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UV-Spectrophotometric Method for the Perindopril Erbumine in Pharmaceutical Formulations Using *Indigo Carmine*

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Abstract: A sensitive, selective and well validated Spectrophotometric method using is based on the formation of extractable colored complex of drug with colouring agent *Indigo Carmine*. Two simple, precise and economical UV methods have been developed for the estimation of Perindopril erbumine in bulk and pharmaceutical formulations. Perindopril erbumine has the Zero order spectroscopic method at 387.2 nm (Method A), Method B applied was Area under Curve (AUC) for analysis of Perindopril erbumine in the wavelength range of 200-400nm. A wavelength maximum was found to be 387.2 nm. The concentration range of 5-40 μg ml^{-1} with linear regression of 0.9996, while the percentage recovery, The LOD and LOQ were 0.32 μg ml^{-1} and 0.49 μg ml^{-1}, respectively. The detection and quantitation limits determined were 0.13 and 0.27 mg ml^{-1} respectively. The result of analysis have been validated statistically and also by recovery studies. From the percentage recovery and specificity studies it was concluded that there was no interference of common additives during the estimation. This proves the suitability of this method for the routine quality control analysis of the Perindopril erbumine in formulation.

Key words: Perindopril erbumine · Indigo Carmine · Zero order spectroscopic method · Area under Curve

INTRODUCTION

Perindopril erbumine 1,(2S,3(infinity)S,7(infinity)S)-1-[(S)-N-[(S)-1-Carboxy- butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACE) inhibitors. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma rennin activity and decreased aldosterone secretion. Literature survey revealed that few analytical methods have been reported for the estimation of PDE; they include immunoassay [2], Spectrophotometric [3, 4], HPLC [5], LC–MS/MSP [6, 7] capillary gas chromatographic method [8]. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [9-23] and Indigo Carmine, Methyl orange [24].Hence an attempt has been made to develop new UV method for its estimation in bulk and pharmaceutical formulations with good accuracy, simplicity, precision and economy.The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective UV method for the estimation of Perindopril erbumine in bulk and its formulations.

MATERIAL AND METHODS

Experimental: UV Visible spectrophotometer (Shimadzu Model, 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Single component tablet formulations of PE (2 mg) were purchased from local market. All chemicals used in this study were analytical grade and used without further purification. Chloroform (s.d. finechem, Bombay, India), Indigo Carmine (s.d. finechem, Bombay, India).

Preparation of Calibration Curve: Standard drug solution (50 μg ml^{-1}) was prepared in double distilled water and was diluted with same, so as to give several dilutions in concentration range 5-40 μg ml^{-1} of drug. To 10 ml of each dilution taken in separating funnel, 10 ml of Indigo Carmine solution was added and shaken gently.

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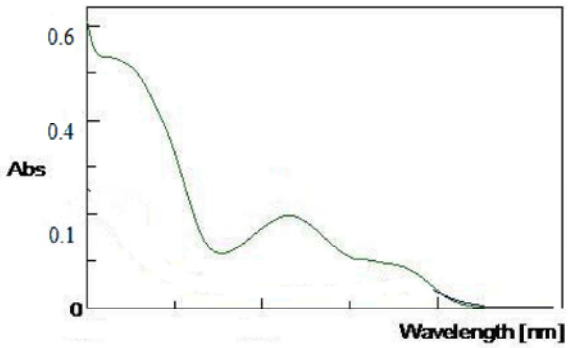

Fig. 1: Zero order spectra (overlain) of Perindopril erbumine

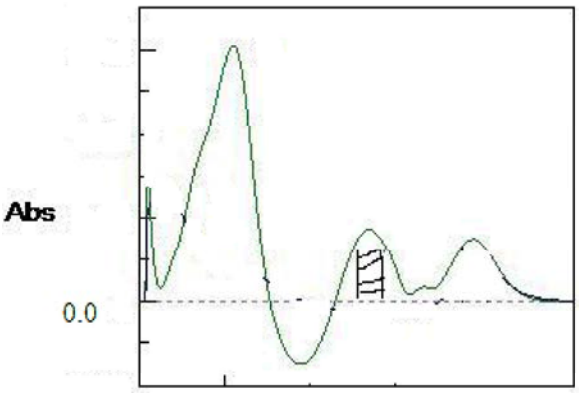

Fig. 2: Area Under Curve derivative spectra (overlain) of Perindopril erbumine

Then 5 ml of ether was added reaction mixture was shaken gently and allowed to stand so as to separate aqueous ether layer. The ether layer was separated out and transferred to 10 ml of volumetric flask. Reaction mixture was extracted further with 50 ml fresh ether. The ether layer was separated out and transferred to 100 ml of volumetric flask. Absorbance of this final extracted chloroform layer was measured at wavelength maxima 580 nm against blank. Calibration curve was plotted between concentration of drug and measured absorbance and combined it with previously extracted chloroform layer containing complex. Absorbance of this final extracted ether layer was measured at wavelength maxima 569 nm against blank. Calibration curve was plotted between concentration of drug and measured absorbance.

Preparation Standard Solutions: 1 mg ml^{-1} stock solution of Perindopril erbumine was prepared by dissolving 20 mg of Perindopril erbumine in appropriate volume of double distilled water and made up to 100 ml in volumetric flask and used as stock solution.

Preparation of Sample Solution: Twenty Perindopril erbumine tablets were powdered and an accurately weighed quantity powder equivalent to 20 mg of Perindopril erbumine from each brands were dissolved in methanol. The excipients were separated by filtration using Whatmann filter paper (No.41) and the filter paper washed three times with distilled water for effective liberation of drug from the core. Filtrate and washings of the tablet samples were transferred into 100 ml flask and diluted to the mark with absolute ethanol and the spectrophotometric procedure was followed.

Reagent Preparation: 1.5 gm of Indigo Carmine was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration (0.5%w/v).

Varying aliquots (0.5-3.0 mL) of standard 500 µg mL^{-1} Perindopril erbumine solutions were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 5.0 mL with ether. To each flask were added 1 mL of 0.5 M acetic acid and 1.0 mL of bromate-bromide mixture (30 µg mL^{-1} in $KBrO_3$) by means of micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 5 mL of 50 µg mL^{-1} indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 569.0 nm against a reagent blank after 30 min. In either method, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Method A- Zero Order Spectroscopic Method:[25-26]: The solutions were scanned in the range from 400-200 nm (method A) and the peaks were observed at 352 nm and 387.2 nm. The wavelength selected for the analysis of the drug was 387.2 nm (Figure 2). The drug followed the Beer's- Lamberts law in the range of 5-40 µg ml^{-1}. Using the calibration curve the concentration of the sample solution can be determined.

Method B- Area under Curve Method (AUC): [25-26]: The AUC (Area under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 339nm and 324 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis

of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (100 µg ml^{-1}) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm and the calibration curve was plotted. All the three method were checked by analyzing the samples with known concentration. As the result obtained were satisfactory, the method was applied for the pharmaceutical formulations.

Analysis of the Tablet Formulation: Twenty tablets of brand were weighed and triturate to fine powder. Tablet powder equivalent to 2 mg of Perindopril erbumine was weighed and then dissolved and further diluted with quantity sufficient with methanol. It was kept for ultrasonication for 30 min; this was filtered through Whatmann filter paper no. 41 to get the stock solution of 50 µg ml^{-1}. Various dilutions of the tablet solution were prepared and analyzed for six times and the concentration was calculated by using the calibration curve for three methods.

Method Development: Aliquots of stock transferred into a series of separating funnel then 1 ml of Indigo Carmine reagent and 2 ml phosphate buffer of pH 4.1 was added, then the solutions were allowed to stand for few minutes, followed by accurately measured quantity (10ml) of methanol and extracted well to give concentration 5-40 µg ml^{-1}, all the solutions were passed through dried sodium sulphate to remove water. Solutions were scanned between 400-800nm which shows ë-max at 472 nm. The above ë-max was used for its analysis of Perindopril erbumine in formulation. Formed ion pair complex was obeying Beer's law in the range of 5-40 µg ml^{-1}.

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ). Linearity various aliquots were prepared form the stock solution (0.3g ml^{-1}) ranging from 10-50g ml^{-1}. The samples were scanned in UV-Vis Spectrophotometer using 0.1N methanol as blank. The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (10mg) and the amount of pure drug was varied that is 0.5mg, 1.0mg and 1.5 mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery.

Limit of Detection (LOD): The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.15-0.50 g ml^{-1}. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Limit of Quantification (LOQ) is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve.

RESULTS AND DISCUSSION

The effect of Indigo Carmine concentration on the reaction was checked out at room temperature and away from direct sunlight. The reaction of Perindopril erbumine was dependent on the concentration of dye used. A concentration of 0.1% (w/v) was selected as the optimum reagent concentration. The absorbance of the solution was measured after 25 minutes after adding reagent and up to 2 hrs.the reaction was slow and the formed colour was stable up to 5 hrs. The developed methods for simultaneous estimation of Perindopril erbumine were validated as per ICH guidelines [27]. To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. From that total amount of drug found and percentage recovery was calculated. To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. The results are presented in Table 2. This method utilizes the active analogue principle that lies at the spectroscopic method [24]. The absorbance of final sample solution was measured against methanol as blank at 466 nm. The amount of Perindopril erbumine was computed by adding the absorbance value in simultaneous equation. The method was validated by recovery study were carried out by the addition of different amount of drugs to pre analyze solution (50 µg ml^{-1}). From the stock solution of 10 µg ml^{-1} of each

Table 1: Analytical and regression parameters of the proposed methods

Parameter	Indigo Carmine
Beer's law limits	5-40
?max, nm	387.2 nm
Molar absorptivity	4.8×10^3
Sandell sensitivity	0.6512
Limit of detection	0.418
Limit of quantification	0.257
Regression equation *	
(a) Intercept	0.0954
(b) Slope	0.0537
Correlation coefficient, (r)	0.9996

Table 2: Accuracy of the proposed method

Method	Label Claim	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added	Amount of Drug recovered	%Recovery	RSD(%n=6)
A	2	2.01	50	20	2.12	101.12	0.23
			100	40	1.98	199.98	0.98
			150	60	2.06	100.06	0.43
B	2	2.05	50	20	2.09	100.09	0.98
			100	40	2.10	100.10	1.10
			150	60	1.98	99.99	0.27

Table 3: Intraday, Interdays, data of tablet formulation

		Interday precision %COV		
Method	Intra day precision %COV (n=6)	Day 1[a]	Day 2[a]	Day 3[a]
A	1.756	1.254	0.957	0.592
B	1.975	1.564	1.0143	0.832

COV: Coefficient of variance

Table 4: Robustness and day to day variation of the method

Parameters Studied	Recovery (%±RSD
Indigo CarmineConcentration(%,w/v)	
5.00	99.98±0.16
40.00	100.18±0.76
Wave length	
400 nm	96.14 ±0.44
500 nm	99.97 ±0.14
600 nm	101.05±0.24
Ruggedness(day-to-day variation)	
Day1	101.08±0.11
Day 2	100.05 ±0.16
Day 3	99.86 ±0.32

drug 1ml solution was taken in each of four volumetric flask (10ml), then 2.0, 0.5, 0.2 ml of mixed standard stock solution (50 µg ml^{-1} of Perindopril erbumine) added in three flasks so that remaining one flask contains no added solution. These solutions were scanned at 511 nm. Percentage recovery was found in the range of 99 % to 103%. Robustness and Ruggedness of the method were also studied by altering wavelength of estimation and changing the dye's concentration which were also within the acceptable limit with respect to % RSD (Table. 3).In case of ruggedness difference in the estimation was studied by means of analyzing the samples in two different days by following same procedure and the results were summarized in Table. 3. All the methods A and B for the estimation of Perindopril erbumine in pharmaceutical dosage were found to be simple, accurate

and reproducible. Beer- lamberts' law was obeyed in the concentration range of 5-40 µg ml^{-1} in all these methods (Table 1). The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The %RSD value is less than 2 indicative of accuracy of the method. The developed method was statistically compared using one way ANOVA. The P value was found to be 0.643 and was greater than 0.05. Hence the results of the ANOVA indicate no significant difference between two methods. Hence these methods can be useful in routine analysis of Perindopril erbumine in bulk drug and pharmaceutical formulations. In present research work a UV Spectrometric method has been developed for determination of Perindopril erbumine formulations. The developed method was based on formation of absolute ethanol extractable complex of drug with Indigo Carmine in double distilled water. Wavelength maxima of Perindopril erbumine was found to be at 387.2 nm and linearity was observed in concentration range of 5-40 µg ml^{-1}. Percentage label claim estimated for tablet formulation was found to be in the range of 99.96-99.99 % and respective values of standard deviation were found in the range of 0.218-0.437 for Perindopril erbumine (Table 1). To fix the linearity a calibration curve was constructed by plotting the absorbance as a function of the corresponding concentrations. The regression equation for the results was:

$$A=3.187 * -0.643 \ (r = 0.\ 0.9998)$$

Where A is the absorbance at 577 nm, C the concentration of Perindopril erbumine in µgml^{-1} in the range of 5-40 µg ml^{-1} and r is the correlation coefficient. The molar absorptivity ($å$) was found to be

$$2.176* 0.7532 \text{ lit mol } cm^{-1}.$$

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = ? S.D.a/b, where ? = 3 for LOD and 10 for LOQ, S.D.a is the standard deviation of the intercept and b is the slope. The LOD and LOQ were 0.32 µg ml^{-1} and 0.49 µg ml^{-1} respectively. The detection and quantitation limits determined were 0.13 and 0.27 mg ml^{-1}, respectively. These low values indicated the high sensitivity of the purposed method. Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. The results of analysis and recovery studies are given in Table.1. The accuracy expresses the agreement between the accepted value and the true value. The mean percentage recovery was found to be LOD and LOQ were 99.98-102.11 %, for tablets (Table 1). This value proves the good accuracy of the purposed method. Intra-day precision was calculated from results obtained from fivefold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are listed in Table 2.

CONCLUSION

Based on the data obtained in our study, the proposed UV Spectrophotometric techniques are simple, rapid and precise. These UV Spectrophotometric techniques are quite economical as proven by the use of water only as solvent. Both derivative and non-derivative techniques do not suffer from interference by excipients in the tablet formulation as confirmed by their recovery study.

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