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

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Fingolimod in tablet dosage form.ZobraxEclipseXDB-C18, 150x4.6 mm 5 µm partical size, with mobile phase consisting of water: acetonitrile in the ratio of 60:40 v/v was used. The flow rate was 0.8 ml/min and the effluents were monitored at 215 nm. The retention time& Recovery time was 20.0min&3.273. The detector response was linear in the concentration of 60-360 mcg/ml. The respective linear regression equation being Y= 41219.363x+65555.8. The limit of detection and limit of quantification was 0.15mcg and 0.45mcg/ml respectively. The percentage assay of Fingolimod was 99.72 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Fingolimod in bulk drug and in its pharmaceutical dosage form.

Keywords: Fingolimod, RP-HPLC, Estimation, and Tablets

INTRODUCTION

Fingolimod trade name Gilenya®(Novartis) is an immunomodulating drug, approved for treating multiple sclerosis. It has reduced the rate of relapses in relapsing-remitting multiple sclerosis by over half. Fingolimod is a sphingosine 1-phosphate receptor modulator, which sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction.

EXPERIMENTAL

Instrumentation: Quantitative HPLC was performed on liquid chromatography, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µL, and 2693 pump. ZobraxEclipseXDB-C18, 150x4.6mm was used. The HPLC system was equipped with Empower Software.

Chemicals and solvents: Fingolimod was provided as gift sample byHetro Labs, Hyderabad, India. All the chemicals potassium dihydrogen phosphate*ortho*phosphoric acid were of AR grade and acetonitrile of HPLC grade were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Commercial tablets of Fingolimod were purchased from local market. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

Preparation of phosphate buffer ph 2.5: 2.72 grams of potassium dihydrogen phosphate was accurately weighed into 1000 mL volumetric flask, added about 900 mL of Milli-Q water and sonicated to dissolve and make up to the final volume with Milli-Q water. 1 mL of triethylamine was added and then pH is adjusted to 2.5±0.5 with *ortho*phosphoric acid solution.

Preparation of the mobile phase and diluent: 3. 48gms Di potassium hydrogen *ortho* phosphate(0.02m) in 1000ml of water and by

adjusting the pH to 2.5 with *ortho*phosphoric acid :Acetonitrile(60:40)

Preparation of standard drug solution: A standard stock solution of the drug was prepared by dissolving 300 mg of Fingolimod in 100 ml volumetric flask containing 50 ml of water, sonicated for about 15 min and then made up to 100 ml with water to get 3 mg/ml standard stock solution.

Working standard solution: 5ml of the above stock solution was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 300µg/ml.

Preparation of sample solution: Twenty tablets (Gilenya) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 10mg of the active ingredient, was mixed with 5 ml of water in 10 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding water up 10 ml to obtain a stock solution of 1mg/ml. 3ml of the above stock solution was taken in 10 ml volumetric flask and thereafter made up to 10 ml with mobile phase to get a concentration of 300µg/ml.

Methodology: The HPLC system was stabilized for thirty minutes by passing mobile phase, detector was set at 215 nm, flow rate of 0.8 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six replicates of each standard solutions 60,120,180,240,300&360µg/mL were injected. Calibration graph was plotted by concentration ofFingolimod on X-axis and peak area on Y-axis and linearity curve was shown in Figure 2. The amount of drug present in sample was computed by calibration graph. Chromatographic conditions for estimation of Fingolimod were described in Table 1.

Pharmaceutical formulations: Prepared dilution of Gilenyapharmaceutical formulation0.5mg Novartis is injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph. The assay results in commercial formulations of Fingolimod were described in Table 2.

RESULTS AND DISCUSSION

The objective of the present work is to develop simple, precise and reliable HPLC method for the analysis of Fingolimod in bulk and pharmaceutical dosage forms. This is achieved by using the most commonly employed ZobraxEclipseXDB-C18,150x4.6mm column detection at 215nm. The representative chromatogram indicating Fingolimod is shown in Figure 3.

Parameter fixation: In developing this method, a systemic study of effects of various parameters was under taken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics: Based on nature and solubility characteristics of, reverse phase mode of HPLC was selected Fingolimod for chromatography. Among different RP-HPLC stationary phases tried ZobraxEclipseXDB-C18,150x4.6mmwas found to be optimum

Mobile phase characteristics: In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like acetonitrile with different buffers in different combinations were tested as mobile phase. A mixture oforthophosphoricacid:Acetonitrile buffer in the ratio 60:40 (v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Linearity: Aliquots of standard Fingolimod stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Fingolimod are in the range of 60-360 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 215 nm and a Calibration graph was obtained by plotting peak area versus concentration of Fingolimod (Fig 2).

The plot of peak area of each sample against respective concentration of Fingolimod was found to be linear in the range of 60-360 µg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being Y= 41219.363x+65555.8 The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table I.

Intra-day precision: To study the intra-day precision, six replicate standard solutions (300 ppm) of Fingolimod were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.3 which are well within the acceptable criteria of not more than 2.0.

Inter-day precision: To study the inter-day precision, six replicate standard solutions (300 ppm) of Fingolimod were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.3 which are well within the acceptable criteria of not more than 2.0.

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.

Ruggedness: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC, Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like XDB C18, Hibar C18, Kromasil C18 and Symmetry C18 didn't show any significant change.

Limit of detection and limit of quantification: The detection limit of the method was investigated by injecting standard solutions Fingolimod into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. The limit of detection (LOD) and limit of quantification (LOQ) for Fingolimod were found to be 0.15µg/ml and 0.45 µg/ml respectively.

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 120%, 100% and 80% level of 10 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD was calculated and results are presented in Table 4. Satisfactory recoveries ranging from 84% 86.40% to 112% were obtained by the proposed method. This indicates that the proposed method was accurate.

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

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 results of system suitability parameters were given in Table 5. The analytical method validation was carried out as per ICH method validation guidelines.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the estimation of Fingolimod and can be reliably adopted for routine quality control analysis of Fingolimod in its tablet dosage forms.

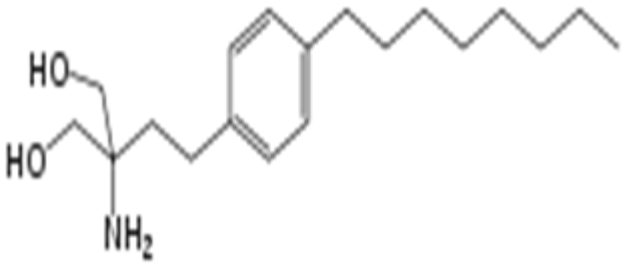

Figure 1: Chemical structure of Fingolimod

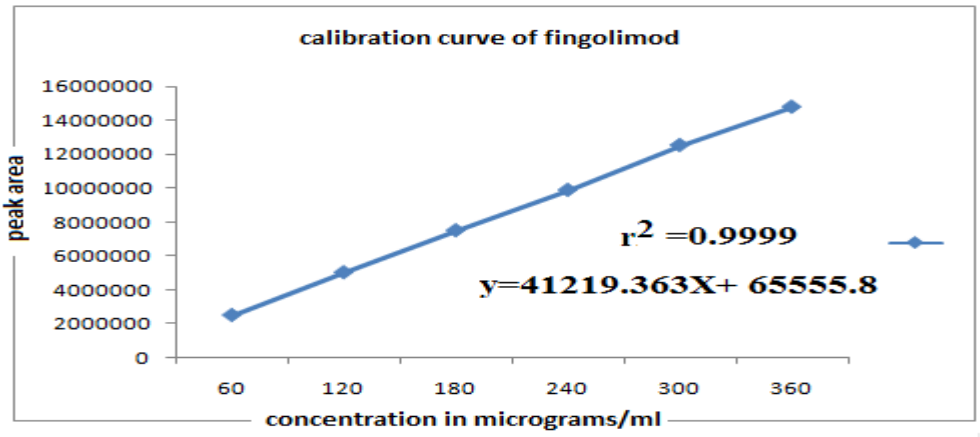

Figure 2: Linearity curve of Fingolimod

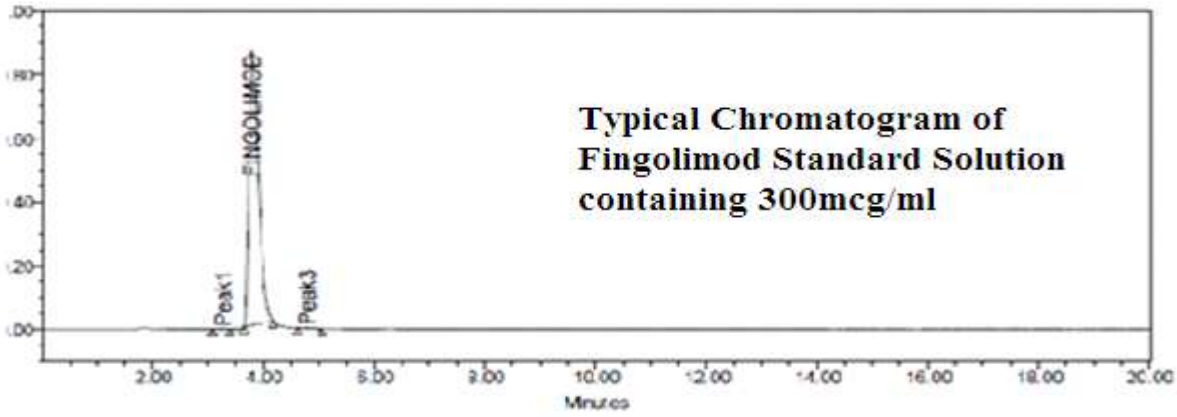

Figure 3: Typical chromatogram of Fingolimod

Tabel 1 : Optimized chromatographic conditions of Fingolimod Parameters:

Table 1: Optimized chromatographic conditions of Fingolimod Parameters	Condition
Mobile phase	orthophosphoricacid:Acetonitrile buffer (60:40 v/v)
pH	2.5
Diluent	Acetonitrile
Column	ZobraxEclipseXDB-C18,150x4.6mm
Column temperature	30°C
Wave length	215 nm
Injection volume	20 µL
Flow rate	0.8 mL/min
Run time	20 min
Retention time	3.796 min

Tabel 2 : Assay results of Fingolimod Formulation:

Table 2: Assay results of Fingolimod Formulation	Label claim	Amount found	%Assay
Gelinya	0.5mg	0.4925	98.5 %

Table 3: Linearity results of Fingolimod

Concentration (µg/mL)	Area
60	2501633
120	5032115
180	7514249
240	9903851
300	12556925
360	14820959

Table 4: Recovery results of Fingolimod

Sample	Amount claim mg/tablet	% found by the proposed method	% recovery
1	0.5	98.82	110
2	0.5	96.42	85.25
3	0.5	88.56	82.24

Table 5: Validation parameters of Fingolimod

Validation Parameter system suitability	Results
Theoretical Plates(N)	2006.6
Tailing factor	1.56
Retention time in minutes	3.787
Resolution Area%	99.87
LOD(mcg/ml)	0.15
LOQ(mcg/ml)	0.45

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