

## Quantitative determination of Epalrestat by RP-HPLC method

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### Abstract

Epalrestat an aldose reductase inhibitor is used in the treatment of diabetic peripheral neuropathy. A simple, sensitive, precise and accurate RP-HPLC method for determination of epalrestat both as a bulk drug and in pharmaceutical formulation has been developed and validated as per the International Conference on Harmonization (ICH) guidelines. Chromatographic separation was achieved using Qualisil C<sub>8</sub> column, detection at 294 nm and mixture of methanol: (0.01 mol L<sup>-1</sup>) potassium dihydrogen phosphate (75:25 v/v), pH adjusted to 4.5 with *ortho*-phosphoric acid as mobile phase. A typical retention time for eparlestat was 6.64 ± 0.02 min. Linearity was observed in concentration range of 2 – 12 µg.mL<sup>-1</sup> with coefficient correlation ( $r^2 = 0.999$ ). The % RSD value for intra-day and inter-day precision was found to be in the range of 0.32 - 0.79 % and 0.12 -1.32 %. The mean % recovery was found to be in the range of 99.47 - 100.30 %. The low values of LOD (0.15 µg) and LOQ (0.46 µg) indicate high sensitivity of the method. The % RSD value for robustness and ruggedness studies was found to be less than 2 %. The amount of drug estimated was found to be in good agreement with label claim. The developed method can routinely be used for analysis of epalrestat in pharmaceutical formulations.

### Keywords:

Epalrestat; RP-HPLC; Validation

### 1. Introduction

HPLC is a flexible analytical technology widely used for the analysis of pharmaceuticals, biomolecules, polymers, and many organic and ionic compounds. HPLC is a premier separation technique capable of multicomponent analysis of samples and complex mixtures. It is highly sensitive and specific detectors extend detection limits to nanogram, picogram, and even femtogram levels [1].

Epalrestat is an aldose reductase inhibitor used in the treatment of diabetic peripheral neuropathy. The dose of epalrestat is 50 mg. day<sup>-1</sup> [2, 3]. Chemically epalrestat is 2-[(5 Z)-5-[(E)-3-cyclohexyl-2-methylprop-2-enylidene]-4-oxo-2-thioxo-3-thiazolidinyl] acetic acid [4]. The chemical structure of epalrestat is shown in Fig 1.

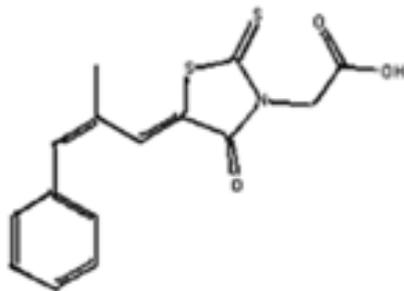
Through literature survey revealed RP- HPLC [5] and HPTLC [6] methods for determination of epalrestat in biological fluids. The resolution of epalrestat stereoisomer has been established by RP-HPLC method using methanol: acetonitrile: water (volume ratio 60:1:50, pH 4.5) [7].

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**Fig. 1.** Structure of epalrestat

However, to our knowledge, no article related to the RP-HPLC method has ever been found for the determination of epalrestat in bulk and in pharmaceutical formulations. Therefore, the study has been undertaken to develop simple, rapid, accurate, reproducible and economical. This method can be used successfully for quality control testing of the drugs from tablet dosage form.

## 2. Experimental

### 2.1 Chemicals and reagents

Epalrestat was supplied by Aristo Pharmaceutical (Mandideep, Bhopal) as a gift sample. Analytical grade solvents and reagents were purchased from Merck specialities Pvt.Ltd. (Mumbai, India). Potassium dihydrogen *ortho*-phosphate was purchased from S.D.fine-chemical Ltd. India. Double distilled water was obtained from all glass double distillation apparatus. Aldonil tablets were purchased from local market; each tablet was labeled to contain 10 mg of epalrestat.

### 2.2 Instrumentation

Analysis was performed on chromatographic system of Shimadzu (Japan) liquid chromatograph comprising LC-10AT-vp solvent delivery system (pump), SPD M-10 A-vp Diode array detector, CTO-10AS- vp as a column oven and a Rheodyne injector with 20  $\mu\text{L}$  loop. Class-M 10A data station was used as a data processor. The chromatographic separation was achieved on Qualisil C8 (5  $\mu\text{m}$ , 25 cm X 4.6 mm i.d.) using methanol: 0.01M potassium dihydrogen phosphate, pH was adjusted to 4.5 with *ortho*-phosphoric acid as mobile phase. Prior to chromatography the mobile phase was filtered using 0.45  $\mu\text{m}$  membrane filter and degassed by ultrasonic vibrations. All experiments were carried out at 30°C and the flow rate of the mobile phase was kept at 1.0 mL.min<sup>-1</sup>. The sample solution of 20  $\mu\text{L}$  was manually injected into column using Hamilton syringe. All determinations were performed at 294 nm.

### 2.3 Stock Standard Solution

Stock standard solution was prepared by dissolving 10 mg of epalrestat in 100 mL methanol that gives concentration of 100  $\mu\text{g.mL}^{-1}$ .

### 2.4 Preparation of Calibration Curve

From stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL were taken in series of 10 mL volumetric flask and diluted up to the mark with mobile phase to obtain concentration in the range of 2 – 12  $\mu\text{g.mL}^{-1}$  for epalrestat. A constant volume 20  $\mu\text{L}$  of epalrestat was injected into column with the help of syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* the drug concentration.

### 3. Validation

#### 3.1 Precision and accuracy

Within the same batch accuracy and precision was performed. The precision was studied as repeatability, intra-day and inter-day precision [8-10].

Repeatability of sample measurement of area was carried out using six replicates of the same concentration ( $10 \mu\text{g.mL}^{-1}$  of epalrestat). The intra-day and inter-day variation for the determination of epalrestat was carried out at three different concentration levels of 4, 6, and  $8 \mu\text{g.mL}^{-1}$ .

Accuracy of the method was assessed as recovery experiments executed out by addition of drug standard solution to pre-analyzed sample solutions at three different levels 80%, 100% and 120%. At each level of the amount, three determinations were performed. This was done to check the recovery of drug at different level in the formulation.

#### 3.2 Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by  $(3.3 \times \text{A.S.D.})/b$  and quantification limit was calculated by  $(10 \times \text{A.S.D.})/b$ , where "b" corresponds to the slope obtained in the linearity study of method.

#### 3.3 Ruggedness and Robustness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analysts using same operational and environmental conditions. Ruggedness studies were performed by analyzing  $10 \mu\text{g.mL}^{-1}$  of sample solution.

Robustness is carried out by introducing various changes in the previous chromatographic conditions *viz* mobile phase composition (methanol: 0.01 mol L<sup>-1</sup> potassium dihydrogen phosphate buffer 74: 26 v/v and 76: 24 v/v); change in flow rate ( $0.9 \text{ mL.min}^{-1}$  and  $1.1 \text{ mL.min}^{-1}$ ); change in pH of mobile phase (4.4 and 4.6); change in column oven temperature ( $28^\circ\text{C}$  and  $32^\circ\text{C}$ ) and the effects on the results were examined. Robustness studies were performed using  $10 \mu\text{g.mL}^{-1}$  of epalrestat.

### 4. Analysis of marketed Formulation

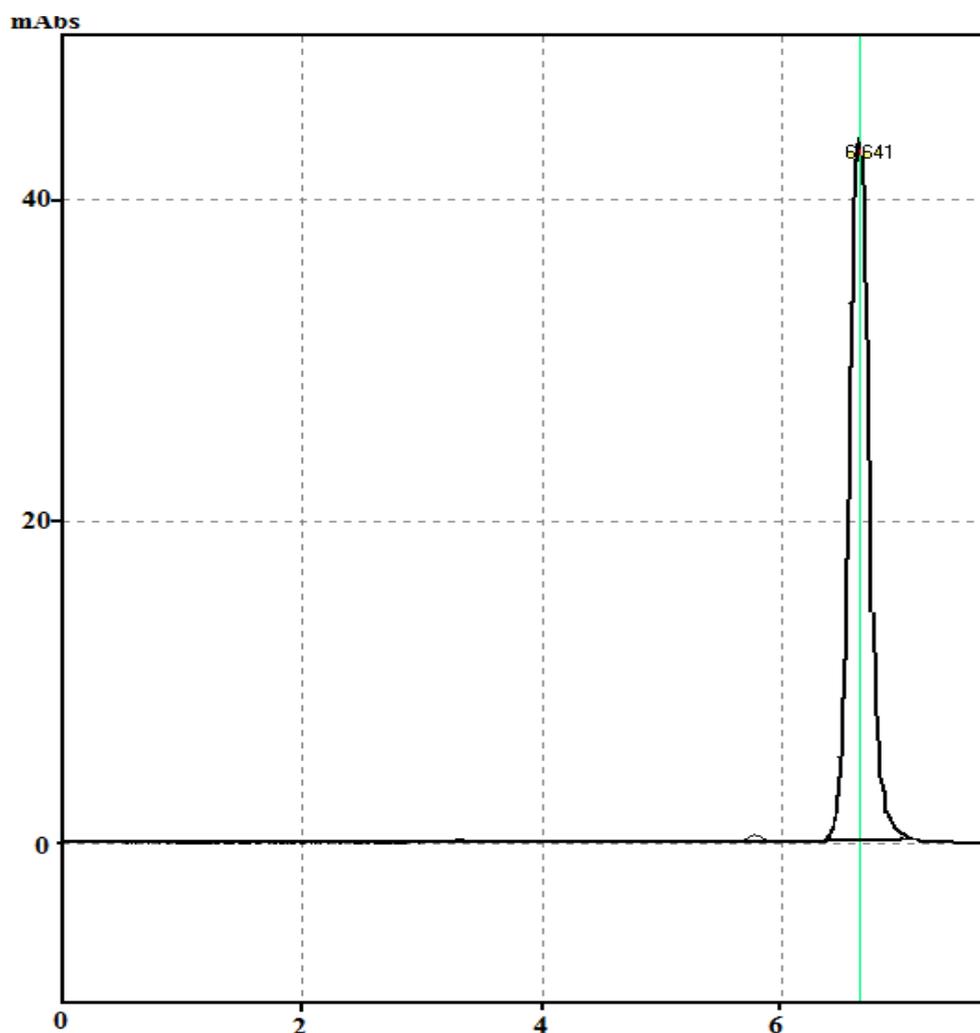
Twenty tablets were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder equivalent to 10 mg of epalrestat was transferred into 100 mL volumetric flask containing 30 mL methanol, shaken manually for 15 min; volume was adjusted to mark using same solvent. The solution was then filtered through Whatmann filter paper no. 41. Aliquot of this solution was diluted to get a final concentration  $10 \mu\text{g.mL}^{-1}$  using mobile phase. A constant volume of  $20 \mu\text{L}$  was injected into column and concentration of epalrestat was determined from linear regression curve.

### 5. Results and Discussion

#### 5.1 Selection and optimization of mobile phase

For optimization of mobile phase; methanol was used first but the broadening of the peak was observed. Therefore, combination of methanol and potassium dihydrogen phosphate buffer ( $0.01 \text{ mol L}^{-1}$ ) in the ratio of 75:25% v/v was tried for resolution of epalrestat. Good resolution and symmetric peak was obtained for epalrestat when the pH of the mobile phase was adjusted to 4.5 and column oven temperature was kept at  $30^\circ\text{C}$ . Under these optimum chromatographic conditions, the retention time for epalrestat was found to be  $6.64 \pm 0.02 \text{ min}$ ,

at a flow rate of 1.0 mL.min<sup>-1</sup>. The detection was carried out at 294 nm. A typical chromatogram is shown in Fig 2.



**Fig. 2.** HPLC Chromatogram of epalrestat 10 µg. mL<sup>-1</sup> in mobile phase methanol: potassium dihydrogen phosphate (75:25 v/v), pH adjusted to 4.5.

### 5.2 Study of linearity curve

The linear regression data for the calibration curve ( $n = 5$ ) showed a good linear relationship over the concentration range of 2 –12 µg.mL<sup>-1</sup> with respect to peak area. The linearity equation  $Y = 74450 X + 8789$ ; slope  $\pm$  SD =  $74450 \pm 504.51$  and Intercept  $\pm$  SD =  $8789 \pm 3415.47$ . No significant difference was observed in the slopes of standard curve.

### 5.3 Validation

The method was validated for accuracy, precision, robustness, ruggedness and sensitivity.

The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The effects on the results were expressed in terms of % RSD which was found to be less than 2; indicates high precision of the method result is shown in Table 1.

**Table 1.** Results of precision studies (Intra-day and inter-day)

Drug	conc. ( $\mu\text{g.mL}^{-1}$ )	Intra- day		Inter- day	
		Amount found ( $\mu\text{g.mL}^{-1}$ )		Amount found ( $\mu\text{g.mL}^{-1}$ )	
		Mean $\pm$ SD	% RSD [n= 3]	Mean $\pm$ SD	% RSD [n= 3]
Epalrestat	4	3.98 $\pm$ 0.03	0.79	3.97 $\pm$ 0.05	1.32
	6	5.99 $\pm$ 0.04	0.67	5.99 $\pm$ 0.03	0.62
	8	7.99 $\pm$ 0.02	0.26	8.001 $\pm$ 0.04	0.53

The accuracy of the method was assessed by % recovery studies. The proposed method when used for extraction and subsequent estimation of epalrestat from pharmaceutical dosage form after spiking with 80 %, 100% and 120 % of additional drug showed recovery in the range of 99 - 101% as listed in Table 2.

**Table 2.** Results of recovery studies

Drug	Initial amount ( $\mu\text{g.mL}^{-1}$ )	Amount added ( $\mu\text{g.mL}^{-1}$ )	Amount recovered $\pm$ SD ( $\mu\text{g.mL}^{-1}$ , n = 3)	Recovery, %	RSD, %
Epalrestat	4	3.2	3.18 $\pm$ 0.03	99.47	1.13
	4	4	3.98 $\pm$ 0.04	99.62	1.24
	4	4.8	4.81 $\pm$ 0.02	100.30	0.58

Robustness of the method was studied by making deliberate variation in four different chromatographic parameters and the effects on the results were examined. The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %. The results are shown in Table 3. The low % RSD value proven robustness of the method.

**Table 3.** Robustness Studies of Epalrestat

Parameter	Theoretical plate (N)	Tailing Factor
Mobile phase composition	5876	1.06
pH of Mobile Phase	4456	1.05
Change in Flow Rate	5367	1.03
Change in column Temperature	2560	1.03

Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions. The % amount of epalrestat by analyst – I was found to be 99.78 (% RSD 0.40) and analyst – II was found to be 99.72 (% RSD 0.78). The LOD and LOQ were found to be 0.15 and 0.46  $\mu\text{g}$ , respectively. The low values of LOD and LOQ indicate enough sensitivity of the method.

System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The retention time ( $T_R$ ), capacity factor ( $K'$ ), theoretical plate (N) and tailing factor (T) were found to be 6.64 min, 0.76, 5974 and 1.03, respectively. The summary of validation parameters are listed in Table 4.

**Table 4.** Summary of Validation Parameters

Parameters	Observation
Linearity range [ $\mu\text{g. mL}^{-1}$ ]	2 – 12
Correlation coefficient[ $r^2$ ]	0.999
LOD [ $\mu\text{g}$ ]	0.15
LOQ [ $\mu\text{g}$ ]	0.46
Precision (% RSD)	
Intra- day [n = 3]	0.32 – 0.79
Inter-day [n = 3]	0.12 – 1.32
Repeatability [n = 5]	0.32
% Recovery $\pm$ SD [n = 9]	99.80 $\pm$ 0.98
Robustness	Robust
Ruggedness ( RSD, %)	
Analyst I [n = 6]	0.40
Analyst II [n = 6]	0.78

## 6. Analysis of pharmaceutical formulation

The retention time for epalrestat extracted from tablet formulation was found to be  $6.64 \pm 0.02$  min. There is no interference from the excipients usually present in the tablets. The % amount of label claim of epalrestat was found to be 100.23 % (% RSD 0.38).

## 7. Conclusion

RP- HPLC technique developed for the analysis of epalrestat is accurate, precise and robust. The method is also, rugged and sensitive. Statistical analysis proves that the method is repeatable and can be used for routine analysis of epalrestat in bulk and in pharmaceutical formulation.

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