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Development of New Validated Analytical Method for the Estimation of Fingolimod in Bulk and Pharmaceutical Formulations

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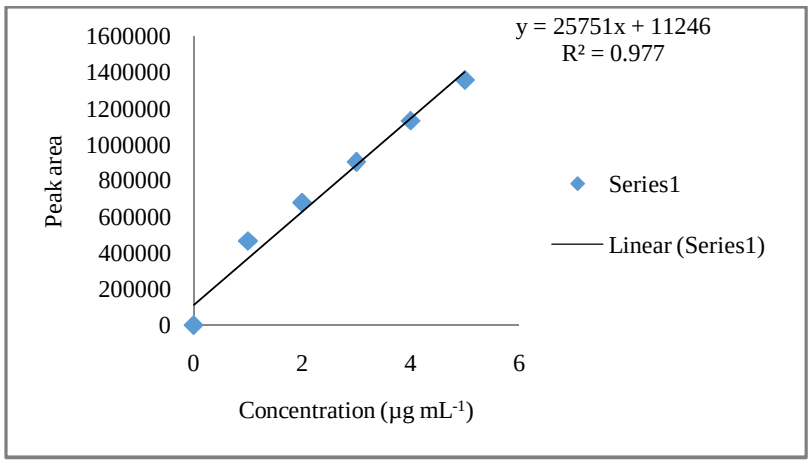
ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of fingolimod in tablet. Chromatographic separation of fingolimod was performed by using a Hypersil ODS column(250×4.6 mm, 5µm) as stationary phase with a mobile phase comprising of Buffer : Methanol 40:60 (v v^{-1}) at a flow rate of 1.2 mL min^{-1} and UV detection wave length at 220 nm and 20 µL sample was injected. The retention time for Fingolimod was 5min. The percentage RSD for precision and accuracy of the method was found to be 0.99%. The limit of detection for Fingolimod was found to be 0.31 µg mL^{-1}. The recovery was calculated by standard addition method. There are few methods developed for the estimation of fingolimod .The proposed method was found to be simple, sensitive and reproducible for the analysis of Fingolimod.

Graphical Abstract



Keywords: Fingolimod, UV detection, Reproducible, Sensitive.

INTRODUCTION

Fingolimod [**1**], chemically designated as 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol an immune modulating drug by functional antagonism sphingosine 1-phospate receptor 1(S1P1). Fingolimod is used for treating multiple sclerosis. It is freely soluble in water and alcohol, also soluble in glycol [**2, 3**]. The molecular weight is 343.93 g mol^{-1} and the empirical formula is $C_{19}H_{33}NO_2.HCL$. Fingolimod is a white powder. There are few methods developed for the estimation of Fingolimod [**4-8**]. The method was developed and evaluated [**9, 10**]. There for the present research work aims to develop a simple, sensitive, accurate and reproducible method.

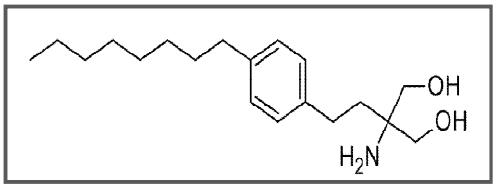


Figure 1. Chemical structure of Fingolimod.

MATERIALS AND METHODS

Chemicals and reagents: Active pharmaceutical ingredient of Fingolimod was obtained.
Reagents used for the Study: Acetonitrile (HPLC grade), Water (Milli-Q grade), ortho phosphoric acid (GR grade), 1-Octane sulfonic acid (GR grade) and Potassium dihydrogen phosphate (GR grade).

Instrumentation and chromatographic conditions: The HPLC (Shimadzu SPD-20A) was used. A column used is Hypersil ODS column (250×4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of Buffer: Methanol 40:60 (v v^{-1}) at a flow rate of 1.2 mL min^{-1} and UV detection wave length at 220 nm and 20 µL sample was injected.

Preparation of standard stock solution: 5mg of Fingolimod standard was accurately weighed and transferred into 250 mL volumetric flask, dissolved and diluted to volume with diluent and mixed well.

Preparation of sample solution: The contents of 10 capsules,equivalent to 5 mg of Fingolimod were accurately weighed and transferred into a 250 mL volumetric flask.160 mL of diluent was added and shaken for 10 min on orbital shaker and sonicated for 20 min with occasional shaking. The solution was cooled to room temperature and diluted to volume with diluent and mixed well. The resulting solution was filtered through 0.45 µm nylon filter.

Preparation of Mobile phase
Buffer preparation: Dissolve accurately 4.4g of dipotassium hydrogen phosphate, 4.4 g of potassium dihydrogen phosphate in 1 Liter of Milli-Q water, adjust the pH to 5.4 with orthophosphoric acid.

Mobile phase preparation: Prepare a filtered and degassed mixture of 40 volumes of the above buffer and 60volumes of Methanol.

RESULTS AND DISCUSSION

Validation of proposed method: The proposed method for the assay of Fingolimod from capsules was subjected to validation to check its suitability for routine analysis.

System Suitability: Prepare Fingolimod standard solution as per test procedure and make 5 injections of standard preparation and establish system suitability as per methodology (Figures 2-4).

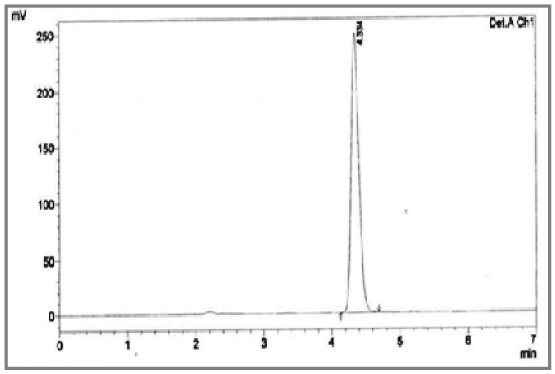


Figure 2. Chromatogram of Standard.

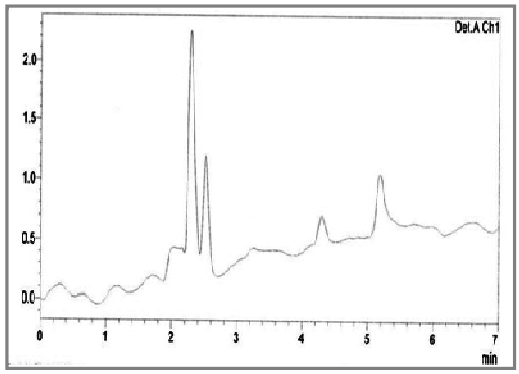


Figure 3. Chromatogram of Blank.

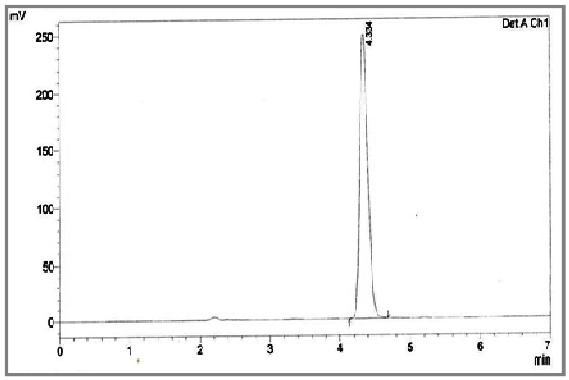


Figure 4. Chromatogram of Sample.

Specificity: Evaluated the interference of blank, placebo with the analyte peak as per the methodology and the results are given below.

Fingolimod identification: Inject the Fingolimod standard solution and tabulate the results.

Acceptance criteria: Chromatogram of Fingolimod should show peak at the retention time of analyte peak.

Observation: Fingolimod peaks were observed at their respective retention times of analyte peaks.

Blank interference: Inject the blank solution.

Acceptance criteria: Chromatogram of blank should not show any peak at the retention time of analyte peak.

Observation: No peak was found.

Placebo interference: Perform the assay on placebo equivalent to the amount that is present in the portion of test preparation

Acceptance criteria: Chromatogram should not show any peak at the retention time of analyte peak.

Observation: No peak was found.

Linearity and Range: To demonstrate linearity of detector response of assay method, inject seven standard solutions with concentration ranging from 1-5 µg mL^{-1} of the test concentration. Plot a graph to concentration versus peak area (Figure 5).

Acceptance criteria: Coefficient of correlation (r) should be not less than 0.999.

Observation: Coefficient of correlation (r) was 0.996 for Fingolimod.

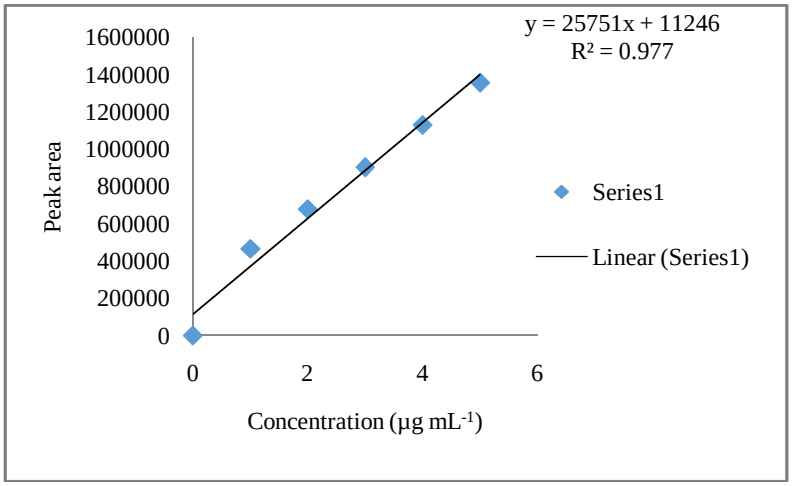


Figure 5. Calibration curve of Fingolimod

Precision

Repeatability: Determine the precision of test method by preparing six test preparations using the product blend by mixing the active ingredient with excipients as per manufacturing formula. Calculate relative standard deviation of assay results (Table 1).

Acceptance criteria: Relative standard deviation of % assay results should be not more than 2.0%the assay of Fingolimod tablets should be not less than 98% and not more than 102%.

Observation: Determine the precision of test method by preparing six test preparations using the product blend by mixing the active ingredient with excipients as per manufacturing formula. Calculate relative standard deviation of assay results.

Acceptance criteria: Relative standard deviation of % assay results should be not more than 2.0%. The assay of Fingolimod tablets should be not less than 98% and not more than 102%.

Table 1. Repeatability of Assay

Sample. No	Area	Amount %
1	1049573	98.7
2	1147014	101.2
3	1098293	98.7
4	1143934	100.9
5	1093453	98.1
6	1072785	98.9
	Mean	99.4
	SD	1.30
	%RSD	1.31

Intermediate precision: To demonstrate intermediate precision of assay method, conduct analyst to analyst variability or along with system to system or column to column variability study.

Acceptance criteria: Relative standard deviation of % assay results should be not more than 2.0% by both the analysts. The assay of Fingolimod tablets should be not less than 98% and not more than 102%

Accuracy: T o validate the accuracy in this method, accurately quantify Fingolimod tablets content at 50%, 100%, 150% and perform assay in triplicate (Table 2).

Acceptance criteria: The mean percent recovery of Fingolimod at each spike level should be not less than 98% and not more than 102%

Table 2. Accuracy

S.No.	Concentration (%)	Area	Amount added mcg mL^{-1}	Amount found $mcgmL^{-1}$	% Recovery	Mean
1	50%	465449	50	50	100	
2	50%	499449	50	53.65	107.3	102.46
3	50%	465889	50	50.04	100.08	
4	100%	904209	100	100	100	
5	100%	904909	100	100.07	100.07	103.7
6	100%	1004209	100	111.05	111.05	
7	150%	1357563	150	150	100	
8	150%	1467563	150	162.15	108.1	101
9	150%	1289563	150	142.48	94.9	

Ruggedness

System to system variability: To demonstrate ruggedness of assay method, carry out system to system variability on two HPLC systems.

Acceptance criteria: Relative standard deviation of % assay results should be not more than 2.0% by both the analysts. The assay of fingolimod tablets should be not less than 98% and not more than 102%

Robustness

Effect of variations: To demonstrate the robustness of test method, check the system suitability parameters by injecting system suitability preparation into HPLC system to pass all the following conditions (Table 3).

Table 3. Robustness

Parameter	% RSD	Tailing factor	Theoritical plates	%Assay difference
Limits	NMT 2.0	NMT 2.0	NLT 2000	±2.0
Flow rate(1.4mL/min)	0.2	1.0	8339	0.1
Flow rate(1.0mL/min)	0.1	1.1	11483	-0.1
Column temp.($40^{o}C$)	0.2	1.0	10075	-0.2
Column temp.($30^{o}C$)	0.1	0.9	7877	-0.1
Organic phase (+5%)	0.1	1.1	13163	0.2
Organic phase (-5%)	0.4	1.3	12401	0.1
P^{H}(+0.2)	0.5	1.3	12657	-0.4
P^{H}(-0.2)	0.1	1.3	12370	0.3

Limit of Detection (LOD): It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

$$LOD = 3.3 \times \sigma / S$$

σ= the standard deviation of y-intercepts of regression line, S = the slope of the linearity curve.

LOD = 0.31 µg mL^{-1}

Limit of Quantification (LOQ): The quantization limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$$LOQ = 10 \times \sigma / S$$

LOQ = 0.94 µg mL^{-1}

The system suitability and system precision are evaluated and found within the limits. A plot is drawn between concentration of component and instrument response. It is found to be linear in the concentration range of 1-5 µg mL^{-1}. Precision and accuracy from the developed method are expressed in 1.3 and 101 respectively. All the system parameters are found to be within the limits.

APPLICATION

The method developed was successfully applied to marketed dosage form and useful for routine analysis.

CONCLUSION

A simple, specific, accurate, and precise RP-HPLC method has been developed and validated for the estimation of Fingolimod. The chromatographic separation was achieved on Hypersil ODS, 250 × 4.6 mm, 5µ, using buffer, methanol (40:60). The correlation coefficient for RP-HPLC method was found to be 0.997. The linearity range was found between 1-5 µg mL^{-1}.

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