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Application of the experimental design method to photostability studies of Karvileks tablet

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ABSTRACT

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This study was examined a photostability of Karvileks (Zdravlje-Actavis, Serbia), packed in the different primary packs. For these examination, the experimental design with 3 independent variables: wavelength of radiation (X_1), duration of radiation (X_2) and type of packs (X_3) was applied. After irradiation of Karvileks, the content of carvedilol and its impurities A, B and C was taken as a response (Y_1, Y_2, Y_3, Y_4). The obtained mathematical equations can be used for determination of carvedilol and its impurities content in any case. Tablets were packed in an opaque plastic container, as well as in red blister. The content of carvedilol was determined using a stability-indicating RP-HPLC method. The obtained models were: $\mathbf{Y_1} = 93.11 + 1.74\,X_1 - 2.19\,X_2 + 2.01\,X_3 + 0.39\,X_1X_2 + 0.39\,X_1X_3$; $\mathbf{Y_2} = 0.22 - 0.02\,X_1 + 0.14\,X_2 - 0.01\,X_3 - 0.01\,X_1X_2 - 0.007\,X_2X_3$; $\mathbf{Y_3} = 0.001 + 0.014\,X_1 + 0.014\,X_2 - 0.001\,X_3 + 0.014\,X_1X_2 - 0.001\,X_2X_3$; $\mathbf{Y_4} = 0.102 + 0.008\,X_1 + 0.064\,X_2 - 0.007\,X_3$. By using an experimental design, the opaque plastic container was selected as an adequate primary pack for Karvileks. This pack provides an adequate protection of the preparation from the effect of sunlight.

Keywords: experimental, design, photostability, Karvileks.

INTRODUCTION

The main use of experimental design is to enable a simultaneous, systematic and rapid evaluation of each factor effect on the selected responses of the parameters. Also, it can be predicted the dependence of the effects between individual factors, identification of the critical factors, as well as its levels[1].

The experimental design has found wide application in the formulation of pharmaceutical products[2-9], optimization of process phases[10], development of analytical techniques[11], as well as in studies of drug stability[12-20].

In this paper, it was investigated product Karvileks (Zdravlje - Actavis, Serbia) from group of beta blockers. Carvedilol, as an active substance of product, is a photosensitive[21]. In literature, it can be found information about chromatographic methods[22-34], which used for determination of carvedilol concentration in biological fluids (blood, plasma and serum). Many methods are focused on the separation of enantiomers[26-30]. P. Sripalakit and coworkers[35] were developed a carvedilol assay in tablet dosage form using HPLC with fluorescence detection. M. Rizwan et. al. were developed a *stability-indicating* LC method[36] to study the kinetics of carvedilol degradation in accordance with ICH regulative[37]. Stojanovic et al.[21] were developed and validated RP-HPLC method for simultaneous separation and determination of carvedilol, as well as its potential impurities in pharmaceutical formulation. They also monitored its photochemical stability.

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The aim of this paper was to examine a photostability of Karvileks (Zdravlje-Actavis, Serbia), packed in different primary packs. For these examination, it was applied the model of experimental design with 3 independent variables factors: wavelength of radiation (X_1), duration of radiation (X_2) and type of packs (X_3). The content of carvedilol and its impurities A, B and C were taken as a response (Y_1, Y_2, Y_3, Y_4), after irradiation of Karvileks.

MATERIAL AND METHODS

Samples. Standard of carvedilol was purchased from NOSCH-a Labs Private Limited, India. Standards of impurities A, B and C were purchased from Merck, Darmstadt, Germany. Karvileks tablets, manufactured by Zdravlje-Actavis, Serbia, is contained a 12.5 mg of carvedilol.

Reagents. Acetonitrile HPLC grade was purchased from Merck, Darmstadt, Germany. All chemicals used were of analytical grade and deionized water was HPLC grade.

Photostability studies. Analyzed tablets were packed in an opaque plastic blister (Al/PVC) and red blister. They are exposed to light to determine the effects of irradiation on the stability of the drugs. All samples for photostability testing

were placed in a light cabinet (Suntest CPS/CPS$^+$, Atlas Material Testing Technology, Germany) and exposed to different wavelength (254 and 540 nm) and duration of radiation (3.5; 7 and 17 days) at 25 °C. Control samples which were protected from light were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as described in the part for preparation of tablets.

Apparatus. The method was performed by Agilent 1100-Series HPLC system (Agilent Technologies, USA), consisting of a HP G13141A variable wavelength UV detector and Agilent 1100-Series auto-sampler using a 20 µL sample loop. The system was controlled and data analyses were performed by Agilent HPLC Data Analysis software. The reproducibility was performed by another LC system, consisting of an Agilent 1100-Series binary pump and Agilent 1100-Series DAD detector (Faculty of Technology, Leskovac). The detector was set at 240 nm and the peak areas were integrated automatically using the Agilent HPLC Data Analysis software program. The separation of carvedilol from its impurities was carried out at 20 ºC using an octylsilyl silica column (4.6 x 100 mm, 5 µm) Agilent Technologies, USA.

Chromatographic conditions. RP-HPLC analysis was performed by isocratic elution with a flow rate of 1 mL/min. A mobile phase was contained a phosphate buffer in water : acetonitrile (65:35 v/v). Procedure for preparation of phosphate buffer is the following: dissolve a 1.77 g potassium dihydrogen phosphate in 650 mL of water. All solvents were filtered through a 0.45 µm millipore filter. Volumes of 20 µL of the solutions and samples were injected into the column. In accordance with the Eur Ph VI[38], the expected retention time of carvedilol was 4.5 min. Retention time of impurities A, B and C were 0.6 min, 3.5 min and 6.7 min, respectively.

Packaging. Two container systems (blister and opaque plastic packs) were used in the stability studies. The first was a blister pack comprising 200 µm PVDC (red and white colour) of grade 40 g/m^2 and of 20 µm aluminium foil. These packs were purchased from Ceduliose (St Maur des Fossés, France). Packs were formed on the Noack DPN 760 blister packers; the plastic film was unwound from the reel and guided though a preheating station on the blister line. The temperature of the pre-heating plates (for the upper and lower plates this is fixed at about 120 °C) was such that the plastic became soft and mouldable and cavities were then formed in a forming station using compressed air. Finally, the aluminium foil was heat-sealed at about 170 °C.

The second tested container was the opaque plastic container. Plastic container content was HDPE (high-density polyethylene). The mass density of HDPE can range from 0.93 to 0.97 g/ml. It is also hard and opaque and can withstand somewhat higher temperatures (120 °C for short periods, 110 °C continuously).

Preparation of standard solutions. 12.5 mg of carvedilol and 10 mg of impurities A, B and C were dissolved in 10 mL volumetric flask in mobile phase, with mixing in the ultrasonic bath for 15 min. 1 mL of this solution was transferred into another clean dry flask of 10 mL. 1 mL of this solution contained 0.125 mg of carvedilol and 0.1 mg of impurities A, B and C.

Sample preparation of product Karvileks. After radiation, tablets were finely broken and all the powder content was transferred into conical flask of 100 mL. The obtained solution was inserted in the ultrasonic bath and sonificated for 15 min. After that, the obtained solution was filtered through qualitative filter paper and then through a membrane filter of 0.45 µm. 1 mL of this solution was transferred into flask of 10 mL and supplemented by mobile phase to the mark. A non-irradiated sample of tablets was used as a blank sample.

Equations for calculating a content of carvedilol and impurities

For determination a content of carvedilol and impurities in the tested samples, it was used a following equation was used:

$$\text{mg Carvedilol/ tablet} = \frac{Ip \times Wst \times K}{1st \times 100} \quad \text{..... (1)}$$

$$\%\ \text{Impurity A} = 2 \times \text{Aanalyzed solution (which correspond to impurity A)} \times 0.5/2 \times \text{Ast} \quad \text{..... (2)}$$

$$\%\ \text{Impurity C} = \text{Aanalyzed solution which correspond to impurity C)} \times 0.05\%/10 \times \text{Astc} \quad \text{..... (3)}$$

$$\%\ \text{other impurity} = \text{Aisp} \times 0.5/\text{Ast} \quad \text{..... (4)}$$

where is:

Ip – peak area of carvedilol in the tested solution

Ist – peak area of carvedilol in standard solution

Wst – measured amount of standard substance of carvedilol

Ast – peak area of standard solution of impurity A

Astc - peak area of standard solution of impurity C

Experimental design. For studying the influence of primary packs on photostability of Karvileks, eight experiments were prepered. For this investigation, tablets were packed in a red blister and opaque plastic container. After radiation, the content of carvedilol and its impurities, as response (Y_1, Y_2, Y_3 and Y_4), were determined using a stability-indicating RP-HPLC method.

The data was statistical analyzed using Statistica 8.0 (Stat Soft, Inc., Tulsa, USA). The values of response and independent variables of model experimental design are presented in Table No.1.

Table 1: The combination of independent variables and the value of dependent variables

Run	X_1 nm	X_2 day	X_3	Y_1 Content of carvedilol (%)	Y_2 Content of Impurity A (%)	Y_3 Content of Impurity B (%)	Y_4 Content of Impurity C (%)
1	254	3.5	red blister	92.1	0.09	0	0.04
2	540	3.5	red blister	94.6	0.08	0	0.04
3	254	17	red blister	87.4	0.401	0	0.173
4	540	17	red blister	90.3	0.362	0.059	0.183
5	254	3.5	plastic container	95.8	0.08	0	0.03
6	540	3.5	plastic container	98.7	0.07	0	0.04
7	254	17	plastic container	90.2	0.371	0	0.132
8	540	17	plastic container	95.8	0.309	0.049	0.176

RESULTS AND DISCUSSION

Environmental factors, such as temperature, humidity and light, are the main causes of drug degradation.

The effect of sunlight on the drug can have a negative impact on its quality, safety and pharmacotherapy. For these reasons, all researches in the direction of photodegradation a Karvileks (Zdravlje Actavis, Serbia) are justified.

In the literature can be found information about monitoring photochemical stability of Karvileks by long-term methodes[21]. The product was kept at room temperature, exposed to the effect of sunlight. The percentage of degradation products was measured every 20 days until 100th day.

In addition to long-term methods for investigating photostability of carvedilol, Rizwan et al.[36] was applied an accelerated aging test in accordance with ICH regulative. For this investigation, methanolic solutions of carvedilol were prepered, which were exposed to the sunlight (72 h) and UV radiation at a wavelength of 254 nm for 3 h.

Having in the mind the fact that carvedilol is a photo-sensitive substance, the influence of different primary packs, as well as effect of wavelength and duration radiation on tablet stability was tested.

For studying a stability of product, it can be applied several methodologies. In this paper, methodology of experimental design was applied for investigation an influence of wavelength (X_1) and duration (X_2) of radiation and type of packs (X_3) on product stability of Karvileks (Zdravlje-Actavis, Serbia). The advantage of this method is simultaneous, systematic and rapid evaluation of the independent variables effect to the appropriate response (the content of carvedilol and impurities). By using the experimental design, the interaction between variables can be defined. The statistical analysis is the best indicator of the critical independent variables. Also, the limits of variables can be predicted using the experimental design.

The coefficients of proposed model were calculated, which represent the relationship between response and independent variables.

The mathematical model is presented in the form of follows equations:

$$Y_1 = 93.11+1.74\,X_1-2.19\,X_2+2.01\,X_3+0.39\,X_1X_2+0.39\,X_1X_3 \quad (5)$$

$$Y_2 = 0.22-0.02\,X_1+0.14\,X_2-0.013\,X_3-0.01\,X_1X_2-0.007\,X_2X_3 \quad (6)$$

$$Y_3=0.001+0.014\,X_1+0.014\,X_2-0.001\,X_3+0.014\,X_1X_2-0.001\,X_2X_3 \quad (7)$$

$$Y_4 = 0.102+0.008\,X_1+0.064\,X_2-0.007\,X_3 \quad (8)$$

On the basis of the mathematical model and statistical data using ANOVA, the significance of equation terms can be estimated. When the value of p is lower than 0.05, the equation term is the significant. These terms have a significant impact on the content of carvedilol and its impurities in the tested preparation. The equation 5-8 has the values of correlation coefficient of 0.993, 0.999, 0.980, and 0.963, respectively.

The main effects of variables on the content of carvedilol and its impurities (A, B, C) are shown in Figs. 1-4.

Fig. 1: Standardized main effect Perto charts for the content of carvedilol

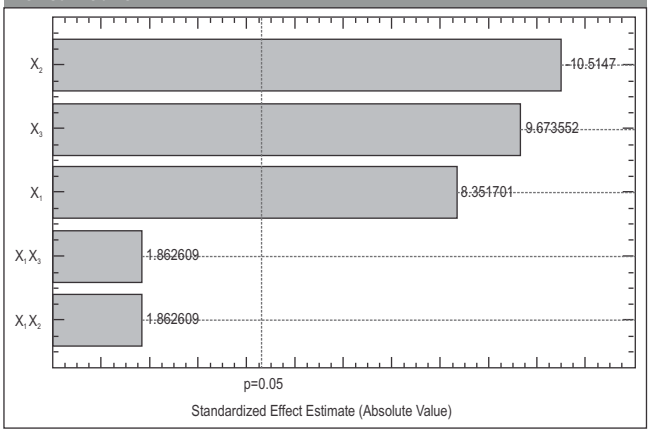


Fig. 2: Standardized main effect Perto charts for the content of Impurity A

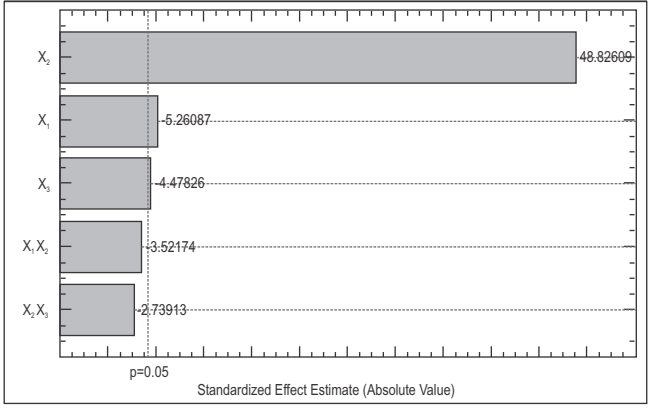
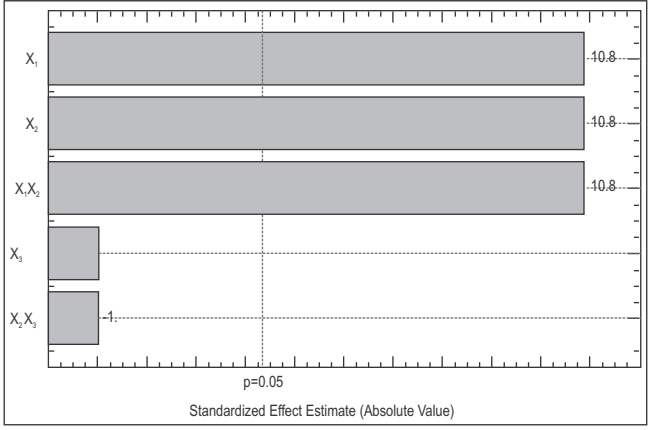
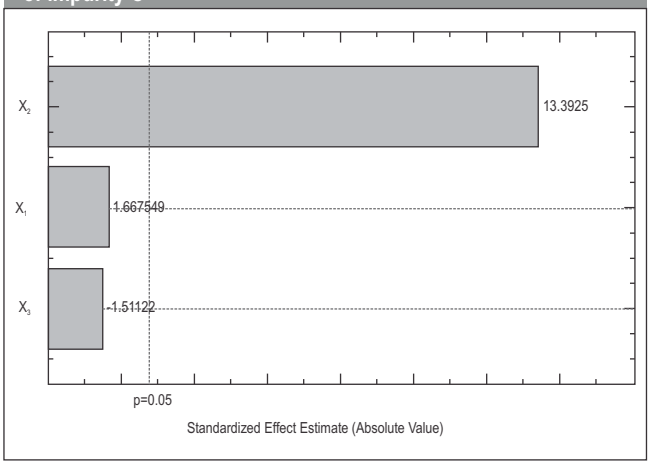


Fig. 3: Standardized main effect Perto charts for the content of impurity B



In the case of a change just one independent variable (Fig. 1), it was noted that the duration of radiation (X_2) had a dominant influence on the content of carvedilol. Type of packs (X_3) had less impact, while wavelength of radiation (X_1) had a negligible effect compared with the previously discussed factors. The greatest influence on the change of impurities

Fig. 4: Standardized main effect Perto charts for the content of impurity C



content had duration of radiation (X_2) and type of radiation (X_1).

A relationship between response and independent variables was shown using the contour diagrams (Figs. 5-8). These diagrams are useful for illustrating the influence of factors independent variables on the change of carvedilol content and its impurities in the irradiated samples of Karvileks.

The preparation was relatively photostable in the wavelength range from 400 to 550 nm for both types of primary packs (Fig. 5). Because, the content of carvedilol in irradiated samples of Karvileks was over 90%, accordance with ICH Q1B regulative. The carvedilol content was in the range of 92 to 95% for a red blister, while the content (96.56 to 99%) was slightly higher for a plastic container at the same value of independent variable X_1.

The content of carvedilol was of 87 to 92% for red blister and slightly higher (92.5 to 95.75%) for opaque plastic container at the change of variable X_1 in the range of 250 to 400 nm.

Fig. 5: The content of carvedilol in red blister (left) and plastic container (right)

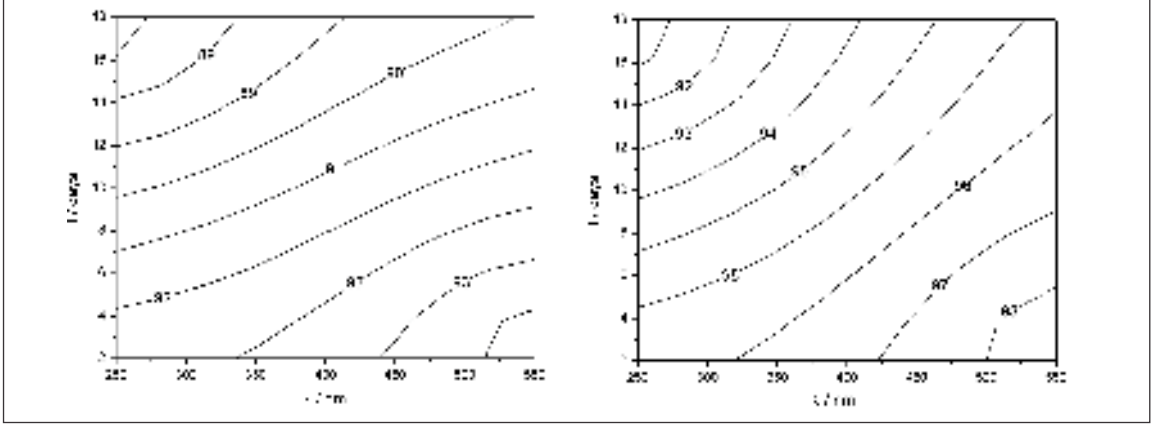


Fig. 6: The content of impurity A in red blister (left) and plastic container (right)

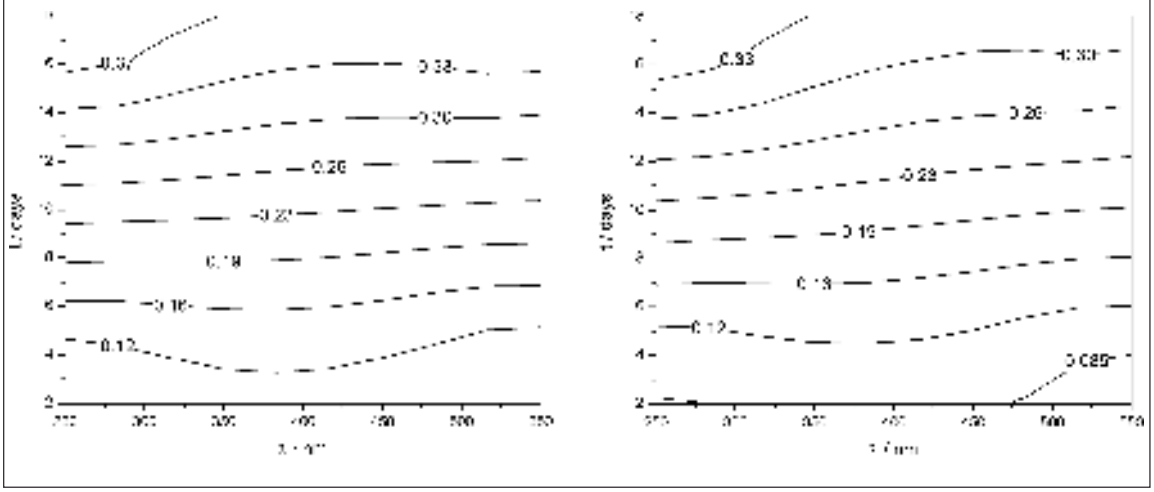


Fig. 7: The content of impurity B in red blister (left) and plastic container (right)

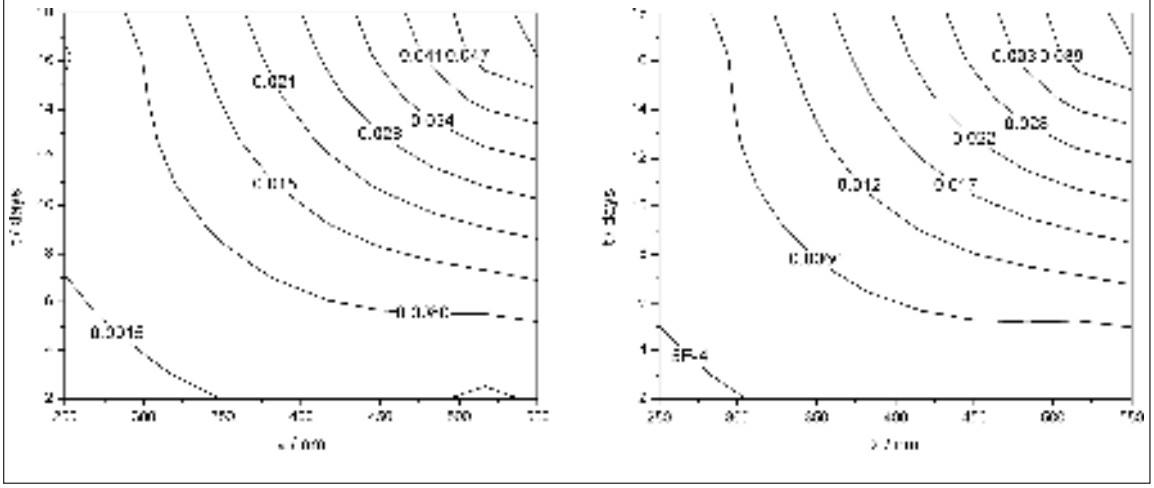


Fig. 8: The content of impurity C in red blister (left) and plastic container (right)

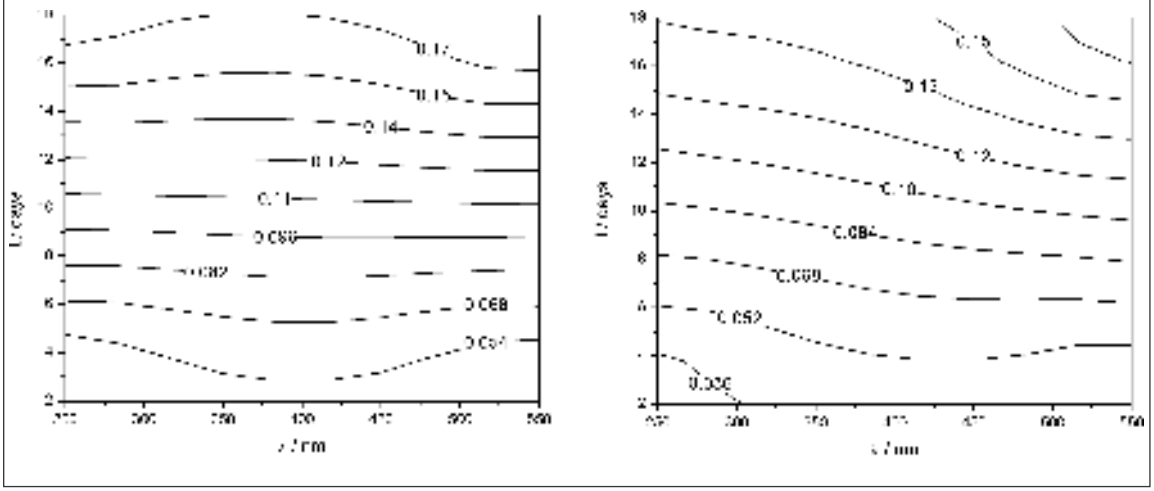


Table 2: The statistical analysis (ANOVA) of response

Variable	df	SS	MS	F value	p value
Y_1 (Content of carvedilol)					
Model	7	97.9	97.9	14.209	0.20
X_1	1	24.2	24.2	36.524	0.10
X_2	1	38.3	38.3	57.892	0.08
X_3	1	32.4	32.4	49.000	0.09
X_1X_2	1	1.2	1.2	1.816	0.41
X_1X_3	1	1.2	1.2	1.816	0.41
X_2X_3	1	0.0	0.0	0.047	0.86
$X_1X_2X_3$	1	0.7	0.7	1	0.50
Error	1	0.7	0.7	-	-
Y_2 (Content of Impurity A)					
Model	7	0.2	02	14.471	0.03
X_1	1	0	0	27.677	0.12
X_2	1	0.2	0.2	23.84	0.01
X_3	1	0	0	20.055	0.14
X_1X_2	1	0	0	12.403	0.18
X_1X_3	1	0	0	1	0.5
X_2X_3	1	0	0	7.503	0.22
$X_1X_2X_3$	1	0	0	1	0.50
Errora	1	0	0	-	-
Y_3 (Content of Impurity B)					
Model	7	0	0	-	0.0001
X_1	1	0	0	116.64	0.06
X_2	1	0	0	116.64	0.06
X_3	1	0	0	1	0.5
X_1X_2	1	0	0	116.64	0.06
X_1X_3	1	0	0	1	0.5
X_2X_3	1	0	0	1	0.5
$X_1X_2X_3$	1	0	0	1	0.5
Error	1	0	1	-	-
Y_4 (Content of Impurity C)					
Model	7	0	0	0	0.0001
X_1	1	0	0	7.11	0.23
X_2	1	0	0	458.67	0.03
$X_3$1		0	0	5.840	0.25
X_1X_2	1	0	0	3.361	0.32
X_1X_3	1	0	0	3.361	0.32
X_2X_3	1	0	0	2.507	0.36
$X_1X_2X_3$	1	0	0	1	0.50
Error	1	0	0	-	-

The obtained results indicate that Karvileks show a greater stability in VIS field compared with UV.

In addition, the wavelength and duration of radiation had an influence on the photostability of product. It was noted from diagram that the product was relatively photo stable for 7 days of duration of radiation, in the case of a red blister and VIS radiation (400 to 550 nm), or 13 days for plastic container. Also, it was confirmed that the type of packs had a significant influence on stability of Karvileks. For both wavelength of radiation (254 and 540 nm), the content of carvedilol was higher using a plastic container compared with a red blister.

The obtained contour diagrams, where are presented the content of pharmacopoeia impurities A, B and C (Figs. 6-8), there are zones where the impurities content was less than 0.1%.

With contour diagrams can be noted that duration of radiation and type of packaging had the greatest impact on content change of impurities, which was confirmed using statistical analysis.

The type of packs and duration of radiation have the highest influence on the change of impurities content, what is confirmed using ANOVA test.

The content of impurity A, when the product was packed in a red blister, was less than 0.1% for radiation in the range of 480 to 550 nm and for 3 days of duration of radiation. Using of a plastic container, the content of the same impurity was remained less than 0.1% using radiation in wider range of wavelength (425 to 550 nm) and longer duration of radiation (6 days).

The content of impurity C was less than 0.1% for a red blister, when the radiation wavelength was in the range of 250 to 550 nm for 9h. But the same content of impurity C was achieved after 11 days of radiation for a plastic container.

Unlike to the impurities A and B, the content of impurity B was less than 0.1% in all investigated cases. The obtained low values were indicated on a high photostability of this impurity.

CONCLUSION

Using of the experimental design, it was found that the dominant influence on the content of carvedilol and its impurities had duration of radiation (X_2). It was found that the greatest change of the carvedilol content and its impurities was been during simultaneous change of the wavelength and duration of radiation (X_1X_2). The most adequate primary packs for Karvileks compared with red blister. On the basis of the content carvedilol and its impurities, it was confirmed that the opaque plastic container is an adequate pack for protection a product from the effect of daylight. In this case the preparation was shown as a relatively photostable.

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