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RESEARCH ARTICLE

Analysis of Epalrestat in Bulk and Tablet Formulation by Difference Spectrometry

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ABSTRACT

A simple, rapid, sensitive and cost effective Difference spectrophotometric method has been developed for the analysis of Epalrestat in bulk and its tablet formulation using two different pH conditions produced by 0.1N HCl (acidic) and 0.1N NaOH (basic). The spectral characteristics of Epalrestat were found to be different in acidic and basic medium. For the difference spectrophotometry, the difference spectra of Epalrestat was scanned in the UV - Visible spectrophotometer between 200 - 800nm by putting acidic solution of Epalrestat in the sample cell and basic solution of the same concentration of Epalrestat in the reference cell. Difference spectra showed the difference absorbance of Epalrestat between basic and acidic conditions. The proposed method was validated according to ICH guideline. Beers' law was obeyed in the concentration range of 1-6 µg/ml at 404 nm which was selected as an analytical wavelength for determination. The correlation co-efficient was found to be 0.9980. The % recovery of Epalrestat was in the range of 96 - 104.4%. The coefficient of variance for intraday and interday precision was found to be 0.207 - 1.98 and 0.912 - 1.43 respectively. Limit of detection and limit of quantification were 0.482 µg/ml and 1.462 µg/ml respectively. The proposed method was applied for determination of Epalrestat in the marketed formulation in which % assay was found to be 104.5%. The method was found to be, accurate, precise, repeatable and specific.

KEYWORDS

Epalrestat, HCl, NaOH, Difference spectrophotometry

INTRODUCTION

Epalrestat is an aldose reductase inhibitor used in the treatment of diabetic peripheral neuropathy. Chemically it is 2-[(5 *Z*)-5-[(E)-3-cyclohexyl-2-methylprop-2-enylidene]-4-oxo-2-thioxo-3-thiazolidinyl] acetic acid[1]. The recommended dosage of oral Epalrestat is 50 mg 3 times daily before meals[2]. Epalrestat is recommended for use in patients with high glycosylated haemoglobin levels (indicating failure to control hyperglycemia), despite standard pharmacological and non pharmacological intervention[3].

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The Extensive review revealed that many analytical methods like UV spectrometry[4], RP-HPLC[5], HPTLC[6], LC-MS/MS[7] and Rapid High-Performance Liquid Chromatography-Tandem Mass Spectrometry have been reported for estimation of epalrestat in dosage form and biological fluid[8]. Determination of stereoisomers of epalrestat by liquid chromatography has been reported. But no any simple UV spectrophotometric method is available for analysis of epalrestat.

So, it was thought of interest to develop simple, precise, and economical spectrophotometric methods for estimation of Epalrestat in bulk and in tablets. The developed methods were

validated for its linearity, accuracy, and precision, limit of detection (LOD) and limit of quantification (LOQ) according to the ICH guidelines. (Q_2R_1).

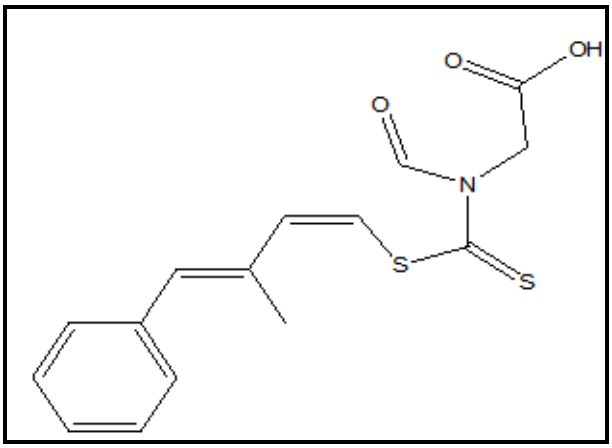

Figure 1: Structure of Epalrestat

MATERIALS AND METHOD

- Epalrestat was procured as a gift sample from Zydus cadila, Ahmedabad.
- Epalrestat tablets (Aldonil tablets Zydus Medica, India) were procured from local market.
- Hydrochloric acid (LR Grade, Sulab Reagents, Vadodara)
- Sodium hydroxide (LR Grade, Sulab Reagents, Vadodara)
- Acetonitrile (AR Grade, Merck specialities Pvt. Ltd., Mumbai)

Instrument

- A Shimadzu model 1800 double beam UV/Visible spectrophotometer with spectral width of 1 ± 0.2 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cells
- SHIMADZU AUX 220 Analytical balance

Preparation of Standard Stock Solution

Epalrestat (10mg) was weighed accurately and transferred into 25 ml volumetric flask. Acetonitrile (15ml) was added, shaken for 15 minutes and dilute up to the mark with Acetonitrile.

Preparation of Hydrochloric acid (0.1N)

Hydrochloric acid (0.85 ml) was transferred into 100 ml volumetric flask and diluted up to the mark with distilled water.

Preparation of Sodium hydroxide (0.1N)

Sodium hydroxide (0.4 gm) was weighed accurate and transferred into 100 ml volumetric flask, dissolved and diluted up to the mark with distilled water.

Working Standard Solution

Standard stock solution (6.25 ml) was taken in 25 ml volumetric flask and dilute up to 25 ml with Acetonitrile to get a concentration of 100 µg/ml.

Preparation of Sample Solution

Twenty tablets (Aldonil 50® Zydus Medica, India) were weighed accurately and powdered. Powder equivalent to 10 mg of Epalrestat was transferred to 25 ml volumetric flask. Acetonitrile (15 ml) was added and sonicated for 15 minutes and diluted up to 25 ml with Acetonitrile. The solution was filtered through Whatman filter paper No. 41. Aliquot (6.25 ml) was diluted to 25 ml with Acetonitrile.

Selection of Wavelength for Determination

Stock solution (0.5 ml) was taken in duplicate 10 ml volumetric flasks, and volumes were made up with 0.1N HCl and 0.1N NaOH respectively to prepare standard solutions containing 5 µg/ml Epalrestat in 0.1N HCl and 0.1N NaOH. The above solutions were scanned in the range of 200 nm to 800 nm to obtain difference spectra by keeping acidic form (i.e. Epalrestat in 0.1N HCl) in sample cell and basic form (i.e. Epalrestat in 0.1N NaOH) in reference cell using 0.1N HCl (in sample cell) and 0.1N NaOH (in reference cell) as blank. The maximum absorbance was observed at 404 nm which was selected for analysis.

RESULTS AND DISCUSSION

The satisfactory results were obtained with 0.1N HCl (in sample cell) and 0.1 NaOH (in reference cell). The wavelength of maximum absorbance of Epalrestat in 0.1N HCl (sample)

and 0.1N NaOH (reference) was found to be at 404 nm.

Method Validation

The proposed method was validated according to ICH guideline (Q_2R_1) for it's routine applicability.

Linearity

Working solutions (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml equivalent to 1, 2, 3, 4, 5 and 6 µg/ml were transferred in a series of duplicate 10 ml volumetric flasks and volumes were made up with 0.1N HCl and 0.1N NaOH respectively to prepare series of standard solutions containing 1-6 µg/ml Epalrestat in 0.1N HCl and 0.1N NaOH. All the above solutions were scanned in the range of 200 nm to 800 nm to obtain their difference spectra by keeping acidic form (i.e. Epalrestat in 0.1N HCl) in sample cell and basic form (i.e. Epalrestat in 0.1N NaOH) in reference cell. Difference absorbance at 404 nm was noted for each solution. Calibration curve of difference absorbance versus concentrations was plotted, and the regression equation was calculated.

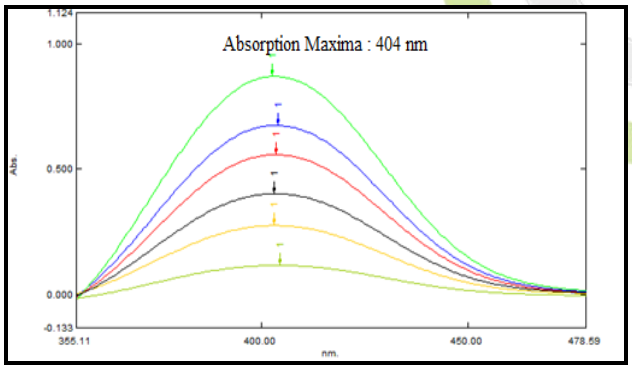

Figure 2: Spectra of Epalrestat 1 – 6 µg/ml

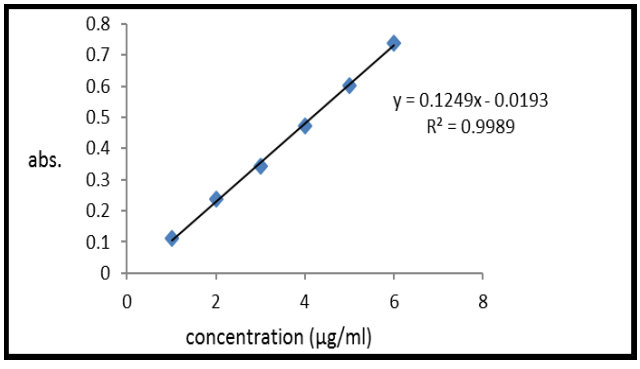

Figure 3: Calibration curve of Epalrestat

Accuracy (% Recovery)

The accuracy of the method was performed by calculating % recovery of Epalrestat by the standard addition method. Known amounts of standard solutions of Epalrestat were added at 50%, 100% and 150% levels to pre-quantified sample solutions of Epalrestat (2 µg/ml). At each level of the amount, three determinations were performed. The amount of Epalrestat was estimated by applying obtained values to regression equation. The % Recovery was found to be in the range of 96 – 104.4%.

Table 1: Results of drug recovery data of Epalrestat

Amt taken (µg/ml)	Amt added (µg/ml)	Amt recovered (µg/ml)	Recovery ± S.D % (n = 3)	% RSD
2	0	1.91	95.5 ± 1.31	1.28
2	1	3.1	104.4 ± 1.90	1.82
2	2	4.0	100.5 ± 1.42	1.41
2	3	4.8	96 ± 1.42	1.48

Precision

The intra-day and inter-day precision of the proposed method was done by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations over the calibration range of Epalrestat (2, 4 and 6 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Repeatability

The precision of the methods was assessed by repeated scanning and measurement of the absorbance of solutions (n = 6) of Epalrestat (4 µg/ml) without changing the parameters for the methods. The % RSD was found to be 0.72 that confirmed the repeatability of the proposed method.

Table 2: Results of Intra-day precision and Inter-day precision study

Epalrestat (µg/ml)	Intra-day precision (n = 3)		Inter-day precision (n = 3)	
	Mean Diff. abs ± S.D.	%RSD	Mean Diff. Abs ± S.D.	%RSD
2	0.303 ± 0.0085	1.89	0.270 ± 0.0038	1.40
4	0.598± 0.0070	1.17	0.528 ± 0.0076	1.43
6	0.821± 0.0017	0.20	0.811± 0.0074	0.91

Table 3: Result of Repeatability study of Epalrestat

Drug	Epalrestat Diff. absorbance
1	0.549
2	0.543
3	0.553
4	0.546
5	0.547
6	0.552
Mean	0.547
S.D.	0.00392
% RSD	0.72

Analysis of Tablet Dosage Form

The proposed UV spectrophotometric method was successfully applied for determination of Epalrestat in tablet dosage form. The percentage of Epalrestat was found to be satisfactory, which was comparable with the corresponding label claim. No any interference due to excipients was found in the assay results. Each result was average of 3 determinations.

Limit of Detection and Limit of Quantification

In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Detection limit was calculated by 3 σ / S and Quantification limit was calculated by 10 σ /S, where σ is the standard deviation of intercept, S is the slope. The limit of detection and limit of quantification were found to be 0.482 µg/ml and 1.462 µg/ml respectively.

Sandell's Sensitivity

It is the concentration of the analyte (in µg/ml) which gives absorbance of 0.001 in a cell of path length 1 cm. and is expressed as µg/cm^2 here the value of sandell's sensitivity was calculated to be 0.1625 µg/cm^2/0.001.

Robustness

Robustness: ability of the method to reproduce results under altered conditions i.e. change in wavelength by ± 2 nm. The Concentration utilize was 4 µg/ml. The % RSD was found to be 1.92.

Table 4: Results of assay of marketed formulation of Epalrestat

Aldonil® tablet	Label claim (mg)	Amount Recovered (mg)	% Amount recovered (n = 3)
1	50	50.55	101.1
2	50	51.05	102.1
3	50	50.72	101.4
Mean			101.5
Standard deviation			0.51
Relative standard deviation			0.50

Table 5: Result of robustness of Epalrestat (at change in Wavelength ± 2 nm)

Absorption maxima (nm)	Absorbance	% Recovered
402	0.491	100
404	0.495	102.5
406	0.491	100
Mean	0.492	100.8
S.D	0.00234	1.94
% RSD	0.47	1.92

CONCLUSION

A UV spectrophotometric method has been developed and validated for the determination of Epalrestat in tablet dosage form. The method was found to be specific as there was no interference of any excipients and impurities. The proposed method was found to be simple, accurate, precise and robust. Hence, it can be used successfully for the routine analysis of Epalrestat in pharmaceutical dosage forms.

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