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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FINGOLIMOD IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The purpose of the investigation was to develop a new RP-HPLC method for the estimation of Fingolimod in pharmaceutical dosage form. Chromatography was carried out on an ODS column (4.6 x 250mm, 5μ particle size) with a isocratic mobile phase composed of Phosphate buffer and acetonitrile (40:60v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 260 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection, limit of quantification, stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention time for Fingolimod was 2.9 min. The relative standard deviation for assay of tablets was found to be less than 2%. The method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of Fingolimod tablets in quality control laboratories and pharmaceutical industries.

1. INTRODUCTION

Fingolimod (INN, trade name Gilenya, Novartis) is an immunomodulating drug, mostly used for treating multiple sclerosis. Fingolimod is a sphingosine-1-phosphate receptor modulator, which sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction. Chemically it is 2-amino-2-[2-(4-octylphenyl)ethyl] propane-1,3-diol. Fingolimod is used in the treatment of the relapsing form of multiple sclerosis. It is derived from myriocin (ISP-1), a metabolite of the fungus Isaria sinclairii. It is a structural analogue of sphingosine and is phosphorylated by sphingosine kinases in the cell (most importantly sphingosine kinase 2).

The molecular biology of phosphofingolimod is thought to lie in its activity at one of the five sphingosine-1-phosphate receptors, S1PR1. It can sequester lymphocytes in lymph nodes, preventing them from moving to the central nervous system for autoimmune responses in multiple sclerosis, and was originally proposed as an antirejection medication indicated after transplantation. It has been reported to

stimulate the repair process of glial cells and precursor cells after injury. Fingolimod has also been reported to be a cannabinoid receptor antagonist, a cPLA2 inhibitor and a ceramide synthase inhibitor.

Various analytical assay methods are reported in the literature for the estimation of Fingolimod. According to literature survey there is no official method for the estimation of Fingolimod by RP-HPLC in capsules dosage forms. Hence, an attempt has been made to develop new method for the estimation and validation of Fingolimod in capsules formulation in accordance with the ICH guidelines.

2. EXPERIMENTAL

2.1 Instrumentation

Chromatography was performed with Alliance Waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and with class Empower-2 software.

2.2 REAGENTS AND CHEMICALS

The reference sample of Fingolimod was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial capsules were purchased from the local pharmacy.

2.3. Preparation of standard stock solution

Accurately Weighed and transferred 5 mg Fingolimod working Standards into 10ml clean dry volumetric flasks separately, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above each stock solution, 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

2.4 Preparation of Working Standard Solutions

Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 & 1.5 ml were pipetted out from the stock solution and transferred into a 10 ml volumetric flask and volume was made up to 10 ml with diluent. This gives the solutions of 12.5, 25, 37.5, 50, 625, 75µg/ml for Fingolimod.

2.5 Sample preparation

5 capsules were weighed and calculate the average weight of each capsule then the capsule powder weight (2.50 mg) equivalent to 0.5 mg of Fingolimod was transferred into a 10ml volumetric flask, 5ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 2ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

2.6 Method validation

Parameters such as systems suitability, Linearity, accuracy, specificity, LOD & LOQ and robustness were performed according to the ICH guidelines.

3. RESULTS AND DISCUSSION

3.1 Method development

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Phosphate buffer and Acetonitrile as mobile phases, in which the drug did not responded properly. The organic content of mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, Buffer and Acetonitrile were taken in isocratic ratio 40:60 and with flow rate of 1.0 mL/min were employed. ODS column (250 mm x 4.6 mm, 5µ) was selected as the stationary phase to reduce the tailing of the peak. 260 nm was selected as the detection wavelength for PDA detector. The retention time was found to about 2.9 min and the results were shown in Table 1 and Figure 2.

3.2 Method Validation

3.2.1 System suitability

A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The analytical method validation was carried out as per ICH method validation guidelines.

3.2.2 Linearity

The linearity range was found in the range of 12.5-75 µg/ml. The response for the drug was linear and the correlation coefficient was found to be 0.9999 and the results are given in Figure 3.

3.2.3 Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as Repeatability and intermediate precision. All the results were with in the limits.

3.2.4 Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Fingolimod in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Fingolimod.

3.2.5 Limit of detection and limit of quantification

A calibration curve was prepared using concentrations in the linearity range (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Fingolimod were 0.04 and 0.12 µg/ml, respectively.

3.2.6 Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard ppm. The solutions were analyzed in triplicate at each level as per the proposed method. Satisfactory recoveries ranging from 98% to 102% were obtained by

the proposed method. This indicates that the proposed method was accurate.

3.2.7 Robustness
Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

3.2.8   Assay
The Content of Fingolimod in the capsules was found by the proposed method. RSD values for Fingolimod are found to be 0.39.

4. CONCLUSION
A new precise accurate and simple HPLC method was developed and validated for the estimation of Fingolimod in capsules dosage form. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of Fingolimod capsules in QC laboratories and industries.

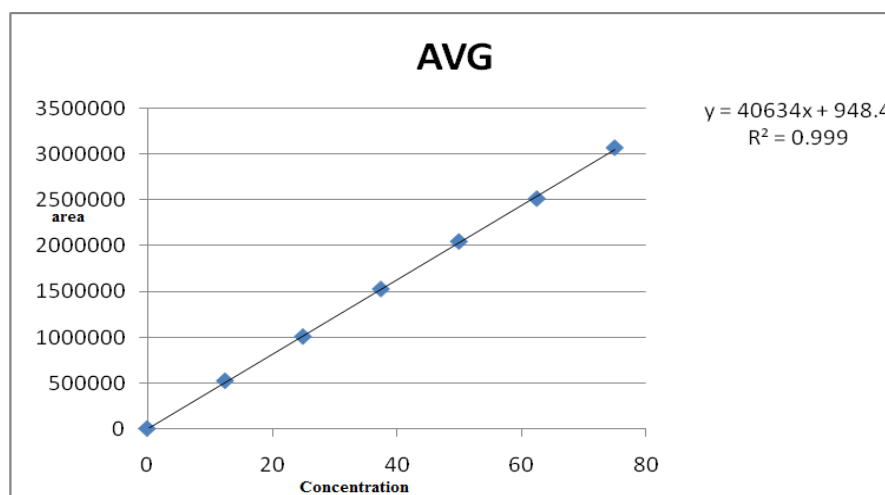**Table 1: Summary of validation parameters**

S. No.	System suitability	Results
1	Linearity range (ppm)	25 - 150 µg/ml
2	Correlation coefficient	0.9999
3	Theoretical plates (N)	5060
4	Tailing factor	1.4
5	LOD (µg/mL)	0.04µg/mL
6	LOQ (µg/mL)	0.12µg/mL
7	Regression Equation	Y=40634x + 948.4
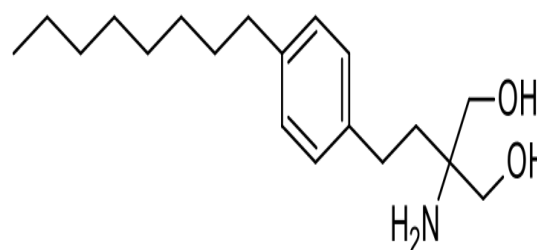
Fig. 1: Structure of Fingolimod
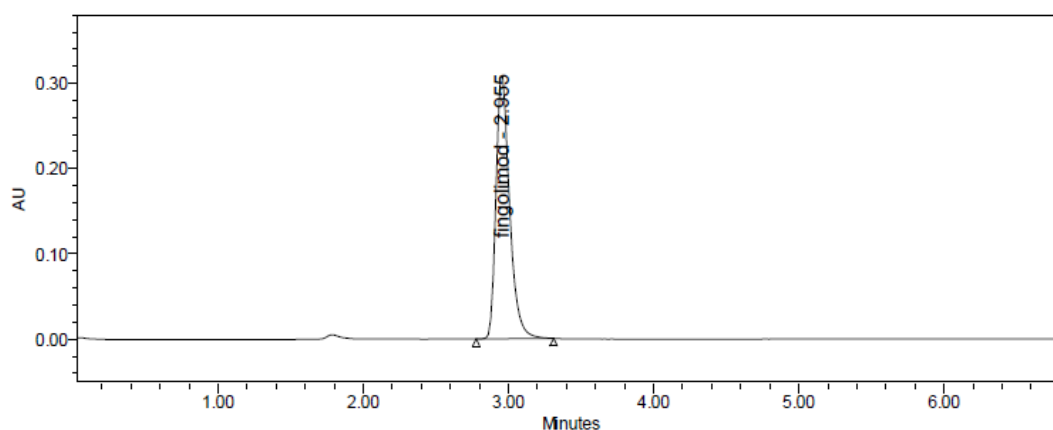
Fig. 2: Chromatogram of Fingolimod Standard

Fig. 3: Linearity curve

$y = 40634x + 948.4$

$R^2 = 0.999$

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