

Study of the Influence of Primary Packaging on Photostability of Tablets Containing Carvedilol

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ABSTRACT

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The aim of this work was to select an appropriate primary packaging and analysis of its influence on stability, of tablets containing Carvedilol (Karvileks, Zdravlje-Actavis, Serbia). The influence of different primary packaging materials and doses radiation was investigated. Karvileks tablets were put in an opaque plastic container, red and white blister packs, composed of polyvinyl chloride and aluminum foil (PVC/Al). After radiation, the content was estimated using a validated HPLC method. Tablets, packaged in different primary packaging materials, were exposed at different doses of UV and VIS radiation. In case of blister packs, the content of active substance was lower than 85% compared with an initial content. Tablets packaged in the opaque plastic containers, the content of carvedilol was not lower than 90%, after radiation. The study showed that the opaque plastic containers provide better photo protection than red and white blister packs for solid oral dosage forms of Karvileks.

Keywords: Karvileks, photostability, HPLC, packaging, tablets.

INTRODUCTION

Carvedilol, a third-generation β -blocker, is a unique cardiovascular drug with multiple pharmacological activities as demonstrated in the treatment of hypertension, angina pectoris, and congestive heart failure¹. It is nonselective β -adrenergic blocking agent^{2,3} with unique antioxidant activities^{4,5} as well as α_1 -adrenoceptor blocking effects⁶ leading to vasodilating actions. It has been reported in vitro studies that the β -blocking potency S-carvedilol is about 100 times greater than that of R-carvedilol, whereas the α -blocking activity of both enantiomers is at equal potency⁷. Carvedilol is marketed under various trade names including Coreg (GSK), Dilatrend (Roche), Eucardic (Roche), and Carloc (Cipla) as a generic drug, Karvileks (Zdravlje-Actavis)⁸.

Several chromatographic methods have been developed for analysis of carvedilol in biological fluids such as whole blood, plasma, and serum⁹⁻²². Most methods have focused on separation of the enantiomers¹²⁻¹⁶. In the literature were described a HPLC methods for the dissolution test²³ and the quantitative determination of carvedilol in pharmaceutical

dosage forms²⁴. One stability-indicating UV spectroscopic method was reported by Imran et al.²⁵. Galanopoulou and coworkers²⁶ have developed and validated of a new HPLC method, enabling the determination of carvedilol and its potential impurities and other degradation products.

In the literature can find information about monitoring of Karvileks photochemical stability using long-term method²⁷. The percentage of degradation products was measured every 20 days for 100 days.

In addition to long-term method for studying photostability of carvedilol, M. Rizwan et al.²⁸, was applied the accelerated aging test in accordance with ICH guidelines²⁹. For this study, the methanolic solutions of carvedilol were prepared. These were exposed to the sunlight (72 h) and UV radiation at a wavelength of 254 nm for 3 h. The obtained results of this study indicate that carvedilol is relatively photostable compound.

In this paper was tested the effect of different primary packaging materials, as well as the type and dose of radiation on the photostability of the tablets containing Carvedilol (Karvileks, Zdravlje-Actavis, Serbia) in accordance with ICH guidelines²⁹. The content of active substance in the investigated samples was monitored by the RP-HPLC method. This method is prescribed in the monograph Ph. Eur Vi³⁰.

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MATERIALS AND METHODS

Samples. Standard of carvedilol was purchased from NOSCH-a Labs Private Limited, India. Standards of impurity A, B and C were purchased from Merck, Darmstadt, Germany. Karvileks tablets (Zdravlje Actavis, Serbia) contained 12.5 mg of carvedilol, as an active substance.

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

Packaging and dose radiation. Commercially available tablets of Karvileks were packed in red blister. For this investigation tablets of Karvileks were packaged in the opaque plastic containers and blister packs (red and white color), composed of polyvinyl chloride and aluminum foil (PVC/Al). The values of strength and duration of the applied UV and VIS radiation are shown in Table 1.

Table 1: The values of applied strength and duration of UV and VIS radiation

Type of dose	Dosage strength	Duration of radiation (day)
UV		
I	200 Wh/m ²	3.5
II	400 Wh/m ²	7
III	1000 Wh/m ²	17
VIS		
I	1.2 milion lux h	5.4
II	2.4 milion lux h	10.7
III	6 milion lux h	27

Apparatus. The method was performed with an 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 with the Agilent HPLC Data Analysis software. The reproducibility were performed with 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 by Agilent HPLC Data Analysis software program. The separation of active substance carvedilol from its impurity was carried out at 20 °C using an ODS 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/m. A mobile phase comprised of phosphate buffer in water: acetonitrile (65:35 v/v). (Phosphate buffer: dissolve 1.77 g

potassium dihydrogen phosphate in 650 ml water). All solvents were filtered through a 0.45 µm millipore filter. Volumes of 20 µL of the solutions and samples prepared were injected into the column. In accordance with the Eur Ph VI, the expected retention time of carvedilol was 4.5 m, and retention times of impurities were: 0.6 m of the impurity A, 3.5 m of the impurity C and 6.7 m of the impurity B.

Sample preparation:

Preparation of standard solutions. Carvedilol amount of 12.5 mg and 10 mg of each impurities A, B and C were dissolved in 10 ml volumetric flask in mobile phase by mixing in the ultrasonic bath for 15 m. 1 ml of that solution was transferred into another clean dry flask of 10 ml. 1 ml of that solution contained 0.125 mg of carvedilol and 0.1 mg impurities of A, B and C.

Sample preparation of product (Karvileks). After radiation, tablets were fine broken and all amount of the obtained powder was transferred into flask of 100 ml. The obtained solution was inserted in the ultrasonic bath and sonificated for 15 m. After that, the obtained solution was filtered through a qualitative filter paper and then through a membrane filter of 0.45 µm. 1 ml of that solution was transferred into flask of 10 ml and supplemented to the mark by mobile phase. A non-irradiated sample of tablets, as a blank for monitoring the influence of primary packaging on photostability of Karvileks tablets.

Formulas for calculating the content of impurities:

% impurity A = $\frac{2 \times A_{\text{analyzed solution (which released to impurity A)}}}{A_{\text{st}}} \times 0.5 / 2 \times A_{\text{st}}$ (1)

% impurity C = $\frac{A_{\text{analyzed solution (which released to impurity A)}}}{A_{\text{st}}} \times 0.05\% \times A_{\text{st}}$ (2)

% other impurities = $\frac{A_{\text{isp}}}{A_{\text{st}}} \times 0.5 / A_{\text{st}}$ (3)

Where

A_{st} - peak Area of standard solution of impurity A

A_{stc} - peak Area of standard solution of impurity C

Method validation:

The method were validated according to International Conference on Harmonization Q2(R1) guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for method³¹.

RESULTS AND DISCUSSION

Validation of HPLC method:

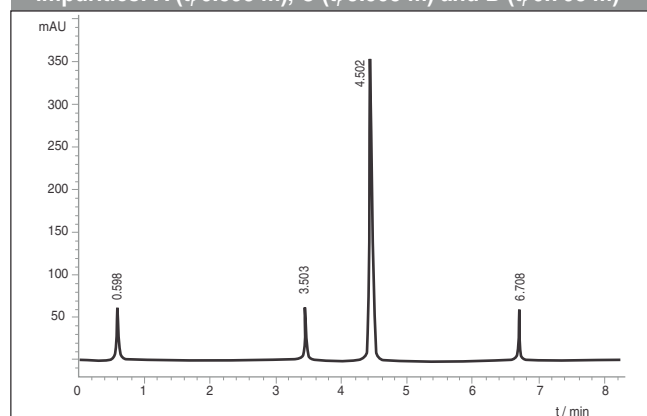
The HPLC method for determination a content of carvedilol, as well as its purity level was prescribed in the monograph Ph. Eur VI. In this study the RP-HPLC method was applied to monitoring photostability of Karvileks (Zdravlje Actavis,

Serbia). In accordance with ICH guidelines, the method is validated before its application to monitoring the stability of preparation.

Specificity of the method was carried out in the presence of excipients and potential impurities, which was prescribed in pharmacopoeia A, B and C. The obtained chromatograms (Fig. 1) indicate that the method provides adequate separation of carvedilol and impurities with resolution factor greater than 1.7.

From the obtained chromatogram (Fig. 1a) at retention time of 4.502 m was identified a peak of carvedilol, but the peaks of impurities A, C and B at retention times of 0.598; 3.503 and 6.708 m, respectively. The retention times of carvedilol and its potential impurities were approximately the same values with those, which are prescribed in Ph. Eur VI. At retention times, which correspond to these standards, were not identified peaks of excipients. On the basis of this, it can be

Fig.1: Chromatogram of standard carvedilol (*t*, 4.502 m) and impurities: A (*t*, 0.598 m), C (*t*, 3.503 m) and B (*t*, 6.708 m)



concluded that the method for the determination of carvedilol and its impurities in the presence of excipients was selective.

The linearity of method was estimated by the method of least squares. Linear relationship between detector response (peak area) and concentration standards of carvedilol was confirmed in the range from 16 to 24 µg/ml. The obtained parameters of regression equation was presented in Table 2.

Method accuracy was estimated by calculating *recovery* values at confidence interval of 5% for carvedilol in the presence of impurities A, B and C. The obtained *recovery* values in all tested cases were about the theoretical value (100%). It was indicated on satisfactory accuracy of the method (Table 2).

Method precision was tested through three levels: repeatability, intermediate precision and reproducibility. The repeatability was estimated using solution of carvedilol, was concentration was 0.1 mg/ml. Number of repeated

measurements was 6. For intermediate precision being used the same solution of carvedilol, and the content of solution was determined over three days. Reproducibility had monitoring on different instruments. Whereas, the obtained values (Table 2) were lower than the theoretical (<2%), the method was appropriate precision.

The calculated values of limit detection (LOD) and limit quantification (LOQ) indicated on the microgram sensitivity of the method (Table 2).

Table 2: The results of method validation		
Validation parameters		Carvedilol
Linearity	Slope (S.E.)	70531 (123.1)
	Y-intercept (S.E.)	13.708 (54.21×10^{-3})
	r	0.9992
	Student's t-test	0.886
Accuracy	Recovery (%)	99.02 ± 0.35
Precision RSD (%)	Intra-day variability	0.681
	day 1	0.682
	day 2	0.893
	day 3	0.991
Sensitivity	Reproducibility	1.421
	LOD (µg/ml)	1.250
	LOQ (µg/ml)	4.125

Photostability study of Karvileks:

The sunlight effect on the drug has a negative influence on its quality, safety and efficiency of pharmacotherapy. From these reasons all the studies, which were carried out in the direction of studying of Karvileks photodegradation products (Zdravljje Actavis, Serbia), are justified.

RP-HPLC chromatograms of Karvileks samples in a red blister packing, obtained after the application of different doses of UV and VIS radiations, are shown in Fig. 2. The peaks from the chromatograms of Karvileks sample were identified at retention times approximately at the same retention times, which correspond to standards of carvedilol and impurities A, B and C. The presence of impurities A and C was confirmed in all irradiated samples of preparation. Presence of impurity B was not identified different doses of UV radiation. It was confirmed in samples irradiated by VIS rays, by the second and third dose of radiation. Unlike to UV radiation, the presence of B impurity was noted, in addition to impurities A and C, for the second and third doses of VIS radiation.

The photostability was monitored for Karvileks, packaged in the white blister. The samples were treated by influence of the different doses of UV and VIS radiation, like in the previous case. The obtained chromatograms were shown in Fig. 3.

The appearance of peaks at retention times, with values approximately 0.6; 3.5; 4.5 ; 6.7 m indicate the presence of impurity A, impurity C, carvedilol and impurity B, respectively. The presence of impurity B was identified in the case of the second and third doses of VIS radiation.

The presence of the same degradation products was confirmed for the irradiated samples of Karvileks packed in the plastic opaque containers (Fig. 4).

After irradiation of formulation, packaged in the different primary containers, the contents of carvedilol (%) were shown as a histogram in Fig. 5.

The % content of pharmacopoeial impurities A, B and C during the photodegradation is shown in Table 3. The decreasing of carvedilol content and increasing of impurities

Fig. 2: Chromatograms of the irradiated product, packed in a red blister, using the different doses of UV and VIS radiation

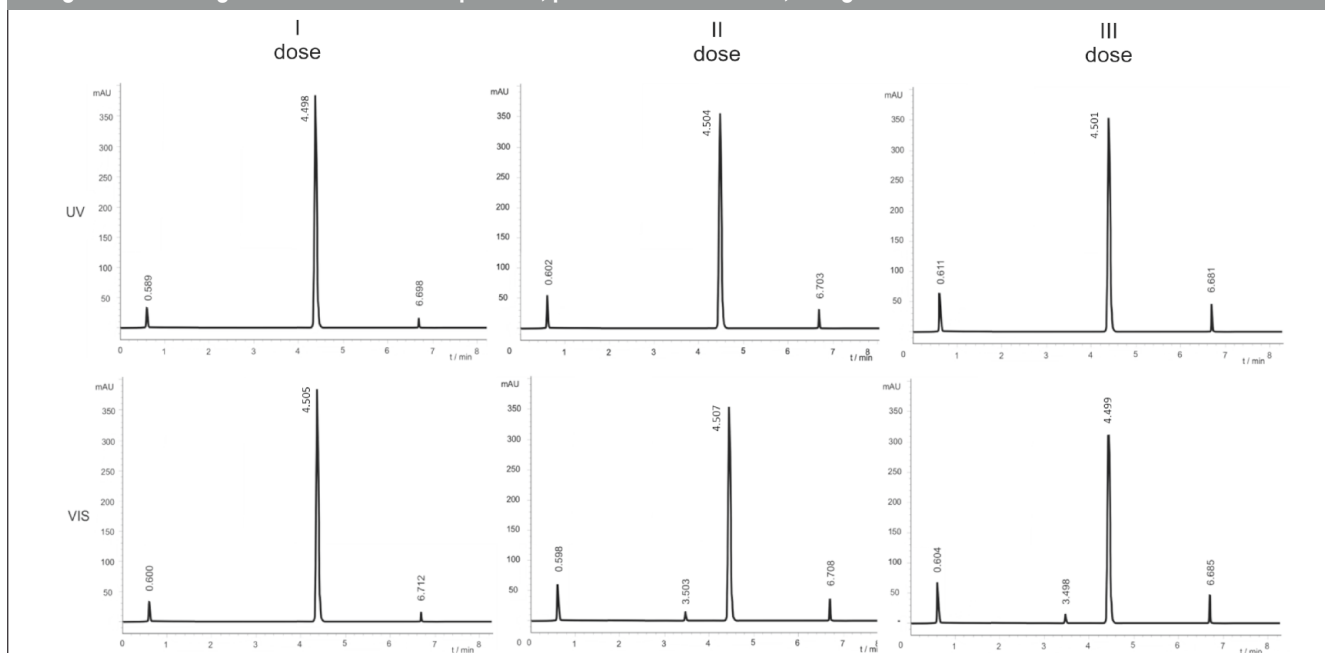
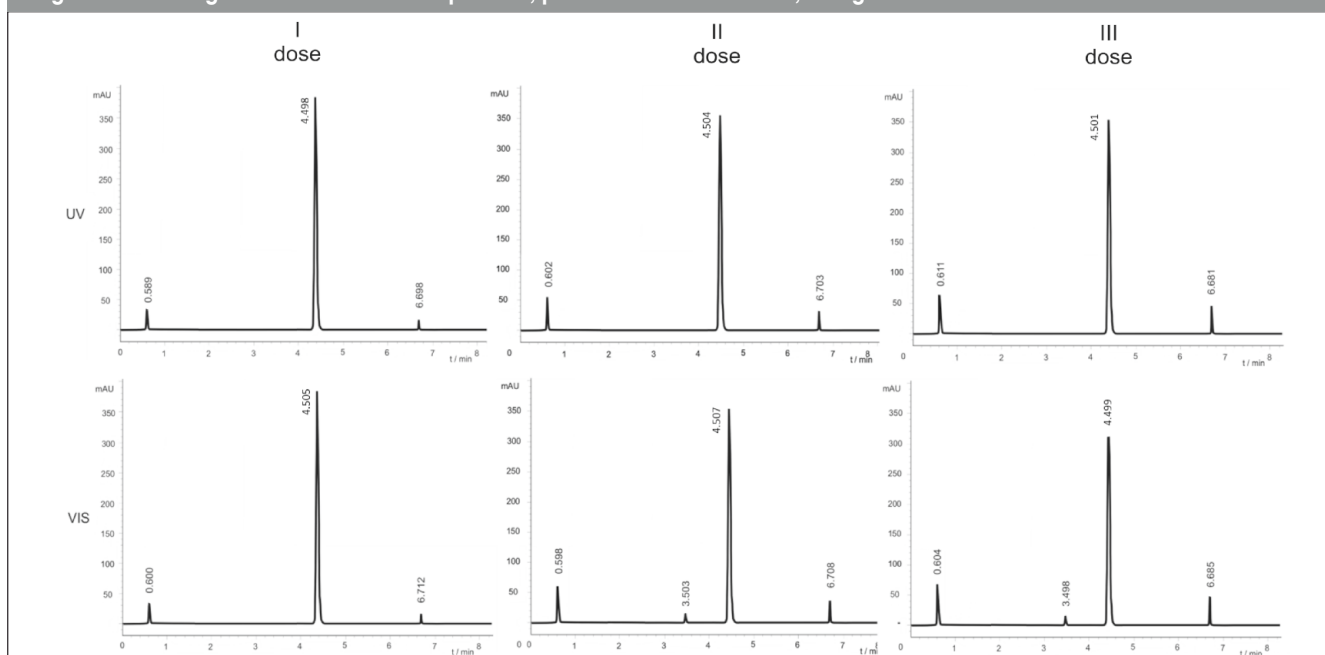


Fig. 3: Chromatograms of the irradiated product, packed in a white blister, using the different doses of UV and VIS radiation



content is higher using UV radiation compared with the effect of VIS radiation. This is expected, because the UV radiation has higher energy compared with the VIS radiation.

From histogram it can be noted that the largest decrease content of carvedilol is in the case, where the preparation is packaged in a white blister. The decrease for the UV radiation was 15.2% and 12.9% for the VIS. The decrease content of carvedilol was slightly less using a red blister compared with a white blister. By using UV rays, the decreasing of carvedilol content was 12.5% compared with the contents of the same substance in unirradiated preparation. That decrease was smaller by using VIS radiation (about 9.7%). Unlike to the blister packs, the least decrease content of carvedilol was confirmed in the plastic containers. This decrease was 9.8% for UV and 4.2% for VIS radiation.

The obtained results of the impurities contents (Table 3) indicate that the most increase has been in white blister, but smaller in the red blister. The least increase of impurities content was been in the opaque plastic container. In all analyzed samples, the content of impurities was less than 1%.

On the basis of these results, it can be concluded that the most appropriate primary packs for packaging of Karvileks is the opaque plastic container. Karvileks is adequate protected from the effects of sunlight and relatively photostable in this packs.

CONCLUSION

The RP-HPLC method was validated for monitoring the influence of primary packaging on photostability of product Karvileks (Zdravlje-Actavis, Serbia). The proposed RP-

Fig. 4: Chromatograms of the irradiated product, packed in an opaque plastic container, using the different doses of UV and VIS radiation

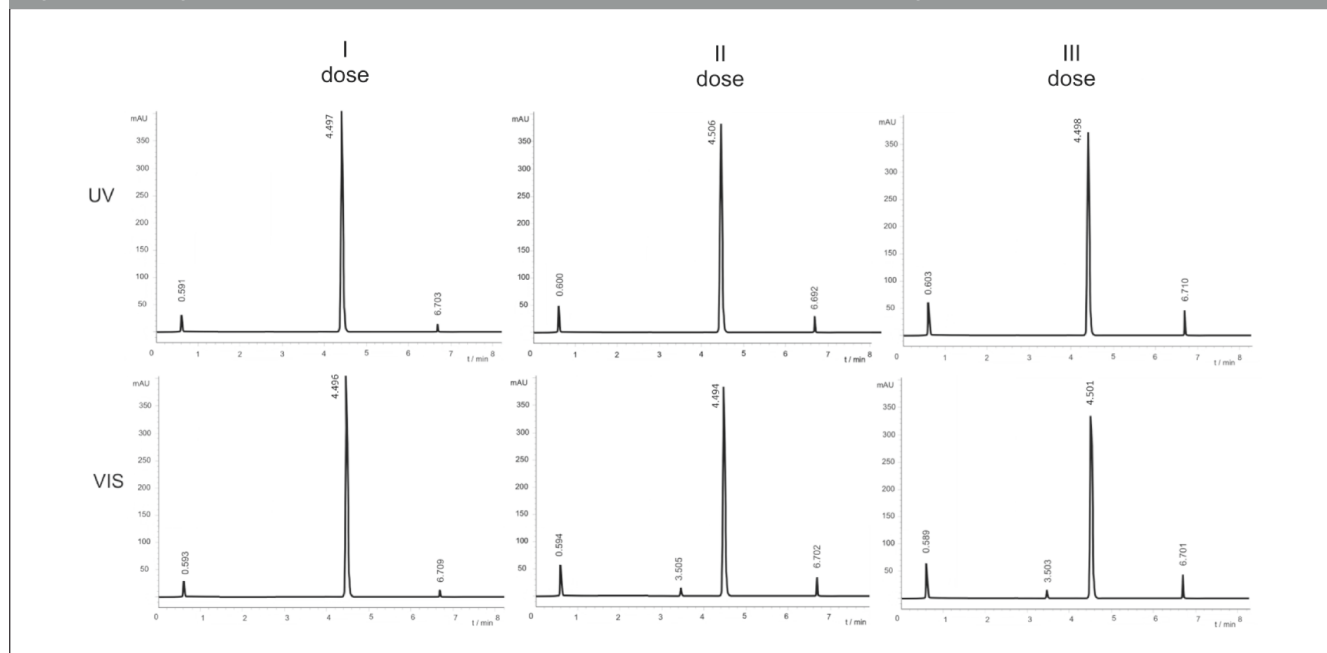
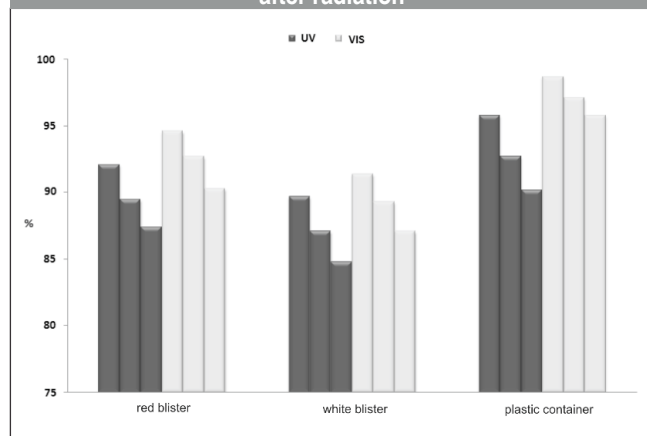


Fig. 5: Carvedilol content (%) in the analyzed packs after radiation



HPLC method was selective, accurate, sensitive and reproducible. After treatment of Karvileks by UV and VIS rays, the presence of pharmacopoeia impurities A, B and C. In all tested cases, the presence of impurities B was identified only in the samples, irradiated by the second and third doses of VIS rays. The decrease content of carvedilol and increase content of impurities for UV radiation was higher than VIS radiation. The least decrease content of carvedilol and increase impurities was noted for an opaque plastic container. The facts show that the most appropriate primary packs for Karvileks is the opaque plastic container. Therefore, the preparation is protected from sunlight using this type of primary packs.

Table 3: Impurities content (%) after radiation

Type radiation	Dose of radiation	PVC/Al red blister			PVC/Al white blister			Opaque plastic container		
		A	B	C	A	B	C	A	B	C
UV	I	0.090	0	0.040	0.102	0	0.045	0.080	0	0.030
	II	0.167	0	0.073	0.198	0	0.079	0.166	0	0.055
	III	0.401	0	0.173	0.501	0	0.184	0.371	0	0.132
VIS	I	0.080	0	0.040	0.087	0	0.048	0.070	0	0.040
	II	0.294	0.050	0.049	0.321	0.058	0.052	0.273	0.041	0.047
	III	0.362	0.059	0.183	0.411	0.061	0.194	0.309	0.049	0.176

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