactory separation of the above-mentioned principles, which are characterized by analogous structures with aromatic rings and similar side chains (Fig. 1). The obtained results demonstrate a satisfactory resolution of about 2.3 between the peaks of fumaric acid and impurity A as well as resolution of 2.4 between the peaks of bisoprolol and impurity G and satisfactory tailing factor of 1.04 for impurity E, which is illustrated by the chromatograms presented in Fig. 2. The stepwise gradient elution was introduced with rapid increasing steps of the organic part of the mobile phase (acetonitrile) in order to enable a faster elution of bisoprolol impurity E, with satisfying symmetry and minimum tailing. The developed method was further subjected to validation in order to demonstrate its analytical performance.

Table 1

Fig. 1

Fig. 2

Validation of analytical method for determination of related substances of bisoprolol fumarate in final dosage form

HPLC method was validated according to the recommendations in the ICH guideline Q2(R1) for validation of analytical procedures (EMA, 2005). The parameters included in the validation were: linearity, accuracy, system repeatability, method repeatability and selectivity/specificity.

Linearity: Linearity was tested in the range from 0.40 mg/mL – 3.00 □g/mL. The obtained results for the response factors for each concentration level along with calculated relative standard deviation of response factors and calculated value for coefficient of determination (R^2) fit within acceptable limits. The values obtained for RSD of response factors was 5.1%, and the obtained value for the coefficient of determination was R^2 is 0.9988. The obtained equation of the calibration curve for the peak area versus

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concentration was y = 5563x + 461.13, and the obtained value for R^2 is 0.9988 that confirms good linear correlation.

Accuracy: The study of analytical recovery was performed in three concentration levels in order to determine the accuracy of the analytical procedure. Placebo was spiked with known concentration of bisoprolol to obtain concentrations of 1 μg/mL, 2 μg/mL, and 3 μg/mL. Results are presented in Table 2, and fit within the acceptance range of 90 - 110%, which confirm that recovery of the analytical method is satisfied in all cases.

Table 2

Specificity/selectivity: Specificity/selectivity was tested by injecting system suitability solution, peak identification solution, placebo solution, standard solution and test solution. Chromatograms obtained from placebo solution, test solution and standard solution confirm that excipients present in the formulation as well as solvents used for preparation do not interfere with the peak of bisoprolol and at the same time do not interfere with the peaks of the specified impurities (bisoprolol impurity A, G, and E).

In the chromatogram obtained for the system suitability solution (Fig. 2), peaks due to fumaric acid, bisoprolol and bisoprolol impurity G can be detected in the order as they appear in the chromatogram. The obtained resolution between bisoprolol and bisoprolol impurity G was 2.4. In the chromatogram of solution for peak identification (Fig. 2), the peaks of fumaric acid, bisoprolol impurity A, bisoprolol, and bisoprolol impurity E can be detected, also mentioned as they appear in the chromatogram. The resolution between fumaric acid and bisoprolol impurity A is 2.3, and the symmetry of the peak corresponding to presence of bisoprolol impurity E is 1.04.

Furthermore, to determine whether the analytical method is stability indicative forced degradation studies were performed. As can be seen in the chromatograms in Fig. 3, the obtained results through forced degradation studies have shown good separation between bisoprolol and degradation products and at the same time satisfactory values for the mass balance between 92.11 and 94.87% (Fig. 3 and Table 3).

Fig. 3

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Table 3

Precision: System precision was quantified as relative standard deviation from five consecutive injections of diluted standard solution. Obtained results for relative standard deviation of 0.18% confirm good reproducibility, which is within limit of acceptance (RSD less than 10%).

Method repeatability was established by five consecutive injections of test solutions that were sampled from the same homogenised powdered tablet mass and prepared according to the analytical procedure. The results presented in Table 4 show the presence of unspecified impurities that were detected and quantified with relative standard deviation less than 10%, while the presence of specified impurities (bisoprolol impurity A, G, and E) was not detected.

Table 4

Conclusion

Chemical moieties that were subject of intere st in this research work share very similar chemical structure. Their separation was considered as challenging, but still achieved in the systematic study performed in which four methods intended for determination of related substances of bisoprolol were demonstrated as satisfactory.

Since quality control laboratories are eager to implement cost/time-effective methods for everyday use which are qualified with high validity and versatility, validation of these analytical methods is necessary and beneficial.

Analytical performance parameters for the developed method for determination of related substances of bisoprolol using Zorbax SB C18 150 x 4.6 mm, 3.5 µm have confirmed good results in point of all validation parameters which confirms that this method is suitable for its intendance.

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Резиме

Нов валидиран RP-HPLC-UV DAD метод за определување на бисопролол фумарат и сродни супстанци во таблети

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Целта на ова истражување е развој и валидација на ефикасен аналитички метод за сепарација и определување на сродни супстанци на бисопролол во готов производ со реверзно-фазна HPLC со UV-DAD. Во претходна студија на различни стационарни фази и услови за елуирање утврдено е дека со повеќе октадецилсилански фази може да се постигне разделување на критичните парови на бисопролол и неговите деградациони продукти. Задоволителна резолуција е добиена со градиентно елуирање со 0,2% перхлорна киселина во вода и ацетонитрил и реверзно-фазни колони од типот: Hypersil 3 BDS C18 (100 x 4 mm, 3 μm); Zorbax SB C18 (150 x 4,6 mm, 3,5 μm); Acquity UPLC BEH C18 (50 x 2,1 mm, 1,7 μm), и Xterra MS C18 (100 x 4,6 mm, 3,5 μm). Во овој труд се презентирани резултатите од валидацијата на методот за определување на сродни супстанци на бисопролол со употреба на колона од типот Zorbax SB C18. Со определување на параметрите за валидација линеарност, точност, прецизност и селективност е потврдено дека методот е соодветен за намената и применет е за карактеризација на примероци од студија на форсирана деградација на таблети од бисопролол.

Клучни зборови: бисопролол, RP-HPLC-UV DAD, валидација, сродни супстанци

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Table 1. Summary of results obtained for resolution, tailing factor and run time

Experimental conditions	R_S Fumaric acid-Imp. A	R_S Bisoprolol-Imp. G	Tailing factor, Imp. E	Run time/min
Zorbax SB C18 150 x 4.6 mm, 3.5 μm; Gradient elution: 0.2% (*v/v*) perchloric acid and acetonitrile Flow: 1.2 mL/min	2.3	2.4	1.04	18
Xterra MS C18 100 x 4.6 mm, 3.5 μm; Gradient elution: 0.2% (*v/v*) perchloric acid and acetonitrile Flow: 0.23 mL/min	2.6	2.4	1.17	13
Hypersil 3 BDS C18 100 x 4.0 mm, 3 μm; Gradient elution: 10 mM phosphate buffer pH 3.0 with 0.2% (w/v) potassium hexafluorophosphate and acetonitrile Flow: 1.0 mL/min	4.3	2.3	1.03	21
Acquity UPLC BEH C18 50 x 2.1 mm, 1.7 μm; Gradient elution: 0.2% (*v/v*) perchloric acid and acetonitrile Flow: 0.23 mL/min	5.6	2.4	1.06	12

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Table 2. Analytical recovery of bisoprolol

Standard addition (μg/mL)	Measured concentration (μg/mL)	Recovery (%)
0.979	0.921	94.04
1.958	1.794	91.59
2.742	2.521	91.95
	Average	92.53
	Relative standard deviation, %	1.41

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Table 3. Mass balance calculation results

	Normal	Acid	Alkaline	Oxidative	Thermal	Light
% content of active substance	94.59	91.50	82.45	81.47	78.94	85.22
% degradation products	0.28	3.23	10.40	10.64	14.14	9.09
total (sum of active substance and degradation products)	94.87	94.76	92.85	92.11	93.08	94.31

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Table 4. Method repeatability results

	Imp. A (%)	Imp. G (%)	Imp. E (%)	Unspecified impurity RRT/0.61 (%)	Unspecified impurity RRT/0,79 (%)	Unspecified impurity RRT/0,90 (%)	Unspecified impurity RRT/1.25 (%)
Test solution 1	nd*	nd	nd	0.036	0.019	0.089	0.035
Test solution 2	nd	nd	nd	0.037	0.019	0.087	0.034
Test solution 3	nd	nd	nd	0.036	0.019	0.086	0.036
Test solution 4	nd	nd	nd	0.037	0.018	0.089	0.036
Test solution 5	nd	nd	nd	0.037	0.02	0.087	0.036
Average	/	/	/	0.04	0.02	0.09	0.04
RSD, %	/	/	/	1.50	3.72	1.53	2.53

*nd – not detected

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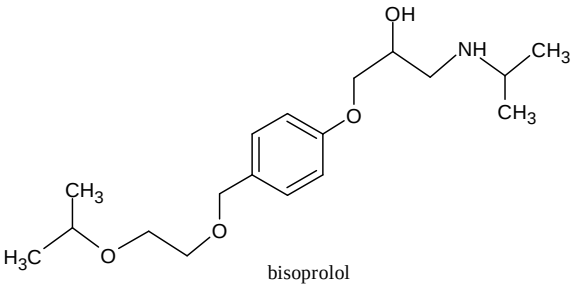
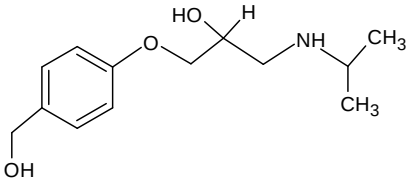
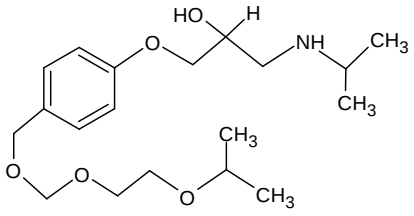


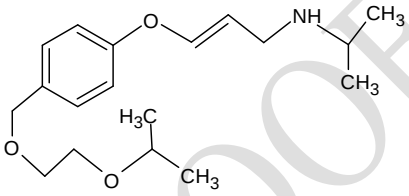
bisoprolol



bisoprolol impurity A



bisoprolol impurity G



bisoprolol impurity E

Fig. 1. Chemical structures of bisoprolol, bisoprolol impurity A, bisoprolol impurity G, and bisoprolol impurity E.

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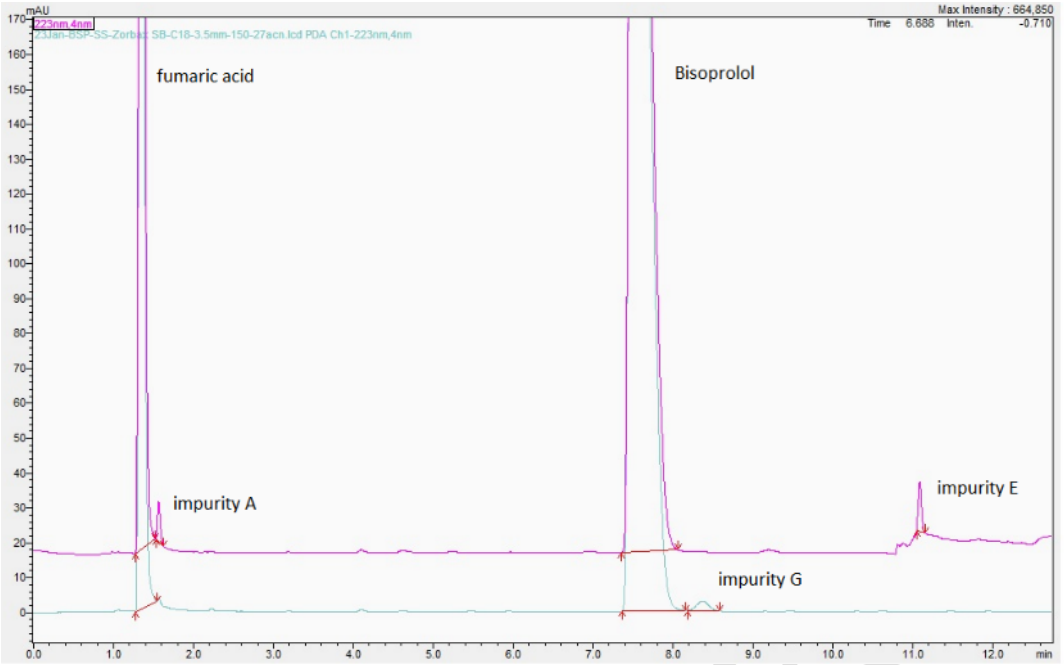

Fig. 2. Chromatograms of solution for peak identification and solution for system suitability obtained using Zorbax SB C18 150 x 4.6 mm, 3.5 μm, and gradient elution with acetonitrile and 0.2% (*v/v*) perchloric acid; 1.2 mL/min, 20 °C.

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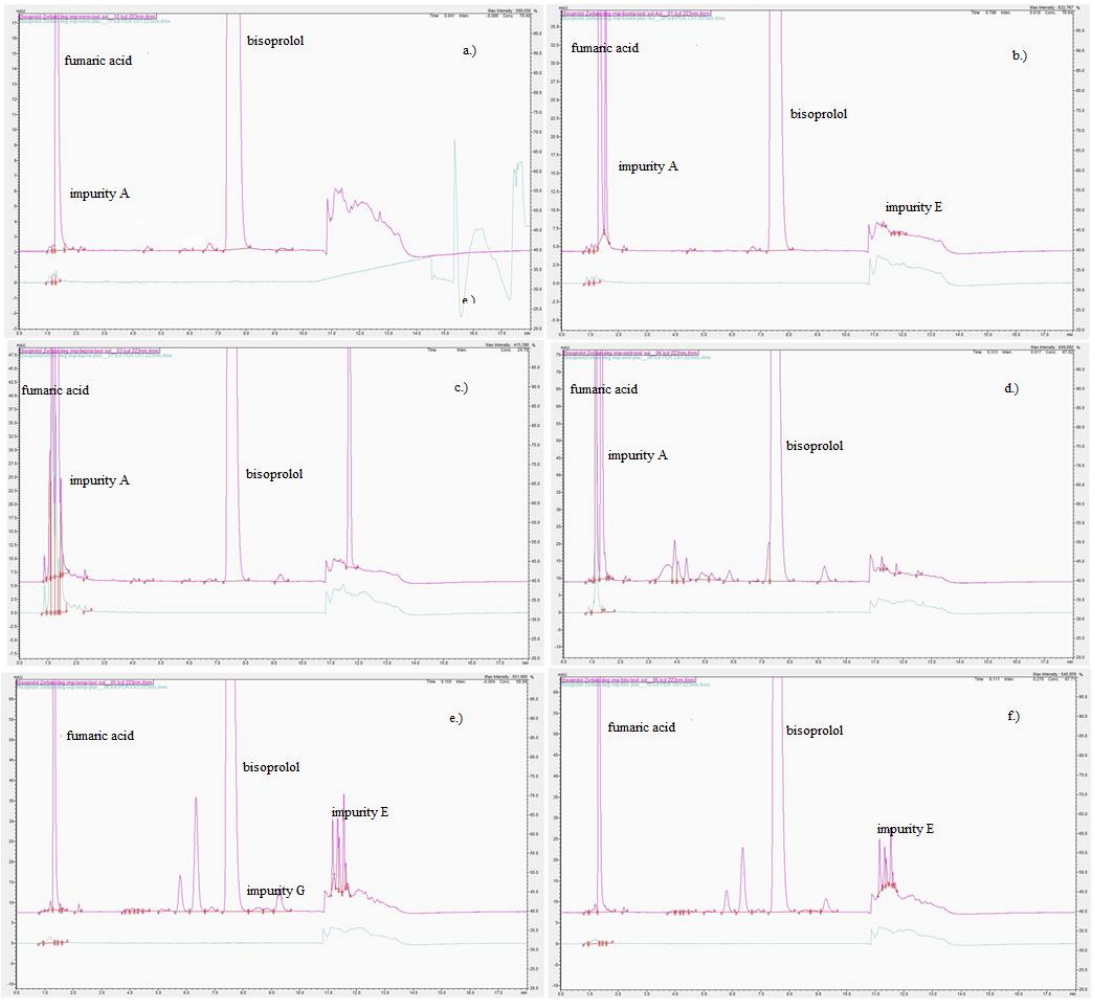

Fig. 3. UV-DAD chromatograms of test vs placebo solution in: a) normal conditions; b) acid degradation; c) alkaline degradation; d) oxidative degradation; e) thermal degradation; and f) photodegradation.

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