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Chemometric Assisted UV-Vis Spectroscopic Study of Photostability of Some Beta-Blockers With Multivariate Curve Resolution-alternating Least Square (MCR-ALS) Method Using Soft and Hard Modelling Approach.

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ABSTRACT

The present work focuses on the use of simple UV-Vis spectrophotometric methods along with chemometric tools to understand the photodegradation and degradation kinetics of five Beta blockers namely atenolol, bisoprolol, metoprolol, propranolol, and sotalol. All the molecules were exposed to sunlight for 24 hours and UV spectra were recorded between 200 -400 nm for a specific interval of time. Amber colour and transparent glass material were used to study the penetration effect of sunlight. The recorded UV absorbance spectra were subjected to Multivariate curve resolution alternating least square method using soft and hard modelling constraints to get an understanding of the number of pure components and their concentration in the reaction mixture. Atenolol, Bisoprolol, Metoprolol, and Sotalol were found to be stable even after exposure to direct sunlight, but Propranolol shows photodegradation through a two-step mechanism Photodegradation kinetics was studied for Propranolol using kinetic constrain under study conditions. ALS optimization shows percentage lack of fit value was found to be 0.0073 with 99.98% variance explained. The standard deviation of residual against experimental data was found to be 0.0129 which suggests the statistical significance of the developed model. The rate constants of the first (k1) and second (k2) steps were calculated as 0.00315 h^{-1} and 0.0149 h−1 respectively.

1. Introduction

Photostability of drug molecules is a very important aspect when it comes to the formulation of the drug molecule. Photostability of the drug molecule is related mainly to its chemical structure and the presence of some characteristic functional groups. Photochemical reactions can lead to the decomposition of the drug molecule which may result in the formation of some degradation products that may be harmful to the human being. The study of photodegradation reaction is very essential at the time of formulation development and selection of the packaging material for the formulation. These photochemical reactions may change the physical or chemical nature of the molecule and the formulation. Drug molecules can undergo decomposition by absorbing photons that are emitted by sunlight may be in the form of UV -VIS light or Infrared radiations.

Most of the time chromatographic methods like high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), and high performance thin

layer chromatography (HPTLC) is used for identification of the drug degradation. Multivariate curve resolution alternating least square MCR-ALS is a chemometric approach that can be used to demonstrate the stability of the drug molecule using spectrophotometric data. It helps to understand multiple sources of variability in spectroscopic measurements and can be used to investigate complex chemical reactions by assessing the number of constituents involved in the reaction process, along with their concentration profile. Kinetic constrain when added as a hard modelling tool predict the kinetics of the photodegradation reaction with determination of rate constants (k).[1-4]

The beta blockers are primarily used in the therapy of cardiovascular diseases. Beta blockers are the primary choice of drugs for cardiovascular diseases.[5] The first beta-blocking drug used in cardiovascular therapy was Propranolol,[9]. It functions as a nonselective beta blocker with membrane stabilizing properties. Bisoprolol, Atenolol, Metoprolol and Sotalol is [6-9] are the four members of that fall under the category of beta1-adrenergic receptor blockers. They have a similar chemical structure and are capable of selectively blocking beta2-adrenergic receptors found in the lungs and vascular smooth muscle. All currently available beta blockers share an amino-alkanol side chain and an aromatic group, which is phenyl in the case of all drugs except Propranolol which possess naphthyl ring as a structural feature.[10]

Vamsi et al. in their review on photocatalysis of beta blockers enlisted various studies carried out on different beta blockers alone or in comparison with use of different catalytic reagents[10]. The current study is mainly focused on the use of simple UV Spectroscopy along with MCR-ALS as a chemometric approach to study the photostability of five beta blockers. To the best of our knowledge chemometrics assisted photo stability study of different beta blockers was not reported in the literature.

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is a chemometric method which aims towards resolution of number of components in an unknown sample. The MCR algorithm performs an approximation of variation in the X axis related data with bilinear two-factor matrices having reduced size along with pure response profile. ALS performs optimization of spectral and concentration profiles of species present in reaction mixture simultaneously with a iterative fashion. This method can be applied to environmental samples, chemical mixtures, chromatographic and spectroscopic data.[11].

According to published research, combining soft and Hard modelling MCR-ALS approaches can reveal information about the reaction kinetics and also reduces the rotational ambiguity effect of soft modelling.

The first step of MCR-ALS process is to build up the data matrix, D. In the rows of this data matrix represents different samples or sampling points whereas columns represents absorbance spectra , bilinear relation between the experimental data, the concentrations and the pure spectra of the components is assumed, In matrix form, this bilinear model is expressed by equation (1).

$$D = CST + E \quad (1)$$

where D(I,J) is the matrix of experimental data, of

dimensions I samples (spectra) by J wavelengths; C(I,K) is the matrix of concentration profiles of the different K analytes presents in the samples; ST(K,J) is the spectra matrix, whose K rows contain the pure spectra associate with the K species present in the samples; E(I,J) is the matrix associated to the experimental error This method decomposes the data matrix to product of two matrices: C and ST where C and ST and are concentration and spectral profile matrices, respectively having actual chemical significance.[12-18]

2. Material and Method

Material

Atenolol, Bisoprolol, Metoprolol, Sotalol and Propranolol were procured as gift samples from reputed pharmaceutical manufacturing companies from Maharashtra state. Glass distilled water was used for preparation of all solutions.

A double beam UV-Vis Spectrophotometer (Jasco; model V-650) having a 10-millimeter quartz cell was used to record the UV-Vis spectra. Data was recorded for an interval of 1 nm wavelength. MCR-ALS was performed by utilizing the MATLAB environment (MathWorks, Natick, MA; version 10 a). using the MCR-ALS function of MATLAB which was developed and designed by Tauler along with de Juan. The toolbox can be found by using the link http://www.ub.es/gesq/mcr/mcr.htm on the MCR website.

Method

Standard stock solutions of all drug molecules were divided into four different volumetric flasks with two transparent and two amber colour volumetric flask. One pair of flasks (one amber and one transparent were placed in direct sunlight and one pair inside the room at 25^0C to study effect of sunlight on UV spectra of each drug molecule namely propranolol, atenolol, metoprolol, bisoprolol and sotalol. Samples were pipetted out from each flask after an interval of 30 mins and the solution was subjected to recording of UV spectra between 200-400nm range as all the drug molecules show good absorption between this ranges which makes it easy to record the spectra of all the drug molecules. Readings were recorded for 24 hours over a period of 4 days. For development of model data in the range of 220 nm to 300 nm was selected.[19]

The data obtained from the UV spectra was used to study the susceptibility of an analyte towards photodegradation. MCR-ALS toolbox was used which employs an algorithm to solve the basic bilinear MCR model. Initially rough estimate of number of components can be obtained with different methods like principal component analysis (PCA)[20-23]. Assessment of singular data can provide a preliminary information about the number of chemical components present in the sample. First, singular value decomposition (SVD) was applied to the data to estimate the number of components. SVD is based on a algorithm where in matrix D is broken down in to the product of three; an orthogonal matrix U, a diagonal matrix S, and the transpose of an orthogonal matrix V[24] as following:

A = USVT

This step is followed by Evolving factor analysis (EFA) which was performed in forward and backward direction. Final EFA results gave the confirmation about the number of components contributing to the sample.

EFA is a local rank analysis method that detects the emergence and decay of the components in the data set and provides concentration profiles assuming a sequential order of emergence-decay for all components in the system. EFA provides a set of initial estimates and relevant additional information, such as the windows of existence of the components in the system. Forward EFA starts with the calculation of the eigenvalues (EVs) of the first row and is continued far all the rows in the data matrix along with generation of loading and score matrix. Thus PCA is performed on complete data set. The point where EV raise above the noise level indicate appearance of new substance which can increase rank by one. Similarly the number of EVs which are above the noise level shows the number of compounds evolved.

In analogy to forward EFA, backward EFA starts with measurements from the last row and EVs for

whole data matrix is calculated. In this case An increase of an EV above the noise level indicates disappearance of the compound from the system. Thus, an EFA graph provides information on both the appearance and the disappearance of the analytes.[25]

Soft modelling gives the values of concentration profile of all species present in the sample and it was used to calculate the rate of reaction based on initial and final concentration of each species. Hard modelling constrain of kinetics was applied to study the exact value of rate constant. The combined soft and Hard modelling gives the estimates of number of species along with their spectral profile and rate constants for each reaction step.

Hard-modelling approaches of fitting multivariate absorption data are based on mathematical relationships, which describe the measurements quantitatively. In chemical kinetics, the analysis is based on the kinetic model or reaction mechanism, which quantitatively describes the reactions and all concentrations in the solution under investigation. In combined soft and hard modelling approach kinetic profiles in concentration submatrix is used as input for a non-linear multivariate kinetic fit. Fitting is directly done on the concentration profiles and there are no spectral contributions to be considered. The resulting fitted kinetic profiles update the soft-modelled ones, and the rate constants values can be obtained for each step involved in the reaction.[16]

3. Results and Discussion

Figures 1-5 show changes in the spectra of the beta blockers studied according to the different exposure conditions figure at left represent sample treated inside room (room temperature) whereas figure to the right represents sample exposed to direct sunlight outside the room. If we look at the absorbance spectra, most of the beta blocker molecules studied, did not show any significant change in the shape of spectra, (Fig 1 to 4). Only a small decrease in absorbance was observed suggesting slight degradation of the drug molecule without formation of any new chemical species or degradation product except in the case of propranolol.

However, UV absorbance spectra of propranolol solution which was exposed to direct sunlight in the transparent flask, showed clear changes in the shape of the spectra absorbance of solution. At 248 nm absorbance goes on decreasing, while at the same time absorbance increases at 280 nm. An isosbestic point was observed at 276 nm (Figure 5,6). This observation indicates that there is formation of new product or degradation product. In order to understand the number of species formed during degradation of Propranolol solution, a chemometric approach through multivariate curve resolution- Alternating Least square was used.

First step of SVD shows presence of three chemical entities in the reaction mixture which can be confirmed based on eigenvalues. This result was confirmed by evolving factor analysis (EFA)s with three principal components to be considered for the pure variable detection. Initial estimates step also shows three separate spectra's with different concentration profiles. This data was further pushed for the forward and backward evolving factor analysis (EFA) (Figure 7)

The graph in the forward and backward EFA demonstrated that three lines intersect with each other. The result was confirmed by the EFA plot, as a large gap is shown in the forward and backward EFA plot between the first three eigenvalues and the remaining, (Figure 7). This is one more proof that three chemical species may be present during the photodegradation reaction of the drug under investigation. This experimental study suggests that the photodegradation of propranolol may be preceded in two steps. Figure 8 shows EFA based concentration profiles of chemical species present in the sample. The plot of concentration profile suggests two step mechanism of photocatalysis of propranolol like A→B→C in transparent flask up on exposure to direct sunlight, where A is pure propranolol and B and C are the intermediate and final products, respectively.

In the final step of optimization spectral and concentration profiles are optimized simultaneously in iterative way. Figure 9 represent the concentration profile of the species present in the sample during course of reaction against time in minutes. The re-

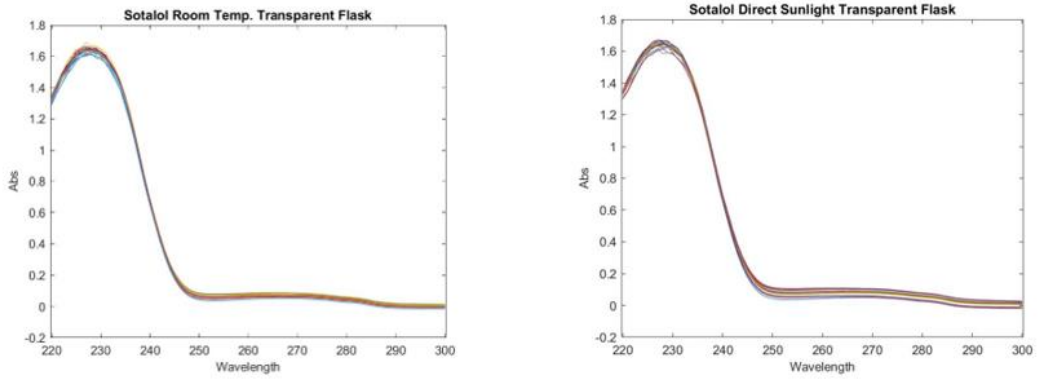

Figure 1: *Absorbance Spectras of Sotalol within room and direct exposure to sunlight outside*

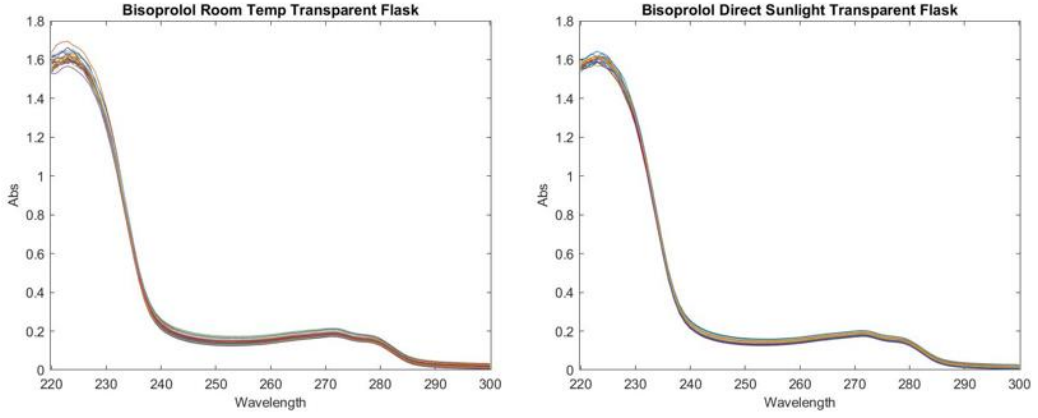

Figure 2: *Absorbance Spectra of Bisoprolol within room and direct exposure to sunlight outside*

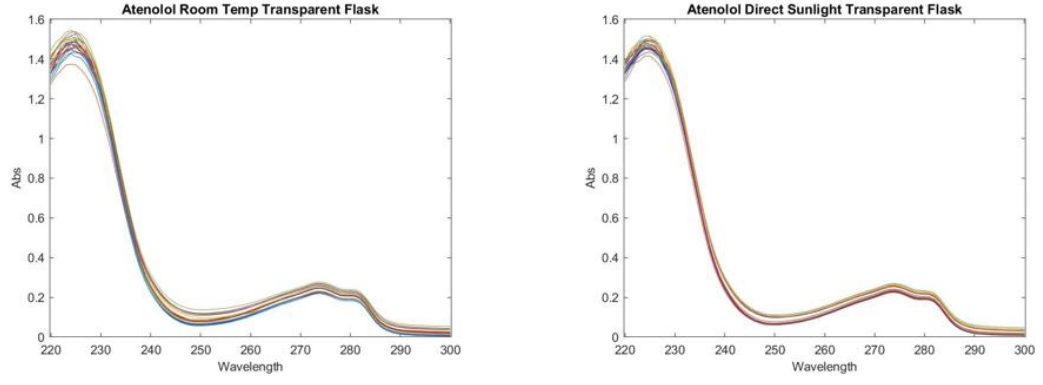

Figure 3: *Absorbance Spectra of Atenolol within room and direct exposure to sunlight outside*

sults of optimization are statistically very significant and are shown in Table 1.

Results of the MCR-ALS shows that the photodegradation of propranolol follows an consecutive two step reaction with the value of (k1) 0.00315 h^{-1} and (k2) 0.0149 h–1 as the rate constants obtained after use of combined soft and hard modelling approach for first and second step of reaction respectively.

It was observed that only propranolol shows major photodegradation along with formation of new species or degradation product in the reaction. In view of this we have studied the chemical structures of all five beta blockers (Figure 10)

As observed in figure 10, the chemical structures of atenolol, bisoprolol, metoprolol, and sotalol contain benzene as their backbone structure and only propranolol consists of naphthalene as an aromatic ring in its structural formula. It can be said that propranolol reacts more rapidly than beta blockers like atenolol, bisoprolol, metoprolol and sotalol. Salvatore et al.[26] have proposed that naphthalene skeleton in propranolol structure is responsible for initiation of photolysis reaction. Uwai et al [27] showed that when the propranolol was used in a solid state, the hydrolysis of the acetal did not appear to proceed. Thus, the C –O bond of the naphthyl ether was cleaved by

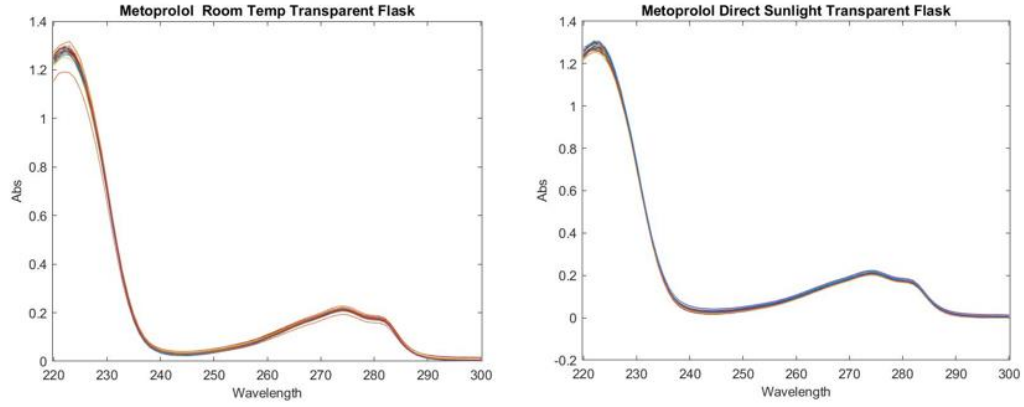

Figure 4: *Absorbance Spectra of within room and direct exposure to sunlight outside*

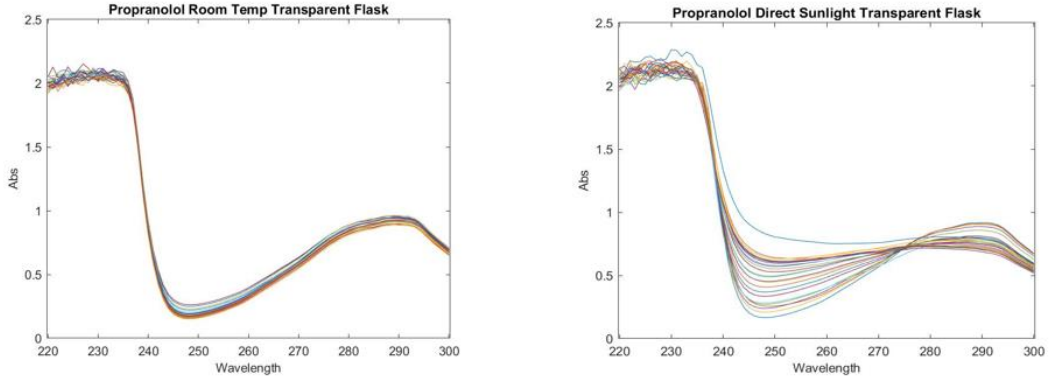

Figure 5: *UV -Absorbance Spectra of Propranolol within room and direct exposure to sunlight outside*

Sr. No.	Factor	Results
1	Standard Deviation of Residuals Vs Expt. Data	0.0129
2	Lack of Fit (LOF in %)	0.0073
3	Percent of Variance Explained	99.98

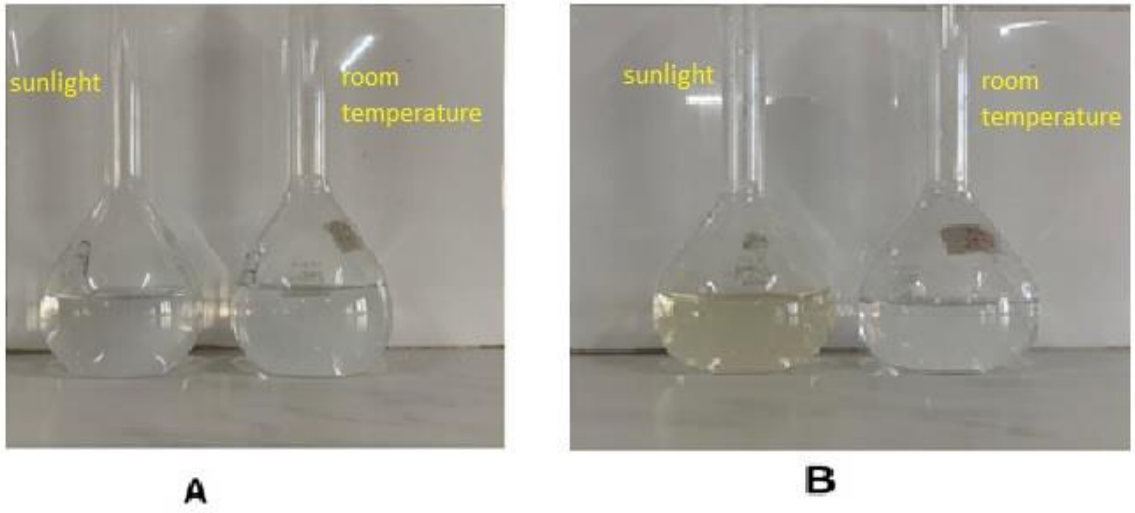

Figure 6: *Physical changes in the Propranolol solution A. Initial Sample solutions B. Sample solution on exposure to direct sunlight.*

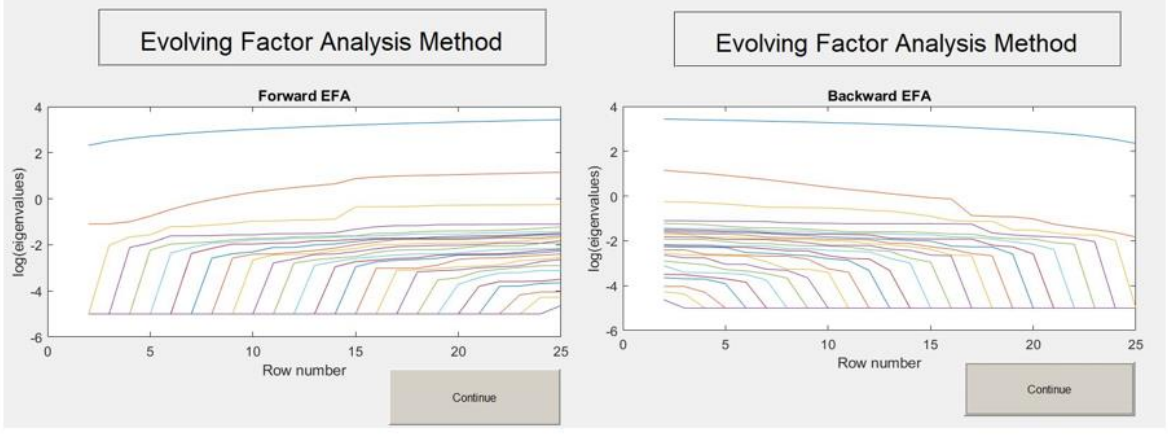

Figure 7: *Forward Evolving Factor Analysis and Backward Evolving Factor Analysis*

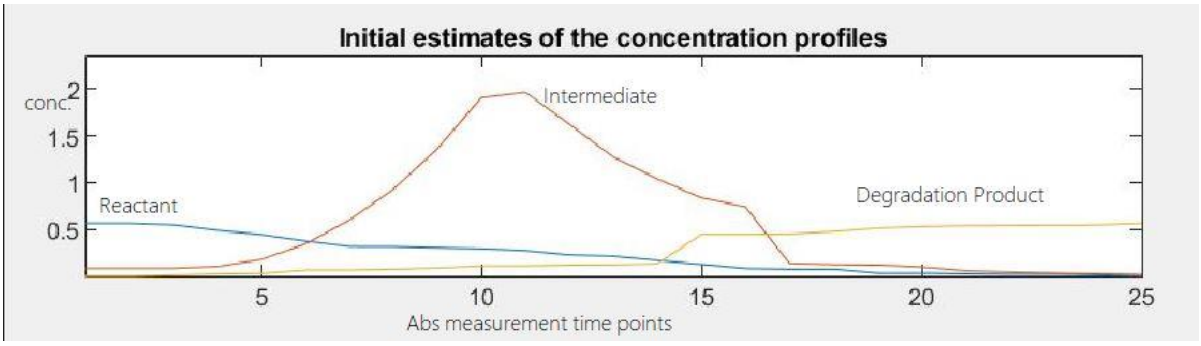

Figure 8: *Concentration profile obtained by EFA*

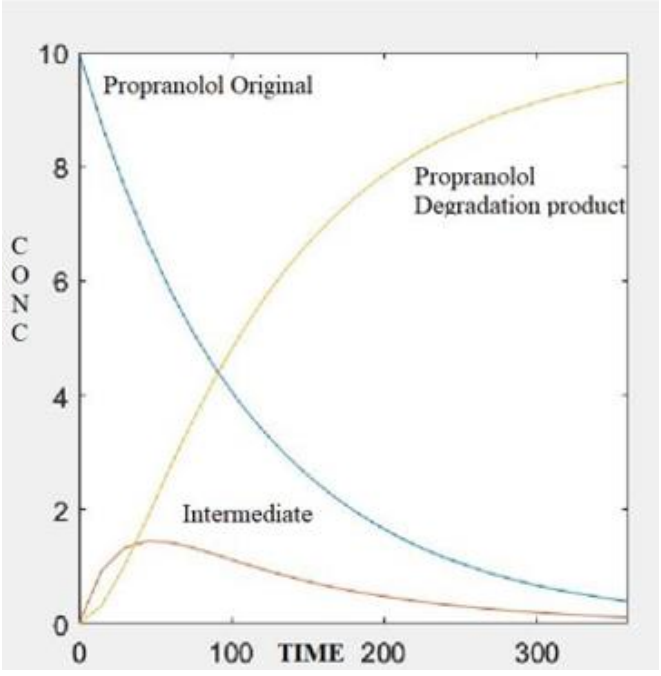

Figure 9: *Results of ALS optimization*

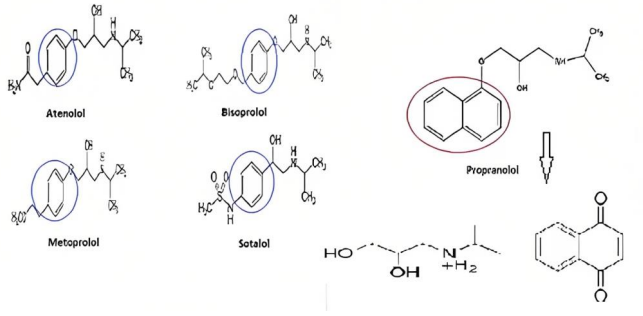

Figure 10: *Chemical Structures of studied beta-blockers and possible breakdown of Propranolol*

a thermally activated crossing of the bonding generating 1-naphthol and the remaining side chain as shown in figure 10. [27,28]

4. Conclusion

Five members of beta blockers were evaluated for their susceptibility to the solar photodegradation in transparent and amber colour containers along with variations in temperature. Clear changes in the absorption spectral profile of propranolol was observed, other beta blocker molecules showed very negligible decrease in the concentration on direct exposure to sunlight in both type of containers. Combined Soft and Hard modelling MCR-ALS resolve pure spectra and kinetics of photocatalysis of propranolol with the rate constants was obtained, it was shown that propranolol undergoes photodegradation via a two-step mechanism with formation of an intermediate species. Concentration profile obtained through MCR-ALS analysis shows that initial concentration of propranolol decreases along with formation of reaction intermediate, and a new final product with increasing concentration can be seen.

This behaviour of the propranolol was due to presence of a naphthalene moiety in the chemical structure as compared to other members which contain benzene moiety in structure.

We can conclude that simple UV spectrophotometric method with the aid of chemometrics can be used as an alternate qualitative tool for HPLC to study stability of drug molecules under various stress conditions. □

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