

VALIDATION OF DERIVATIVE SPECTROPHOTOMETRIC AND UPLC METHODS FOR QUANTITATIVE DETERMINATION OF EPALRESTAT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: A new, simple, rapid, sensitive, precise, accurate and effective Derivative spectrophotometric and UPLC method's has been developed and validated for the analysis of Epalrestat in bulk and tablet dosage formulation as per United states of pharmacopeia (USP) guidelines.

Methods: In UPLC, the Chromatographic separation was achieved on Agilent C18 column 5 x 2.1mm (1.8micron) and the detection wave length at 293 nm using a mobile phase of 0.5% Ammonium Acetate Buffer, methanol and acetonitrile in the ratio of 40:40:20, pH was adjusted to 3.0 with ortho phosphoric acid. In Derivative spectrophotometric method, the detection was carried out at 388nm, 366nm, 344nm and 322nm for Zero, first, second and third order derivatives, using methanol as a solvent

Results: In UPLC, the retention time for Epalrestat was found to be 4.033 ± 0.01 min. Linearity was observed in the concentration range of 250-1500mcg with a correlation coefficient 0.9998. The % RSD value for precision was found to be 0.07 and the mean recovery was found to be 99.96-129.98%. In Derivative spectrophotometric method, linearity range of 1-5mcg for zero and first orders, 8-16mcg for second and third orders with correlation coefficient of 0.995, 0.9998, 0.9998 and 0.9993 respectively. The mean %recovery for the proposed method was found to be 110.07-129.72, 110.42-130.10, 109.83 130.14, 110.20-130.31% indicating no interferences from the tablet excipients. The LOD and LOQ were in the limits and the percentage purity in the pharmaceutical dosage form is 99.56, 101.54, 89.32, 101.06 and 82.67%.

Conclusion: The amount of drug estimated was found to be in good agreement with label claim. The method was validated and found to be simple, sensitive, accurate and precise as per USP guidelines by UPLC and Derivative spectrophotometry.

Keywords: UPLC, Epalrestat, Derivative spectrophotometry, First order, Second order, Third order, UV Determination of Epalrestat.

INTRODUCTION

Epalrestat is an Aldose reductase inhibitor is used in the treatment of peripheral neuropathy chemically known as 5-[(Z,E)-β-Methylcinnamylidene]-4-oxo-2-thioxo-3- thiazolidine acetic acid and The recommended dosage of oral epalrestat is 50mg 3 times daily before meals. Epalrestat is particularly recommended for use in patients with high glycosylated haemoglobin levels (indicating failure to control hyperglycaemia), despite standard pharmacological and non pharmacological intervention.

The methods for Epalrestat in bulk and pharmaceutical dosage forms was determined by RP-HPLC [1-2] but only few are reported the zero order and area under curve in pharmaceutical formulations Epalrestat [3].

UPLC is the most advanced and innovative technique in the LC, compare to HPLC UPLC has the effective separation with high resolution in short run time, low solvent consumption at high pressure makes the detection is carried out even at small minute traces of samples, Columns in UPLC plays the key role in analysis which are made with BEH, HSS, CSH and 2D technologies [4].

Derivative spectroscopy involves in the conversion of the normal spectrum into its derivatives [5]. Derivatives minimises the interaction of matrix interferences which results in accurate estimation of the API in the dosage form unlike Normal spectrum [6]. As the order of derivatives increase there will be decrease in interferences [7].

The literature reports did not show any Derivative spectrophotometric method for Epalrestat in bulk and pharmaceutical formulation, hence there was a need to develop and validate an analytical method for the Epalrestat in bulk and dosage form. Hence the present study aimed to develop and validate a method Epalrestat in bulk and tablet dosage form by Derivative spectrophotometric method. The structure of Epalrestat was shown in Fig 1.

MATERIALS AND METHODS

Chemicals and Materials

Epalrestat standard was supplied by IDEAL ANALYTICAL AND RESEARCH INSTITUTION, Puducherry-605 110 as a gift sample.

Analytical grade solvents and reagents like Acetonitrile (HPLC grade), ortho phosphoric acid 0.5% Ammonium Acetate Buffer (HPLC Grade), Methanol (HPLC Grade) were supplied by KLR pharmacy college, Milli-Q Water was obtained from all glass double distillation apparatus. Eparel50 (Micro labs Ltd), India tablets was purchased from local market.

Instrumentation

UPLC Analysis was performed on chromatographic system of Thermo fisher Scientific with Chromquest software. Liquid chromatograph comprising Accela 1250 photo Diode array detector (PDA Detector), Agilent C18 5 x 2.1mm (1.8micron) as a column oven and a Rheodyne injector with 20µL loop,

Derivative Spectral and photometric absorbance measurements were done on double beam UV Spectrophotometer with UV Win software, lab India make 3092. 10mm path length quartz cells were used. Digital analytical balance Shimadzu make AUX 220 was used for weighing.

Method validation

Preparation of standard stock solution

A quantity of Epalrestat working standard 25.00mg was accurately weighed and transferred into a 50mL volumetric flask. 5mL of methanol was added and sonicated to dissolve. The volume was made with methanol and mixed well. From the above solution 5ml was transferred to 25mL volumetric flask and the volume was made with methanol, mixed well.

Preparation of sample stock solution

A quantity of Epalrestat working standard 25.00mg (89.08 mg) was accurately weighed and transferred into a 50mL volumetric flask. 5mL of methanol was added and sonicated to dissolve. The volume was made with methanol and mixed well. From the above solution 5ml was transferred to 25mL volumetric flask and the volume was made with methanol, mixed well.

Linearity

Preparation of Linearity solutions for UPLC

From the standard stock solution various aliquots of 0.5, 1, 1.5, 2, 2.5, 3 ml were taken in a series of 10 ml volumetric flasks and diluted up to mark with the methanol to get concentrations in the range of 205 to 1500 mcg/ml.

Preparation of Linearity solutions for Derivative spectrophotometry

Aliquots of solutions 0.1-0.5ml were taken from the standard stock solution into 10ml volumetric flasks. The volume was made up to 10 ml using methanol to obtain the concentrations of 1-5(mcg/ml). The absorbance was measured at 388nm, 366nm, against blank. This was shown in Fig 2, 3.

Aliquots of solutions 0.8ml-1.6ml were taken from the standard stock solution into 10ml volumetric flasks. The volume was made up to 10 ml using methanol to obtain the concentrations of 8-16 (mcg/ml).The absorbance was measured at 344nm, 322nm, against blank. This was shown in Fig 4,5. The results were given in table 1.

Precision

Preparation of Precision sample solution (Prepare in six replicates)

Carefully transferred different concentrations of tablet content into 6 different 50mL volumetric flasks and dissolved in 5mL methanol and made up the volume with methanol. From the above solution 5ml was transferred to 25mL volumetric flask and made up to 25mL, 10mL aliquot was filtered through 10 μ m filter and injected into UPLC system and the absorbance was recorded at 388,366,344,322 nm against blank in UV VISIBLE Spectrophotometer. The precision was determined by repeatability (intraday) and intermediate precision (inter day). Intraday precision was carried out by preparing drug (Epalrestat) solution of same concentration and analysing at three different times in a day. The same procedure was followed for three different days to determine inter d ay precision. Intraday and Intermediate precision was expressed as % relative standard deviation. The results were given in table 2 and 3.

Accuracy

Accuracy can be done by injecting three replicate samples at 100% sample dilutions each at other levels prepared by spiking Epalrestat API Sample solution at 10%, 20%, 30%, to sample stock solution of target concentration level.

Preparation of sample solution (10% standard spiked) Prepare in triplicate

From the above sample stock solution, 5ml was carefully transferred into 25ml volumetric flask. To this, 0.5ml of standard stock solution was added and made up the volume with methanol.

Preparation of sample solution (20% standard spiked) Prepare in triplicate

From the above sample stock solution, 5ml was carefully transferred into 25ml volumetric flask. To this, 1ml of standard stock solution was added and made up the volume with methanol.

Preparation of sample solution (30% standard spiked) Prepare in triplicate

From the above sample stock solution, 5ml was carefully transferred into 25ml volumetric flask. To this, 1.5ml of standard stock solution was added and made up the volume with methanol.

All the above solutions were filtered through 10 μ m filter and 10mL aliquot of each solution was injected into UPLC system and the absorbance was measured at 388nm, 366nm, 344nm and 322nm in UV-VISIBLE spectrophotometer. The results were tabulated in table 4.

Assay

The proposed method was applied for the determination of Epalrestat with a brand name Eparal 50.10mL aliquot was filtered

through 10 μ m filter and injected into system and the spectral and photometric determinations for Zero, first, second and third order derivatives were obtained. The results were tabulated in table 5.

System Suitability

10mL of standard stock solution was injected into UPLC system. The results were tabulated in table 6.

Detection Limit

The Detection Limit of an individual analytical procedure was the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as:

$$LOD = 3.3\sigma / S$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the Analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure was the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The Quantitation Limit (LOQ) may be expressed as:

$$LOQ = 10\sigma / S$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

The results were tabulated in table 7.

Ruggedness and Robustness

Ruggedness was determined by performing the same proposed method on different instruments, by two different analysts and on different days to check the reproducibility.

Robustness was the capacity of a method to remain unaffected by small deliberate variations in method parameters such as temperature, wavelength, pH, solvents. The results were tabulated in table 8.

RESULTS AND DISCUSSION

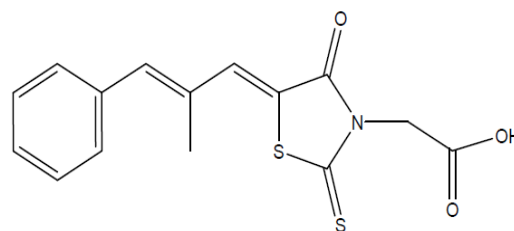


Fig. 1: Structure of Epalrestat

Linearity of Epalrestat

The drug Epalrestat exhibits linearity within the range of 250-1500 mcg in UPLC whereas in Derivative Spectroscopy, the Drug Epalrestat obeys Beer's Lambert's Law in the range of 1 - 5mcg for zero, First and 8-16 mcg for Second, third derivatives respectively and the correlation coefficient was found to be within the limits.

Precision

The two methods performed were precise; In UPLC, the %RSD was found to be 0.07 and it was within the limits.

In Derivative Spectroscopy, the mean %RSD for the intermediate precision were 0.69, 1.49, 1.2, 1.55; the inter day precision were 0.477, 1.756, 1.306, 1.710; and the repeatability were 0.62, 1.26, 1.01, 1.22 for zero, first, second and third order derivatives respectively and all were within the limits.

Accuracy

The Accuracy was carried out according to the guidelines of USP. The recovery studies were performed on Drug Epalrestat at 110-130% in UPLC and Derivative spectroscopy. The mean recovery and %RSD were found to be within the limits and the data was given in table 4.

Assay

The marketed formulation (EPARAL 50) was assayed as per USP guidelines and the data was given in Table 5.

System Suitability

The drug Epalrestat was suitable with UPLC conditions and the mean peak area was found to be 4026089.8 and the %RSD was 0.38 and was within the limits.

LOD and LOQ

The LOD and LOQ data for UPLC and derivative spectroscopy was shown in Table 7

Ruggedness and Robustness

The %RSD for ruggedness carried out by Instrument I and II, Analyst I & II were within the limits whereas for Robustness performed under altered conditions were also within the limits and the data was given in table 8 & 9.

Table 1: Linearity Data of Epalrestat

| Conc (µg/ml) | Peak area | Conc (µg/ml) | Abs 0 order | Abs 1 st order | Conc (µg/ml) | Abs 2 nd order | Abs 3 rd order |
|----------------------|------------|--------------|-------------|---------------------------|--------------|---------------------------|---------------------------|
| 250 | 1001230 | 1 | 0.101 | 0.014 | 8 | 0.064 | 0.023 |
| 500 | 1910006 | 2 | 0.225 | 0.029 | 10 | 0.075 | 0.037 |
| 750 | 2902169 | 3 | 0.356 | 0.044 | 12 | 0.087 | 0.05 |
| 1000 | 3918296 | 4 | 0.499 | 0.059 | 14 | 0.098 | 0.063 |
| 1250 | 4825846 | 5 | 0.63 | 0.075 | 16 | 0.11 | 0.075 |
| 1500 | 5783373 | — | — | — | — | — | — |
| Slope | 38485.1240 | | -0.0374 | -0.0014 | | 0.0178 | 0.0106 |
| Intercept | 22717 | | 0.1332 | 0.0152 | | 0.0023 | 0.0026 |
| r² | 0.9997 | | 0.9995 | 0.9998 | | 0.9998 | 0.9993 |

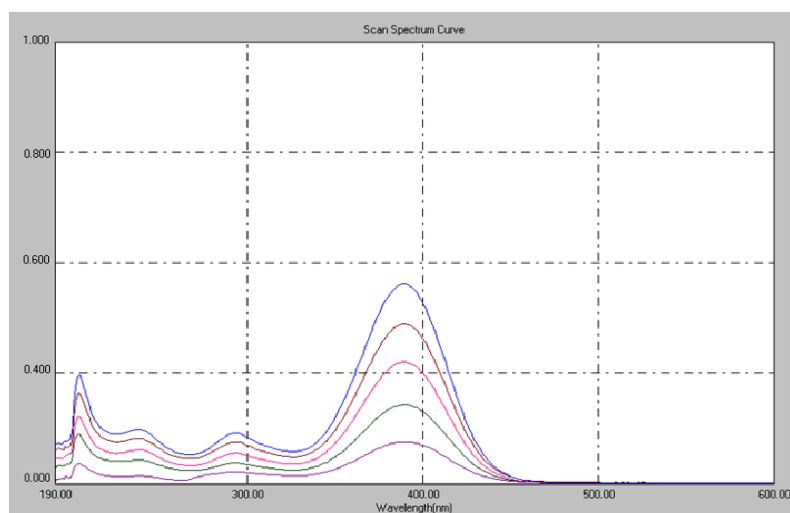


Fig. 2: All spectra of Epalrestat in Derivative spectroscopy (Zero order)

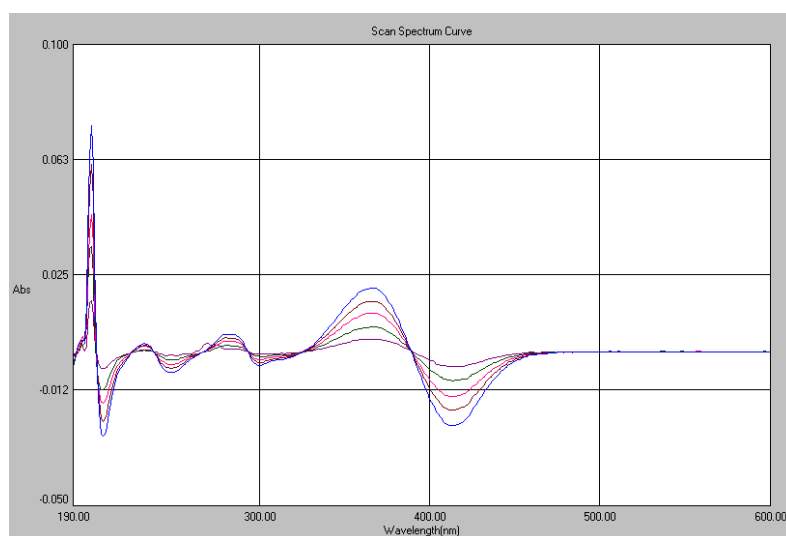


Fig. 3: All Spectra of Epalrestat in Derivative spectroscopy (First order)

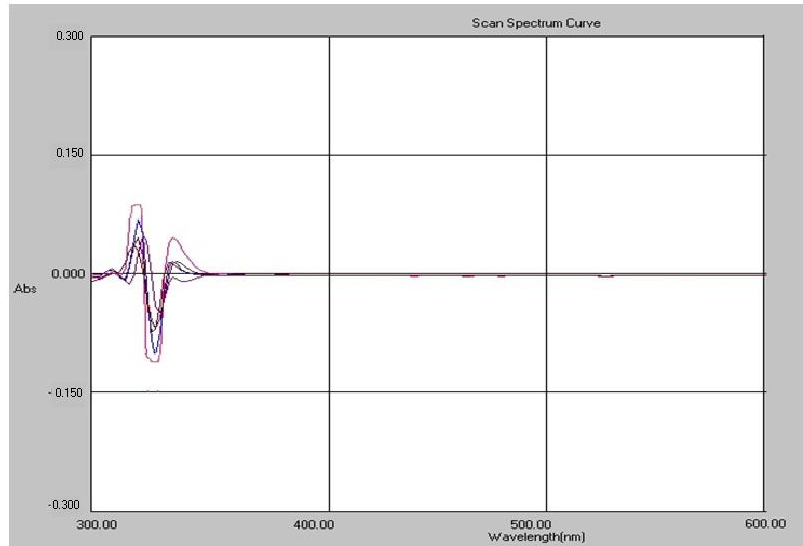


Fig. 4: All Spectra of Epalrestat in Derivative spectroscopy (Second order)

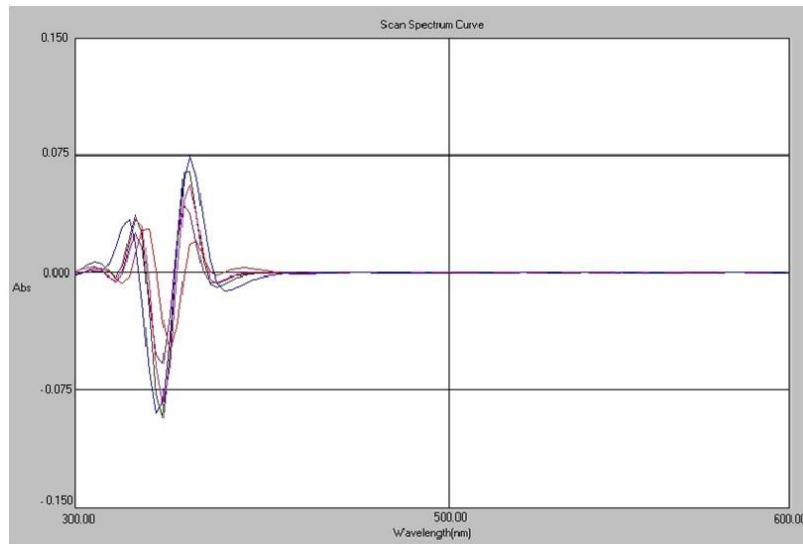


Fig. 5: All Spectra of Epalrestat in Derivative spectroscopy (Third order)

Table 2: Precision data of Epalrestat by UPLC:

| Sample Id | Standard Area | Sample Area | Mg/Tablet | % of Drug |
|-------------|---------------|-------------|-----------|---------------|
| 1 | 4026090 | 3952401 | 49.96 | 99.93 |
| 2 | 4026090 | 4001744 | 50.00 | 100.00 |
| 3 | 4026090 | 4025487 | 50.06 | 100.12 |
| 4 | 4026090 | 4030179 | 50.05 | 100.10 |
| 5 | 4026090 | 3992436 | 49.99 | 99.98 |
| 6 | 4026090 | 4017999 | 50.01 | 100.03 |
| Mean | | | | 100.03 |
| SD | | | | 0.1 |
| %RSD | | | | 0.07 |

Table 3: Inter day and Repeatability Precision data in Derivative Spectroscopy:

| Method | Conc. µg/ml | Avg | Inter day %RSD (Days) | | | Avg | Repeatability %RSD(hrs) | | | Avg |
|-----------------------------|-------------|------|-----------------------|-------|------|-------|-------------------------|------|----|------|
| | | | 1 | 2 | 3 | | 0 | 8 | 16 | |
| Zero order | 3 | 0.69 | 0.340 | 0.462 | 0.62 | 0.477 | 0.95 | 0.91 | 0 | 0.62 |
| 1st order | 3 | 1.49 | 1.33 | 1.97 | 1.97 | 1.756 | 1.92 | 1.88 | 0 | 1.26 |
| 2nd order | 12 | 1.2 | 1.70 | 1.35 | 0.87 | 1.306 | 1.64 | 1.41 | 0 | 1.01 |
| 3rd order | 12 | 1.55 | 1.79 | 1.74 | 1.60 | 1.710 | 1.34 | 1.10 | 0 | 1.22 |

Table 4: Accuracy data of Epalrestat in UPLC and Derivative Spectroscopy:

| Method | Conc Level | Amount Added | Mg/Tab Mean | Amount Recovered Mean | %Recovery Mean | SD | %RSD |
|-----------------------|------------|--------------|-------------|-----------------------|----------------|------|------|
| UPLC | 110 | 0.5 | 54.89 | 0.49 | 109.78 | 0.36 | 0.21 |
| | 120 | 1 | 60.00 | 1.01 | 120.00 | 0.12 | 0.12 |
| | 130 | 1.5 | 64.99 | 1.49 | 129.98 | 0.30 | 0.31 |
| Zero order | 110 | 0.5 | 55.00 | 0.55 | 110.07 | 0.67 | 0.61 |
| | 120 | 1 | 60.30 | 1.30 | 120.26 | 0.25 | 0.21 |
| | 130 | 1.5 | 65.10 | 1.52 | 129.72 | 1.11 | 0.85 |
| 1 st order | 110 | 0.5 | 54.37 | 0.54 | 110.42 | 0.63 | 0.57 |
| | 120 | 1 | 60.10 | 1.01 | 120.70 | 0.96 | 0.80 |
| | 130 | 1.5 | 65.30 | 1.53 | 130.10 | 0.63 | 0.48 |
| 2 nd order | 110 | 0.5 | 54.54 | 0.49 | 109.83 | 0.38 | 0.35 |
| | 120 | 1 | 59.82 | 1.00 | 120.07 | 0.25 | 0.21 |
| | 130 | 1.5 | 64.60 | 1.43 | 130.14 | 0.38 | 0.29 |
| 3 rd order | 110 | 0.5 | 55.90 | 0.52 | 110.20 | 0.37 | 0.33 |
| | 120 | 1 | 60.40 | 1.06 | 120.88 | 0.77 | 0.64 |
| | 130 | 1.5 | 65.61 | 1.59 | 130.31 | 0.74 | 0.57 |

Table 5: Assay data of Epalrestat in UPLC and Derivative spectroscopy:

| Method | Label claim | Standard Area | Sample Area | Mg/tab | Percentage | SD | %RSD |
|-----------------------|-------------|---------------|-------------|--------|------------|------|------|
| UPLC | 50 | 4026089.8 | 3948399 | 49.98 | 99.56 | 0.2 | 0.52 |
| Zero order | 50 | 0.356 | 0.355 | 50.77 | 101.54 | 0.73 | 0.72 |
| 1 st order | 50 | 0.044 | 0.038 | 44.66 | 89.32 | 1.19 | 1.33 |
| 2 nd order | 50 | 0.087 | 0.086 | 50.53 | 101.06 | 1.22 | 1.21 |
| 3 rd order | 50 | 0.050 | 0.040 | 41.33 | 82.67 | 1.65 | 2.00 |

Table 6: System suitability data of Epalrestat in UPLC:

| Sample Id | Standard peak Area of Epalrestat |
|----------------|----------------------------------|
| 1 | 4014196 |
| 2 | 4015089 |
| 3 | 4044651 |
| 4 | 4041178 |
| 5 | 4015335 |
| Avg. peak area | 4026089.8 |
| SD | 15414 |
| %RSD | 0.38 |

Table 7: LOD and LOQ Data of Epalrestat in Derivative spectroscopy:

| Method | LOD | LOQ |
|--------------|----------|----------|
| Zero Order | 0.594595 | 0.18018 |
| First Order | 0.137211 | 0.415789 |
| Second Order | 0.155174 | 0.470225 |
| Third Order | 0.160642 | 0.486792 |

Table 8: Ruggedness Data of Epalrestat in Derivative spectroscopy:

| Method | Ruggedness | | | | | | | | | | | |
|--------------|------------|-------|-------|------------|------|-------|--------------|--------|-------|---------------|--------|-------|
| | Analyst I | | | Analyst II | | | Instrument I | | | Instrument II | | |
| | Mean | SD | %RSD | Mean | SD | %RSD | Mean | SD | %RSD | Mean | SD | %RSD |
| Zero Order | 0.353 | 0.835 | 0.827 | 0.355 | 0.64 | 0.633 | 0.351 | 0.0007 | 0.784 | 0.351 | 0.0004 | 0.609 |
| First Order | 0.0381 | 1.73 | 1.97 | 0.0383 | 1.88 | 2.00 | 0.0403 | 0.0006 | 1.33 | 0.0406 | 0.0009 | 1.66 |
| Second Order | 0.0468 | 1.53 | 1.60 | 0.0463 | 1.04 | 1.11 | 0.0466 | 0.0006 | 1.36 | 0.0463 | 0.0008 | 1.04 |
| Third Order | 0.0860 | 1.65 | 1.64 | 0.0868 | 1.71 | 1.69 | 0.087 | 0.002 | 1.17 | 0.0866 | 0.0002 | 1.94 |

*Average of three Determinations

Table 9: Robustness Data of Epalrestat in Derivative spectroscopy:

| Method | Robustness | | | | | | | | | | | |
|--------------|------------|--------|-------|--------|--------|-------|-------|--------|------|-------|--------|-------|
| | Room Temp | | | 25°C | | | -2nm | | | +2nm | | |
| | Mean | SD | %RSD | Mean | SD | %RSD | Mean | SD | %RSD | Mean | SD | %RSD |
| Zero Order | 0.351 | 0.0005 | 0.164 | 0.351 | 0.0005 | 0.164 | 0.349 | 0.0004 | 0.17 | 0.353 | 0.0007 | 0.167 |
| First Order | 0.0403 | 0.0005 | 1.431 | 0.0406 | 0.0005 | 1.419 | 0.039 | 0.0003 | 1.67 | 0.049 | 0.0005 | 1.423 |
| Second Order | 0.0466 | 0.0005 | 1.237 | 0.0463 | 0.0005 | 1.246 | 0.044 | 0.0004 | 1.32 | 0.056 | 0.0008 | 1.297 |
| Third Order | 0.087 | 0.001 | 1.149 | 0.0866 | 0.0005 | 0.666 | 0.083 | 0.003 | 1.17 | 0.068 | 0.0003 | 0.776 |

*Average of three Determinations

CONCLUSION

The method was validated and found to be simple, sensitive, accurate and precise as per USP guidelines. UPLC and Derivative spectrophotometric methods were developed for the determination of Epalrestat in bulk and tablet dosage form. Epalrestat can be directly determined in tablets in presence of excipients without sample pre treatment procedures by using UPLC and Derivative spectrophotometric methods. The apparatus and reagents used seem to be accessible even for the simple laboratories. Also, no significant difference was found between the proposed UPLC and Derivative spectrophotometric methods. Compare to RP-HPLC, UPLC is the most advanced technique with great resolution in short run time and as the increase in the order of derivative spectroscopy, the interferences from the excipients in the dosage form decreases which leads to the accurate estimation of pure drug in the dosage form. Therefore, developed methods can be recommended for routine and quality control analysis of Epalrestat pure drugs and their pharmaceutical formulations.

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