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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF EPALRESTAT AND PREGABALIN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and accurate spectrophotometric method has been developed for the first time to estimate epalrestat and pregabalin present in combined dosage form. Ethanol was used as the solvent for preparing the standard solution. Without prior separation of the analytes, the quantification of epalresta and pregabalin was performed by measuring the absorbance at 390 nm before derivatization and at 575 nm after the derivatization with ninhydrin reagent, respectively. The linearity was established in the range of 5-15 µg/mL for both drugs. The method was validated as per ICH guidelines and the results were obtained well within the acceptance limits. It was further applied successfully to the combined tablet dosage form without any interference from the excipients.

Keywords: Epalrestat, pregabalin, spectrophotometric, method development, validation

INTRODUCTION

Epalrestat, chemically 2- [(5Z)- 5-[(E)- 3-phenil- 2- methylprop- 2- enylidene]- 4-oxo- 2-thioxo- 3- thiazolidinyl] acetic acid is used to treat diabetic neuropathy. Being an aldose reductase inhibitor, it inhibits high glucose-mediated neutrophilendothelial adhesion molecules **[1, 2]**.

Pregabalin, chemically (3S)-3-(amino methyl)-5-methylhexanoic acid is used in the management of neuropathic pain associated with diabetic neuropathy and also

in the treatment of partial-onset seizures **[3, 4]**.

As per the available literature, spectrophotometric methods were reported for the estimation of EPL and PGB individually **[5-11]** or in combination with other drugs **[12-14]**. To the best of knowledge, no methods were reported earlier for the estimation of EPL and PGB using spectrophotometry, suitable for assay of combined formulations.

Hence, the aim of the present work was to develop and validate spectrophotometric method for the estimation of EPL and PGB, applicable for the quantification of both the drugs present in the combined dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standards of EPL and PGB were received as the gift samples from Symed Labs Ltd., Hyderabad, India and Century Pharmaceuticals Ltd., Vadodara, India, respectively. Spectrophotometric grade ethanol was obtained from Sigma-Aldrich Chemicals Pvt. Ltd. Baglore, India. Analytical grade ninhydrin was procured from Avra Synthesis Pvt. Ltd., Hyderabad, India. PreAldonil tablets of Label claim Epalrestat 150 mg and Pregabalin 150 mg, marketed by Zydus Cardiva, India were purchased from retail pharmacy store.

Instrument and conditions

A double beam UV-Visible spectrophotometer (Shimadzu-1800) having two matched quartz cells with 1 cm path length was used. Medium scan speed and band width of 1 nm was used for the study. Data acquisition was done using UV Probe software version 2.71.

The standard solution consisting of Epalrestat and Pregabalin prepared in ethanol as the solvent was measured for the absorbance at 390 nm to determine the concentration of Epalrestat. The same solution was further derivatized using ninhydrin reagent and then the absorbance was measured again at 575 nm to estimate the concentration of pregabalin.

Preparation of standard solutions

Primary standard solutions of EPL and PGB were prepared (1000 µg/mL) separately in ethanol. The pooled standard solution was prepared by transfer of 10 mL each of the above solution into a 100 mL volumetric flask and diluting to 100 mL using ethanol (100 µg/mL). The pre-derivatized working standard solutions were prepared by appropriate dilution of the pooled standard solution.

Derivatization of standard solution using ninhydrin reagent

The pooled standard solution of 50 mL was mixed with 20 mL of ninhydrin reagent (0.2% w/v in ethanol), heated for 20 min at 75 ^{0}C and was made up to 100 mL with ethanol (50 µg/mL). The solution was

diluted further to prepare the post-derivatized working standard solutions of appropriate concentrations.

Method validation

The developed method was validated as per ICH guidelines **[15]** to evaluate the linearity, range, accuracy, precision, limit of detection and limit of quantitation.

RESULTS AND DISCUSSION

Method development

The standard solutions of EPL and PGB were prepared separately and their absorption spectra were recorded. EPL showed significant absorption at 390 nm and no absorption at 575 nm, whereas PGB did not show any absorption in the range of 210-800 nm. The same solutions were then derivatized with ethanolic ninhydrin reagent and again the spectra were recorded. EPL showed the same absorption at 390 nm and no absorption at 575 nm **(Figure 1)**. After the derivatization, PGB developed blue violet colour, which showed significant absorption at 575 nm **(Figure 2) [7]**. Hence, to estimate both the drugs present in the combined formulation, the spectrophotometric method was developed by determining the absorption of pooled standard solution at 390 nm and at 575 nm and after derivatization for estimating EPL and PGB, respectively present in the combined formulation.

Linearity and Range

A series of pre- and post-derivatized working standard solutions of concentrations 5, 7.5, 10, 12.5 and 15 µg/mL were measured for absorbance at 390nm and 575 nm to evaluate the linearity of the method for EPL and PGB, respectively and the calibration curves were plotted **(Table 1)**. The correlation coefficients found were >0.99, showing the linearity of the method **(Figure 3 and Figure 4)**.

Accuracy and Precision

Accuracy was evaluated by the recovery studies. Sample solution of the formulation (5µg/mL) was spiked with pooled standard solution at 50%, 100% and 150% level and the % recoveries of EPL and PGB were calculated, respectively before and after derivatization with ninhydrin reagent. The % recovery was found in the range of 98.93 tom101.20% **(Table 2)**.

The precision was assessed by measuring the absorbance of pre- and post-derivatized standard solutions (10 µg/mL) for six times on the same day (intra-day precision) and on different days (Inter-day precision). The %RSD values were calculated, which were found to be in the range of 0.37 to 1.62% **(Table 3)**.

Limit of Detection and Limit of Quantitation

The LoD and LoQ were determined for both the analytes based on the calibration curve data using the formulae:

$$\text{LoD} = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$\text{LoQ} = \frac{10 \times \text{Standard deviation}}{\text{Slope}}$$

For EPL and PGB, the limit of detection was found to be 1.05 µg/mL and 1.38 µg/mL and the limit of quantitation was found to be 3.19 µg/mL and 4.19 µg/mL, respectively.

Assay of marketed formulation

The sample solution of marketed tablet formulation having the concentration of 10 µg/mL each of EPL and PGB was prepared using ethanol as the solvent. The absorbance was measured at 390 nm before the derivatization and 575 nm after the derivatization with ninhydrin reagent (0.2% w/v in ethanol) to determine the %purity of EPL and PGB, respectively. The results are shown in the **Table 4**.

Table 1: Linearity Data

S. No.	Concentration, (µg/mL)	Absorbance at 390 nm	Absorbance at 575 nm
1	5	0.772	0.044
2	7.5	1.238	0.065
3	10	1.521	0.094
4	12.5	1.922	0.112
5	15	2.352	0.146

Table 2: Accuracy data

Name of the drug	Concentration of sample (µg/mL)	Concentration of standard added(µg/mL)	Concentration of standard recovered (µg/mL)	% Recovery
Pregabalin	5	2.50	2.48	99.20
		5.00	4.98	99.60
		7.50	7.42	98.93
Epalrestat	5	2.50	2.53	101.20
		5.00	4.97	99.40
		7.50	7.57	100.93

Table 3: Precision data

S. No.	Intraday precision-Absorbance		Interday precision-Absorbance	
	At 390 nm	At 575 nm	At 390 nm	At 575 nm
1.	1.542	0.092	1.432	0.108
2.	1.456	0.094	1.558	0.093
3.	1.464	0.092	1.456	0.098
4.	1.469	0.094	1.442	0.103
5.	1.507	0.096	1.456	0.107
6.	1.478	0.093	1.564	0.093
Mean	8.916	0.094	8.908	0.602
SD	0.033	0.002	0.060	0.007
%RSD	0.37	1.62	0.67	1.11

Table 4: Analysis of marketed formulation data

Name of the drug	Absorbance	Conc. Found, µg/mL	% purity
Pregabalin	0.091	9.92	99.20
Epalrestat	1.567	10.12	101.20

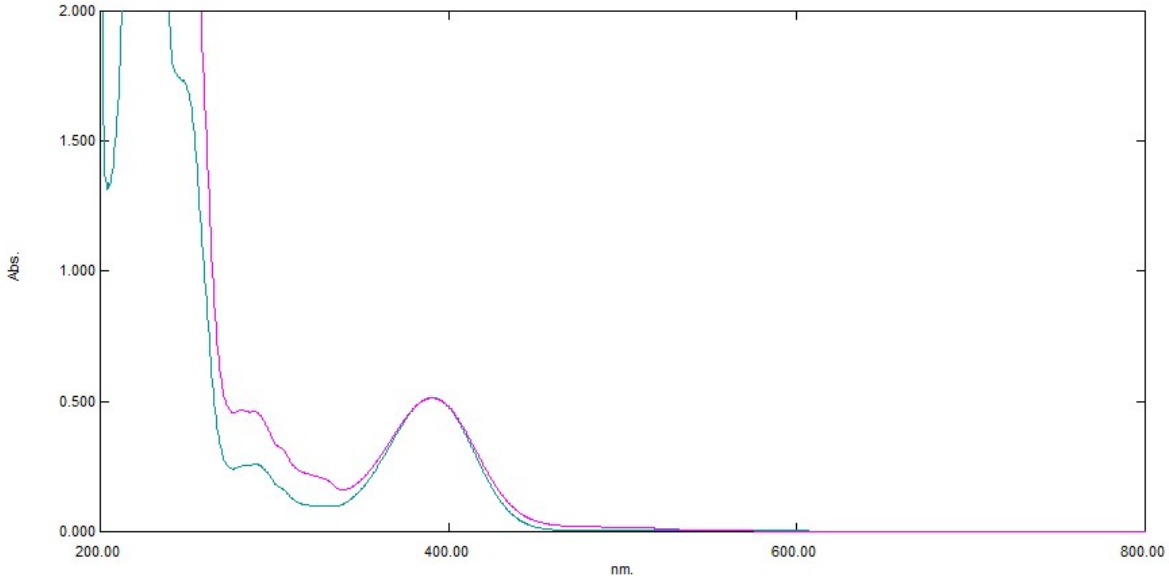

Figure 1: Overlay spectra of Epalrestat before and after derivatization

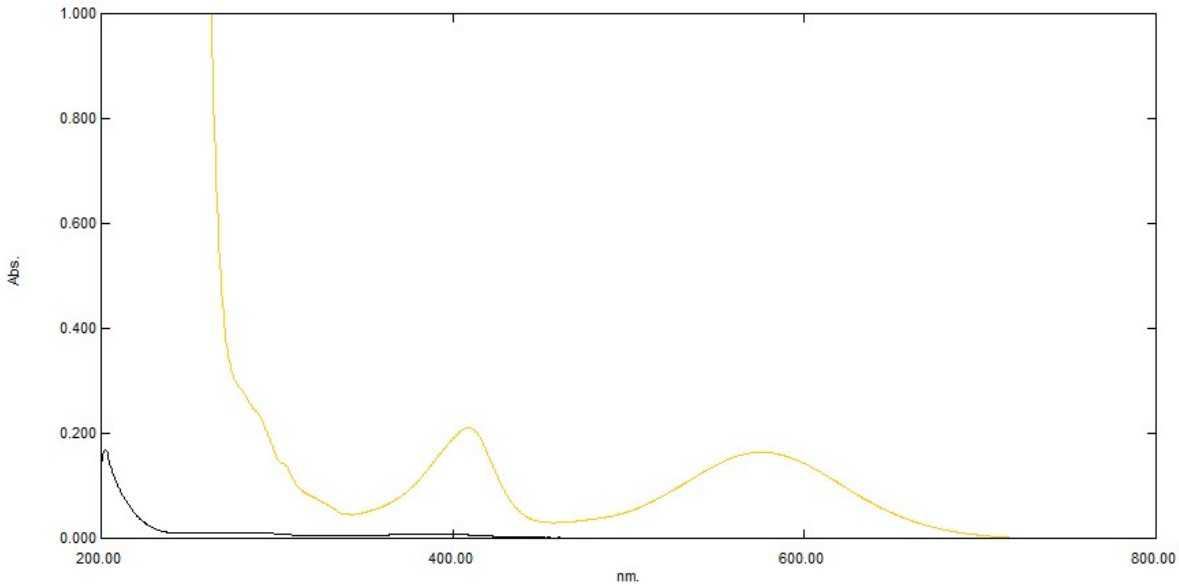

Figure 2: Overlay spectra of Pregabalin before and after derivatization

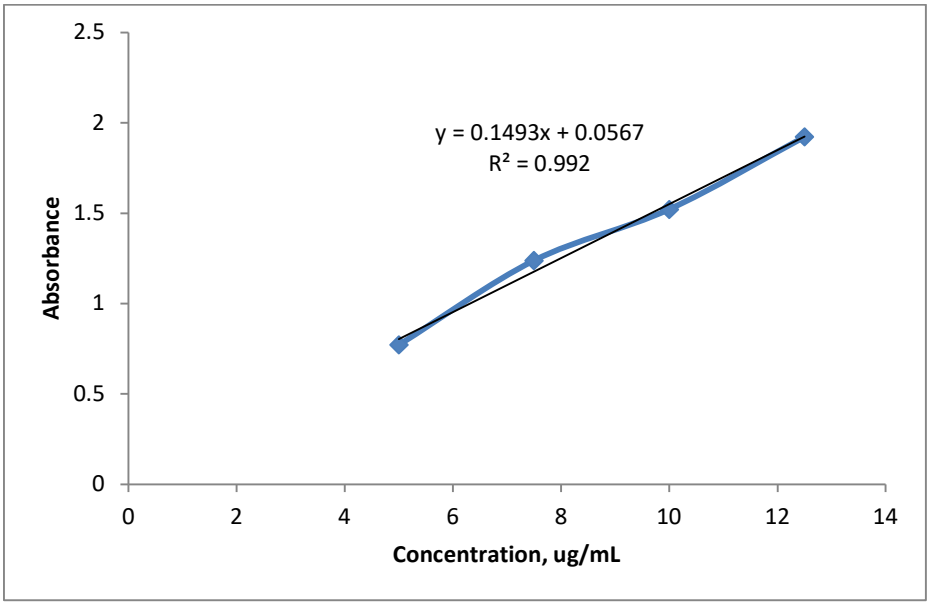

Figure 3: Calibration graph of Epalrestat

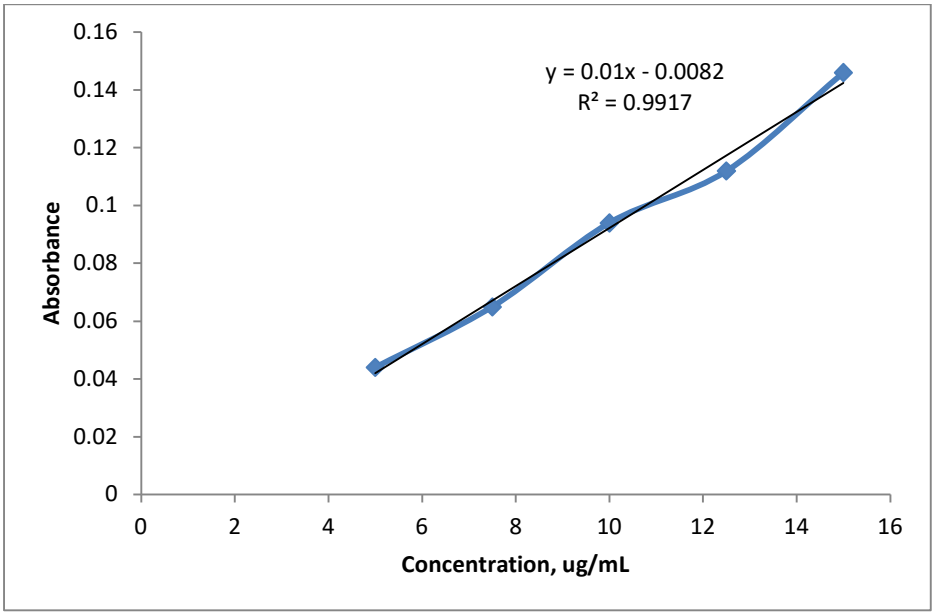

Figure 4: Calibration graph of Pregabalin

CONCLUSION

A simple, precise and accurate spectrophotometric method was developed and validated for the routine analysis of EPL and PGB in tablet dosage forms. The method is applicable for determination of EPL and PGB in combined formulations without any interference of each other and from the excipients. The proposed method is recommended for routine quality control analysis of both the drugs present in the two component formulations.

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