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Research Article

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Development of stability indicating RP-HPLC method for the estimation of Fingolimod in its bulk dosages form as per ICH guideline

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ABSTRACT

An accurate, precise, rapid & economical RP-HPLC method was developed & validated for the estimation of Fingolimod in pharmaceutical dosage forms, using UV detector. Elution was carried out using a mobile phase consisting of Buffer : Water (60 : 40) and flow rate was set on 1.2 ml / min at 319 nm wave length, retention time for Fingolimod was found to be 3.329 min. The method was found to be linear within the range of 40-120 µg/ml. In the linearity study, regression equation and correlation coefficient was found to be y=14744x and 0.999 respectively. This method was Rugged and Robust in different testing criteria, LOD and LOQ was found to be 0.005 µg / ml & 0.17 µg / ml respectively. Accuracy study was done in 3 different concentration level i.e 50, 100, 150% & % recovery of the method was found to be 99.7%, 99.8%, 99.9% respectively in 3 different levels & mean recovery was 99.8 %, so method was accurate. Results of all validation parameter were within the limits as per ICH guideline.

Key words: Fingolimod, Validation, Method development, HPLC, ICH.

INTRODUCTION

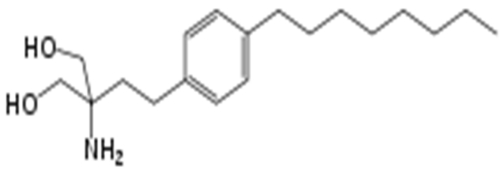

Figure no 1: Shows Chemical structure of Fingolimod

Fingolimod [1] Chemically designated as 2-amino-2-[2-(4-octylphenyl) ethyl] propane-1,3-diol, is a novel oral drug used for the treatment of relapsing-re-emitting multiple sclerosis(RRMS) [2] shown in **Figure no 1.** [3].

According to the information collected from literature there is no method reported for the determination of Fingolimod [4-7] in HPLC. In the present work, we have therefore focused to achieve the optimum chromatographic conditions for the determination of Fingolimod in bulk dosages form. We have described a simple, sensitive and validated HPLC method with total run time less than 6 minutes for the determination of Fingolimod as per ICH guideline [8].The developed method can be applied successfully for quality control and other analytical purposes. The objective or need of the proposed method is to develop simple and accurate methods for the determination of Artemether by RP-HPLC methods in pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Chemicals and reagents: Water for HPLC-milli-Q grade (Merck), Potassium hydrogen phosphate (Merck), Hcl (Grade LR, Finar Chemical Limited), NaOH (Grade LR, SD Fine-Chemical Limited), H_2O_2 (Alpha Pharma Limited).

Apparatus: pH meter (Labindia-pH Analyser), Sonicator (Analytical Technologies Limited- Ultrasonic cleaner), Weighing machine (Afcoset er-200A)

Instruments: HPLC - (Waters, PDA – 2695), UV/VIS spectrophotometer (LABINDIA UV 3200) Column: Phenyl (4.6 x 250mm, 5µm, Make: Agilent), Buffer pH: 7.4, Mobile phase: Buffer : Water(60:40), Flow rate: 1.2ml per min, Pipettes and Burettes Borosil.

Preparation of standard solution: Accurately weighed 10 mg of Fingolimod standard was transfered into a 25 ml volumetric flask and about 10 ml of diluent was added, sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 5 ml of the above stock solution was pipetted into a 25ml volumetric flask and diluted up to the mark with diluents. Filtered through 0.45µm filter.

Preparation of sample solution: Accurately weighed 10 mg of Fingolimod sample was transferred into a 25 ml volumetric flask. 10 ml of diluent was added and sonicated to dissolve it completely and made volume up to the mark with diluent. Further 5 ml of the above stock solution was pipetted into a 25ml volumetric flask and diluted up to the mark with diluent. Filtered through 0.45 µm filter.

Chromatographic conditions:

Column	:	Phenyl (4.6 x 250mm, 5µm, Make: Agilent)
Buffer pH	:	7.4
Mobile phase	:	Buffer : Water (60:40)
Flow rate	:	1.2 ml per min
λ_{max}	:	319 nm

Preparation of Phosphate buffer: Accurately weighted 1.44 grams of K_2HPO_4 was taken in a 1000 ml volumetric flask, dissolved and diluted up to the mark with HPLC water and the volume was adjusted to pH 7.4 with Orthophosphoric acid.

Method Validation:
The suggested analytical method was validated according to international guidelines with respect to following parameters such as, precision, accuracy, linearity, robustness, ruggedness, LOD and LOQ.

Precision: Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility). In order to determine injection repeatability, samples spiked with 5 ml of Fingolimod were injected 6 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and % RSD calculated from the data obtained.

Accuracy: Accuracy was determined in terms of percent recovery Sample solution spiked with the analytes at three different concentration levels 50,100,150 µg/ml of Fingolimod. Another set of standard mixtures at the same concentration levels was also prepared with the diluents. Sample and standard solutions are injected into the HPLC system in triplicate. Percentage recovery of Fingolimod was calculated.

Linearity: The linearity of the method was established by spiking a series of standard of Fingolimod (40-120 µg/ml). Above solutions were injected onto the HPLC system. Calibration curves for standard solutions was constructed by plotting their response (peak area of the analytes) against their respective concentrations. Linear regression was applied and slope (a), intercept (b), correlation coefficient (r) and standard error (Es) were determined.

Limit of detection & Limit of quantification: Detection and quantification limits were determined through dilution method using Signal/Noise approach by injecting a 10µl sample. LOD was considered as the minimum concentration with a signal to noise ratio of at least three (S/N˜3), while LOQ was taken as a minimum concentration with a signal to noise ratio of at least ten (S/N˜10).

Robustness: The robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in organic composition.

Ruggedness: The ruggedness of the method was investigated by evaluating the influence of different analyst, different time intervals.

Degradation studies: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Fingolimod using the proposed method.

Stock Solution Preparation: Accurately weighed and transferred 10 mg Fingolimod sample into a 25ml dry volumetric flask, diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Hydrolytic degradation under acidic condition: 5ml of the above stock solution, 3 ml of 0.1N HCl were added in 25 ml volumetric flask. Then, the above solution was sonicated for 30min and then neutralized with 0.1 N NaOH and made the volume to 25ml with diluent and the solution was filtered with 0.45 microns syringe and placed in vials.

Hydrolytic degradation under alkaline condition: 5ml of the above stock solution, 3 ml of 0.1N NaOH were added in 25 ml of volumetric flask. Then, the above solution was sonicated for 30min and then neutralized with 0.1 N HCL and made the volume up to 25ml with diluent and the solution was filtered with 0.45 microns syringe filters and placed in vials.

Oxidative degradation: 5 ml of the above stock solution, 1 ml of 3 % w/v of hydrogen peroxide added in 25 ml of volumetric flask. Then above solution was sonicated for 30minutes and the volume was made up to the mark with diluents and the solution was filtered with 0.45 microns syringe filters and placed in vials.

Heat induced degradation: 10mg of the Fingolimod standard was weighed and placed in an oven at 105^0c for 6hrs. Then it is taken in a 25ml volumetric flask and the volume was made up to the mark with the diluents. 5ml of the above stock solution was pipetted in a 25ml volumetric flask and the volume was made with diluent and the solution was filtered with 0.45 microns syringe filters and placed in vials.

Sunlight induced degradation: 10mg of the Fingolimod standard was weighed and is exposed to sunlight for about 55hrs and transferred in to 25ml volumetric flask and the volume was made up to the mark with the diluent. Further 5ml of the above solution was pipetted into a 25ml volumetric flask and the volume was made with diluent. The solution was filtered with 0.45 microns syringe filters and placed in vials.

RESULTS AND DISCUSSION

Mobile Phase Preparation: Initially the mobile phase tried was Methanol: Water. Then tried with Water: Phosphate buffer in varying proportions. Finally, the mobile phase was tried with di potassium hydrogen orthophosphate buffer (pH 7.4) and water in proportion 60:40v/v respectively and then it was optimized shown in Figure 2.

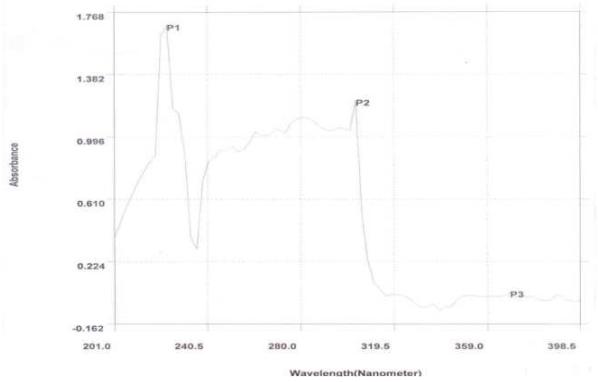

Figure no: 2. Shows spectrum for standard of Fingolimod

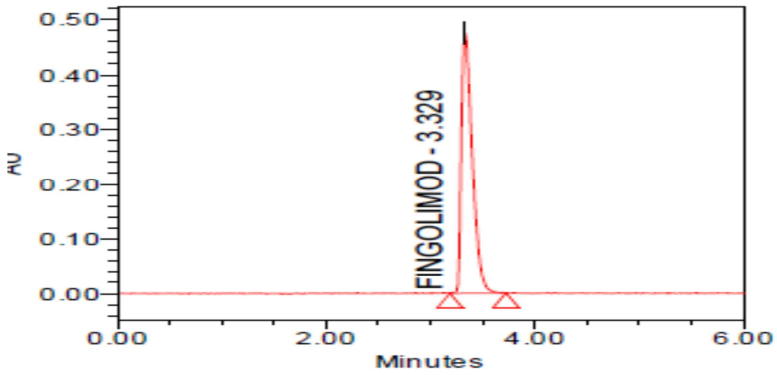

Figure no 3: Shows Chromatogram for Fingolimod in pure form

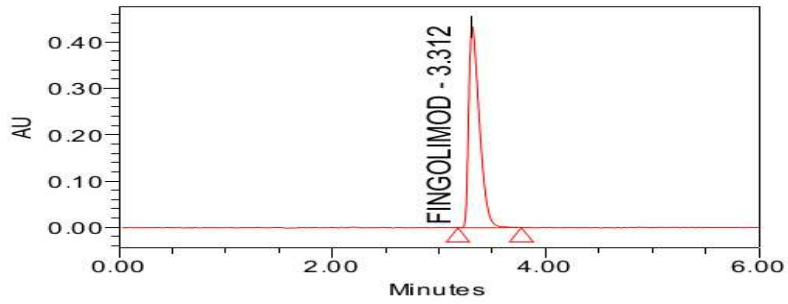

Figure no 4: Shows Sample Chromatogram for Fingolimod

Table no 1: Shows Results for Chromatogram for Fingolimod in pure form

Retention Time	Area	Height	USP Tailing	USP Plate Count
3.329	1464631	231124	1.53	6638

Method validation:

Precision: Precision data representing both repeatability (injection and analysis) and intermediate precision (different analyst) are summarized in **Table no. 2, 3, 4** respectively.

The %RSD values for both Precession & ID Precision were less than 2.0%, which indicates that the proposed method is precise.

Table no 2: Shows Results of Method precision for Fingolimod

S.no	Sample name	RT	Area
1	Precision 1	3.291	1472093
2	Precision 2	3.287	1475017
3	Precision 3	3.288	1473196
4	Precision 4	3.290	1476368
5	Precision 5	3.290	1477460
6	Precision 6	3.291	1470611
Mean			1474124
S.D			2618.748
%R.S.D			0.18

Table no 3: Shows Results of System precision for Fingolimod:

S.no	Sample Name	RT	Area
1	Precision 1	3.359	1460340
2	Precision 2	3.353	1466503
3	Precision 3	3.343	1466796
4	Precision 4	3.335	1462377
5	Precision 5	3.332	1463497
6	Precision 6	3.329	1464631
Mean			1464024
SD			2480.325
%RSD			0.16

Table no: 4 Shows Results of Intermediate precision for Fingolimod

S. No	Sample name	RT	Area
1	Precision 1	3.290	1473165
2	Precision 2	3.287	1475517
3	Precision 3	3.288	1473216
4	Precision 4	3.291	1477368
5	Precision 5	3.288	1475461
6	Precision 6	3.287	1474611
Mean			1475889
S.D			2806.115
%R.S.D			0.19

Accuracy: Average recoveries of Fingolimod are 100.03%, 99.8%, 99.3%, at 50%,100% & 150% concentrations level respectively. The percentage recoveries of the drug is within the limits 99-101%. So the method is accurate, accuracy data for Fingolimod are presented in **Table no. 5**.

Table no 5: Shows Accuracy (recovery) data for Fingolimod

% level	Sample Area	µg/ml added	µg/ml found	%Recovery	%mean
50%	730548	40	39.92	99.8	99.7
50%	731463	40	39.97	99.9	
50%	730232	40	39.87	99.7	
50%	730548	40	39.92	99.8	
50%	731829	40	39.98	99.5	
50%	731646	40	39.98	99.6	
100%	1461279	80	79.85	99.8	99.8
100%	1462743	80	79.93	99.9	
100%	1460181	80	79.79	99.7	
150%	2194023	120	119.89	99.6	99.9
150%	2198781	120	119.98	99.9	
150%	2194572	120	119.96	99.6	
150%	2196585	120	119.95	99.8	
150%	2195121	120	119.95	99.8	
150%	2197500	120	119.97	99.9	

Linearity:
The response was found linear over a concentration range of 40-120 µg/mL of Fingolimod.

The correlation co-efficient were found to be 0.998 for Fingolimod So the method is linear, data is presented in **Table no. 6,** Linearity graph of Fingolimod is given in **Figure no. 5**

Table no 6: Shows Linearity results for Fingolimod

s.no	Sample Name	Concentration in µg/ml	RT	Area
1	Linearity50%	40	3.370	737936
2	Linearity75%	60	3.349	1103009
3	Linearity100%	80	3.335	1474027
4	Linearity125%	100	3.322	1842896
5	Linearity150%	120	3.307	2211006

Limit of detection: The LOD for Fingolimod standard solutions were found to be 0.26 µg/ml given in **Figure no. 6.**

Limit of quantification: The LOQ Fingolimod standard solution was found to be 0.81 µg/ml given in **Figure no. 7.**

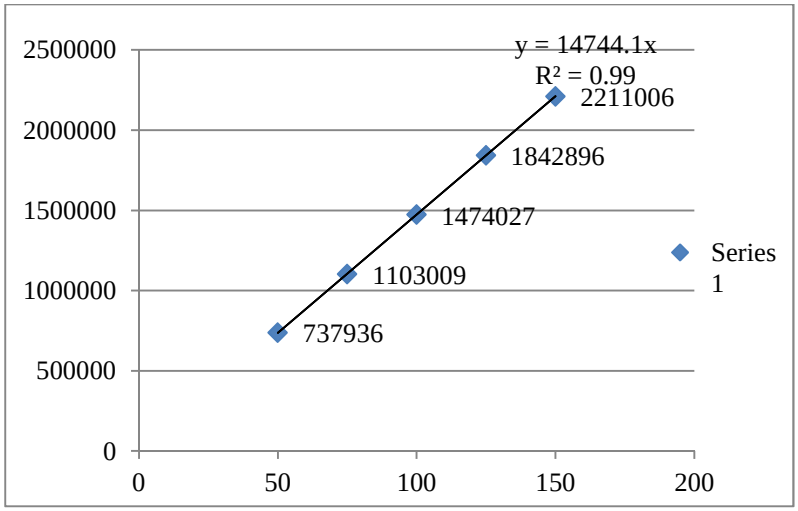

Figure no 5: Shows Calibration graph for Fingolimod

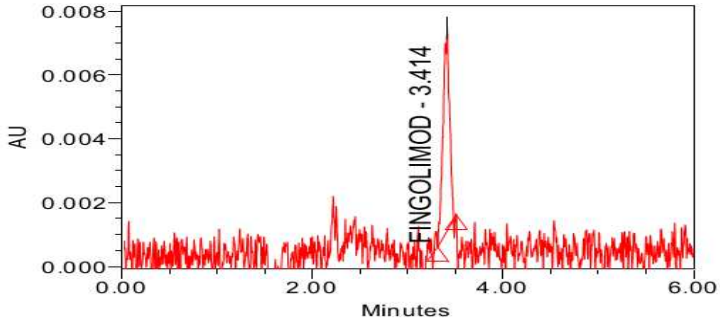

Figure no 6: Shows LOD Chromatogram of Fingolimod

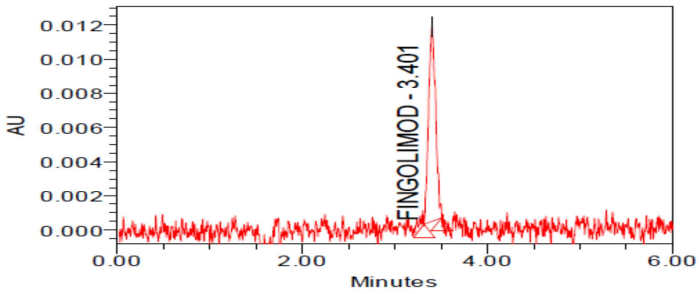

Figure no: 7 Shows LOQ Chromatogram of Fingolimod

Robustness: Minor deliberate changes in different experimental parameters such as flow rate (±5%) and wavelength (±5units) did not significantly affect the retention time & peak area of Rilpivirine indicating that the proposed method is robust which is mentioned in **Table no. 7 & 8.**

Table no 7: Shows Results of effect of flow rate

Flow	RT	Area	USP Tailing	USP Plate count
1ml/min	4.178	1843975	1.613	7039
1.4ml/min	2.805	1188838	1.550	6289

Table no 8: Shows Results of effect of temperature

Temperature	RT	Area	USP Tailing	USP Plate count
Temperature 1	3.343	1743825	1.528	6680
Temperature 2	3.323	1448613	1.576	6686

Forced degradation studies

Table no 9: Shows results of forced degradation studies

Degradation studies	Sample area	% Assay
Acid degradation	1305909	89.2
Base degradation	1368862	93.5
Peroxide degradation	1266381	86.5
Temperature degradation	1405756	96.02
Sunlight degradation	1428887	97.6

Table no 10: Shows Validation summary for Fingolimod

S.NO	Parameter	Acceptance criteria	HPLC
1	Linearity range((µg/ml)	-	40-120(µg/ml)
2	Correlation coefficient	NLT 0.999	0.999
3	No. of Theoretical plates	NLT 2500	6638
4	Method precision	%RSD (NMT 2%)	0.18
5	System precision	%RSD (NMT 2%)	0.16
6	Intermediate precision	%RSD (NMT 2%)	0.19
7	% recovery	98-102%	99.8 %
8	LOD	-	0.26(µg/ml)
9	LOQ	-	0.81(µg/ml)

CONCLUSION

Method development & validation of Fingolimod was done by RP-HPLC method. The estimation was done by using Phenyl C_{18} (4.6 x 150 mm, 5µm, Make: Aligant). Mobile phase was used as Buffer & Water in (60:40) ratio at a flow rate 1.2 ml/min, retaintion time was 3.329 min. at λ_{max} 319 nm. The linearity range of Fingolimod was found to be within 40-120 µg/ml. Mean recovery was 99.8 %, which is within 98-102%. Correlation coefficient value was 0.999, % RSD was 0.18 % which is within the limit. These results show the method is accurate, precise, sensitive, economic & rugged. The HPLC method is more rapid. The proposed method can be successfully applied to estimate bulk drug & Tablet dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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