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RESEARCH ARTICLE

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF EPALRESTAT AND METHYLCOBALAMIN IN TABLET DOSAGE FORM

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ABSTRACT

Forced degradation study was carried out and a newly developed method is applicable to degradation analysis of drug. A chromatographic separation of drug as well as its degradants was achieved using spherisorb CNRP, 4.6 × 250mm, 5μm C18 column with Mobile phase of Acetonitrile: 0.05 M Potassium Dihydrogen Phosphate Buffer pH 4.0 adjusted with ortho phosphoric acid (60: 40, V/V). Drug and degradants were monitored at detection wavelength of 292nm, the flow rate was 1 ml/min, injection volume was 20 μl. Retention time of Epalrestat and Methylcobalamin were about 3.26 min and 4.92 min respectively. Both the drugs were subjected to acid, alkali, oxidation, thermal and photo degradation. The degradation studies indicates Epalrestat to be more susceptible to acid hydrolysis while Methylcobalamin to be more susceptible to photo hydrolysis. The degradation products were well resolved from the pure drug with significant differences in their retention time values.

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INTRODUCTION

Epalrestat[1-3]

It is an Aldose reductase inhibitor (Anti-diabetic drug) appears as yellow to orange crystal powder. It inhibits high glucose-mediated neutrophil endothelial cell adhesion and expression of endothelial adhesion molecules not only through inhibition of a PKC-dependent pathway, but also through increased endothelial NO production. It also reduses glucose to sorbitol. It isfreely soluble in methanol, acetonitrile, and ethanol and sparingly soluble in water.

Molecular formula of Epalrestat is $C_{15}H_{13}NO_3S_2$.
It is chemically5-[(Z,E)- -Methylcinnamylidene]-4-oxo-2-thioxo-3-thiazolidine acetic acid.

The pKa and Log P value of Epalrestat is 3.61 and 2.02 respectively.

Methylcobalamin[4-6]

It is a Vitamin andappears as Bright red crystals. It is Co-factor in the enzyme methionine synthase, which functions to transfer methyl groups for the regeneration of methionine from homocysteine. In Anemia, it increases erythrocyte production by promoting nucleic acid synthesis in the bone marrow and by promoting maturation and division of erythrocytes. It donates the methyl factor for the synthesis of lecithin which is a major component of the myelin sheath and thus repairs nerve damage. It is freely soluble in water, soluble in methanol, ethanol and acetone, insoluble in acetonitrile. Molecular Formula of Methylcobalamin is $C_{14}H_{91}CON_{13}O_{14}P$.

It is Chemically Co -[-(5,6-dimethylbenz-1H-imidazolyl)]-Co - Methylcobamide.

The pKa and Log P value of Methylcobalamin is 0.48 and 1.85 respectively.

Stability Studies[7-8]

A Stability Indicating Assay Method (SIAM) is a "Quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to the degradation."According to FDA guidelines, a SIAM is defined as "A validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities", and the FDA recommends that all assay procedures for stability studies should be stability indicating.

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Purpose of Stability Studies

- SIAM is developed routinely by stressing the API under conditions exceeding those normally used for accelerated stability testing.
- In addition to demonstrating specificity in SIAM, stress testing, also referred to as forced degradation, also can be used to provide information about degradation pathways and products that could form during storage and helps to facilitate formulation development, manufacturing, and packaging.
- Stressing the API in both solutions and in solid-state form generates the sample that contains the products most likely to form under most realistic storage conditions, which is in turn used to develop the SIAM.
- In simplest terms, the goal of the SIAM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no co elution.
- Generally, the goal of these studies is to degrade the API 5-10 %. Any more than this and relevant compounds can be destroyed, or irrelevant degradation products produced.
- Literature survey reveals that these drugs have been analyzed individually and in combination by many analytical methods, but not a single HPLC method is reported for the estimation of API & its degradants products during force degradation studies for combination of both drugs.

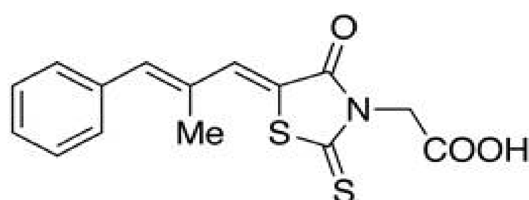

Figure 1 Structure of Epalrestat

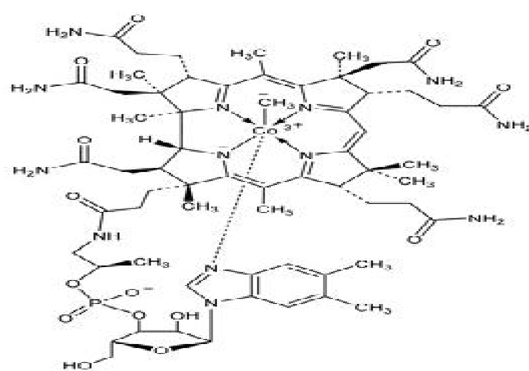

Figure 2 Structure of Methylcobalamin

MATERIALS AND METHOD

Instruments

Agilent technologies 1200 infinity series HPLC system with PDA detector. Sperisorb CNRP C18 (250×4.6, 5µm particle size), Syntronics- µ pH system 361, analytical balance of Libror AEU-210 (Shimadzu).

Chemicals

Epalrestat and Methylcobalmin standards were supplied byZydusCadila, Ahemdabad. Acetonitrile of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Potassium Dihydrogen Phosphate,Orthophosphoric acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (50%) of AR grade was obtained also from E. Merck (India) Ltd., Mumbai and milli Q water. Epalrica-M tablets (Containing 150mg Epalrestat and 1.5 mg Methylcobalamin) were procured from local market.

HPLC conditions

A chromatographic separation of drug was achieved using spherisorb CNRP, 4.6 × 250mm, 5µm C_{18} column with Mobile phase of Acetonitrile: 0.05 M Potassium Dihydrogen Phosphate Buffer pH 4.0 adjusted with ortho phosphoric acid (60:40, V/V). Drug and degradants were monitored at detection wavelength of 292nm, the flow rate was 1 ml/min, injection volume was 20 µl. Retention time of Epalrestat and Methylcobalamin were about 3.26 min and 4.92 minutes respectively.

Preparation of Mobile Phase

Prepare 0.05M Potassium Dihydrogen Phosphate by dissolving 6.8 gm of Potassium Dihydrogen Phosphate in 1000 ml of water. Adjust pH 4.0 with OPA solution. This solution was sonicated for 5 min for degassing and filtered through 0.45 µ Millipore filter.

Preparation of Standard Solution

50 mg of Epalrestat and 50 mg of Methylcobalamin were taken and transferred to 50 ml volumetric flask separately and volume was made up with diluent (Stock solution-1000 µg/ml Epalrestat and 1000 µg/ml Methylcobalamin). 5 ml from Epalrestat and Methylcobalamin stock solution was taken into 50 ml volumetric flask and volume was made up by diluent (Working standard solutions-100 µg/ml Epalrestat & 100 µg/ml Methylcobalamin). Final concentration was made by taking 2 ml and 1 ml from 100 µg/ml from both working standard solutions into 10 ml volumetric flask and volume made up to mark with diluent (20 µg/ml Epalrestat and 10 µg/ml Methylcobalamin).

Preparation of Sample Solution

For the analysis of tablet Formulation (EPALRICA-M Tablet, Epalrestat-150 mg and Methylcobalamin-1.5 mg), Twenty tablets were weighed, powdered. Tablet powder equivalent to 50 mg of Epalrestat and 0.5 mg of Methylcobalamin was transferred to 50 ml volumetric flask and Methylcobalamin (24.5 mg) was added in the same flask. About 30 ml of diluent was added and sonicated for 15 min. The volume was made up to the mark with diluent (1000 µg/ml of Epalrestat and 500 µg/ml of Methylcobalamin). The solution was filtered through 0.45 µ Millipore filter. An aliquot (5 ml) was diluted up to 50 ml with diluent, so final concentration were 100 µg/ml and 50

µg/ml for Epalrestat and Methylcobalamin respectively. The solution (2 ml) was diluted up to 10ml with diluent. (Epalrestat 20 µg/ml and Methylcobalamin 10 µg/ml).

Forced degradation studies

Forced degradation studies were performed at a 20 µg/mL concentration of Epalrestat and 10 µg/mL Methylcobalamin in tablets for identification of the stability-indicating property and specificity of the proposed method. A peak purity of Epalrestat and Methylcobalamin peaks were conducted using a PDA detector on stress samples.

All solutions under forced degradation studies were prepared by dissolving the drug product in a small volume of stressing agents. After degradation, these solutions were diluted with mobile phase to yield a stated concentration approximately. Conditions employed for performing the stress studies are described below.

Table 1 Degradation Summary

Type	Solutions	Area		% Degradation	
As Such	Epalrestat	287735		-	
	Methylcobalamin	68405		-	
	Epalrestat + Methylcobalamin(Sample)	286782	68216	-	-
Acid 0.1 N HCL at Room Temperature for 2 hrs	Epalrestat	230157		20.29	
	Methylcobalamin	56228		17.80	
	Epalrestat + Methylcobalamin (Sample)	221968	55079	23.13	19.48
Base 0.1 N NaOH at Room Temperature for 2 hrs	Epalrestat	240019		16.25	
	Methylcobalamin	55357		18.62	
	Epalrestat + Methylcobalamin (Sample)	254906	54196	11.67	20.33
Peroxide 3% H_2O_2 at Room Temperature for 3 hrs	Epalrestat	239568		17.03	
	Methylcobalamin	51395		24.86	
	Epalrestat + Methylcobalamin (Sample)	247051	50499	14.44	26.17
Thermal At 60°C for 3hrs	Epalrestat	274061		5.04	
	Methylcobalamin	53146		22.30	
	Epalrestat + Methylcobalamin (Sample)	272661	54183	5.52	20.79
Photolytic In sun light for 1 hr	Epalrestat	231996		19.66	
	Methylcobalamin	49115		28.19	
	Epalrestat + Methylcobalamin (Sample)	245507	47683	14.19	30.29

Acid Degradation

Pipette out 2 ml from 100 µg/ml Epalrestat and 50 µg/ml Methylcobalamin from sample solution, 1 ml of 0.1N hydrochloric acid was added and it was kept at room temperature for 2 hours. After that it was neutralized by adding 1 ml of 0.1N NaOH & volume was made up to 10 ml with diluent. For analysis of standard solution 20 µg/ml Epalrestat standard solution and 10 µg/ml Methylcobalamin standard solution kept under acid degradation same as sample solution. After making final dilutions the standard and sample solutions were injected into HPLC and the peak area and peak shapes were observed.

Base Degradation

Pipette out 2 ml from 100 µg/ml Epalrestat and 50 µg/ml Methylcobalamin from sample solution, 1 ml of 0.1N sodium hydroxide was added and it was kept at room temperature 2 hours. After that it was neutralized by adding 1 ml of 0.1N HCL & volume was made up to 10 ml with diluent.

For analysis of standard solution 20 µg/ml Epalrestat standard solution and 10 µg/ml Methylcobalamin standard solution kept under base degradation same as sample solution. After making final dilutions the standard and sample solutions were injected into HPLC and the peak area and peak shapes were observed.

Peroxide Degradation

Pipette out 2 ml from 100 µg/ml Epalrestat and 50 µg/ml Methylcobalamin from sample solution, 1 ml of 3% H_2O_2 was added and it was kept at room temperature 3 hours. After that volume was made up to 10 ml with diluent. For analysis of standard solution 20 µg/ml Epalrestat standard solution and 10 µg/ml Methylcobalamin standard solution kept under peroxide degradation same as sample solution. After making final dilutions the standard and sample solutions were injected into HPLC and the peak area and peak shapes were observed.

Thermal Degradation

Tablet powder equivalent to 50 mg of Epalrestat and 0.5 mg of Methylcobalamin was taken and Methylcobalamin (24.5 mg) was added to the powder. It was kept in hot air oven at 60°C for 3 hours. After that powder transferred to the 50 ml volumetric flask and diluted to obtain final concentration of 20 µg/ml of Epalrestat and 10 µg/ml Methylcobalamin. For analysis of standard solution 20 µg/ml Epalrestat standard solution and 10 µg/ml Methylcobalamin standard solution kept under thermal degradation same as sample solution. After making final dilutions the standard and sample solutions were injected into HPLC and the peak area and peak shapes were observed.

Photolytic Degradation

Twenty tablets (EPALRICA-M Tablet) were weighed, powdered. Tablet powder equivalent to 50 mg of Epalrestat and 0.5 mg of Methylcobalamin was taken and Methylcobalamin (24.5 mg) was added to the powder. It was exposed to direct sun light for 1 hour. After that powder transferred to the 50 ml volumetric flask and diluted to obtain final concentration of 20 µg/ml of Epalrestat and 10 µg/ml Methylcobalamin.

For analysis of standard solution 20 µg/ml Epalrestat standard solution and 10 µg/ml Methylcobalamin standard solution kept

under photo degradation same as sample solution. After making final dilutions the standard and sample solutions were injected into HPLC and the peak area and peak shapes were observed.

RESULT

The degradation studies indicates that Epalrestat is more susceptible to acid hydrolysis while Methylcobalamin is more susceptible to photo hydrolysis.

The degradation products were well resolved from the pure drug with significant differences in their retention time values.

% Degradation under various conditions are described in table - 1.

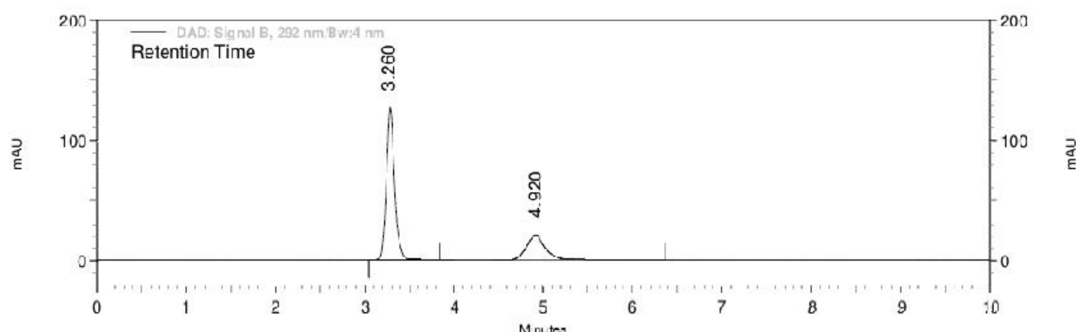

Figure 3 Chromatogram of Standard Mixture

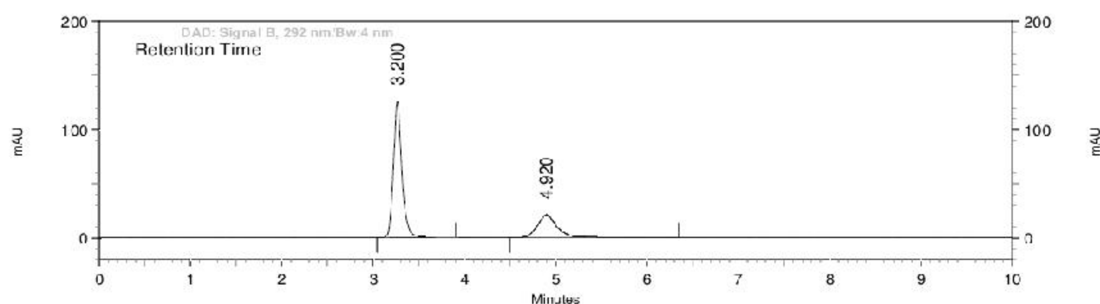

Figure 4 Chromatogram of Sample

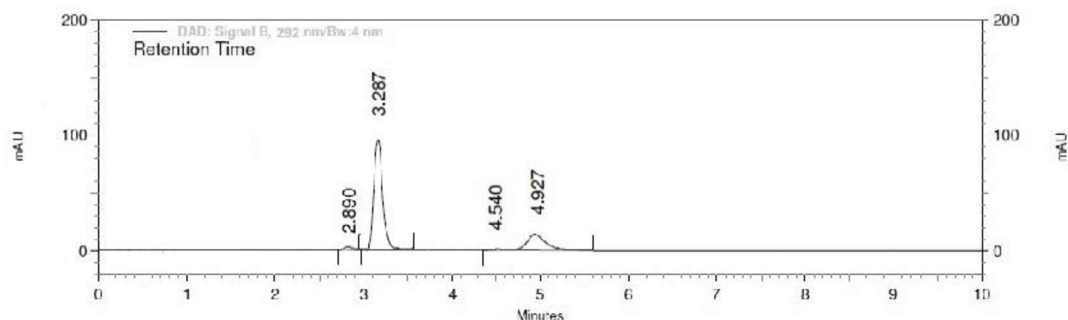

Figure 5 Chromatogram of Sample for Acid Degradation

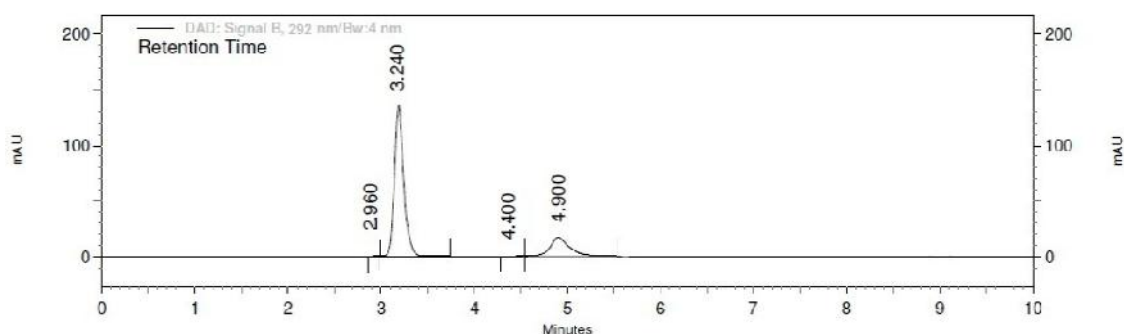

Figure 6 Chromatogram of Sample for Base Degradation

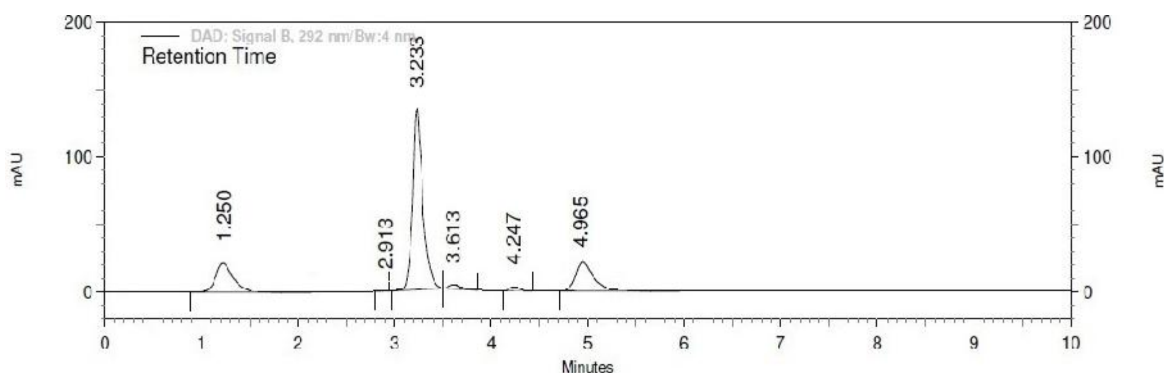

Figure 7 Chromatogram of Sample for Peroxide Degradation

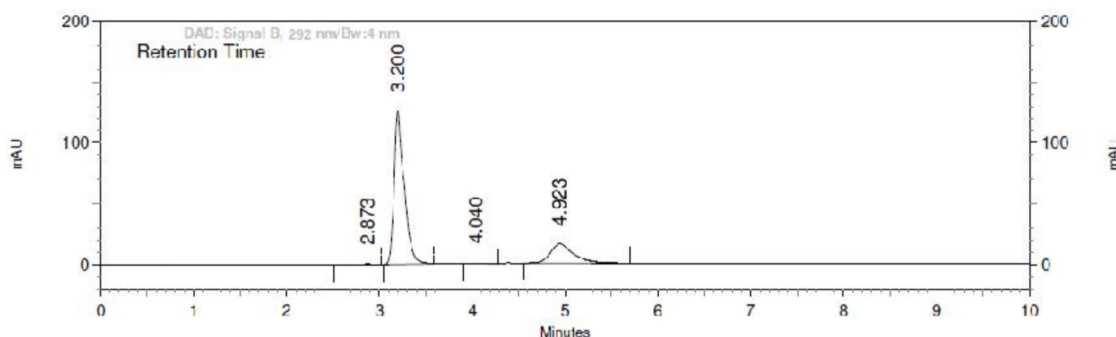

Figure 8 Chromatogram of Sample for Thermal Degradation

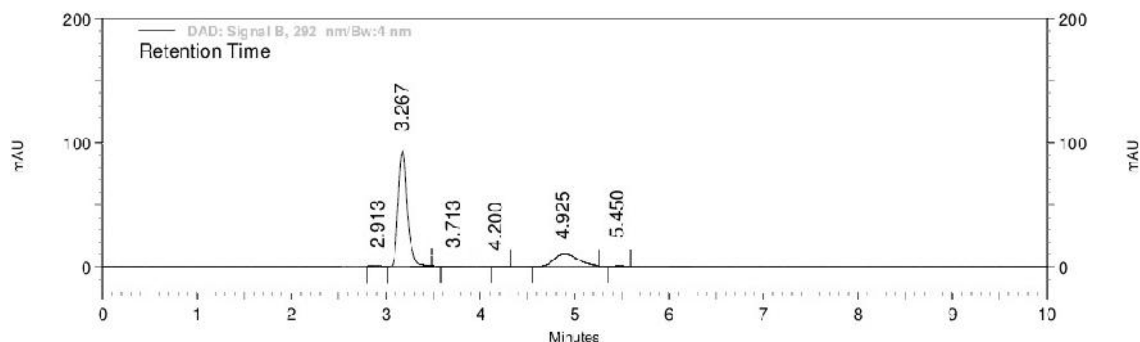

Figure 9 Chromatogram of Sample for Photo Degradation

DISCUSSION

Hence, from the result it can be concluded that the developed HPLC method is simple and rapid as it separates components with good chromatographic criteria. Method has short run time and all degradants are well separated from drug. Moreover, the method is quite sensitive, economic, fast and reliable to qualify as well as to quantify components in microgram quantities.

Here, Specific method gives spectrally pure active peak. Nothing is co-eluted in drug peak and there is no interference in peak purity. The degradants products are separated from the peak of drug with significant difference.

CONCLUSION

The simplicity of the method allows its application in the laboratory for routine quality check as well as for the stability studies for the formulated product. Stability indicating power of method has been proved.

Acknowledgement

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