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Original Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR QUANTIFICATION OF FINGOLIMOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, stability indicating RP-HPLC method was developed and validated for the assay determination of Fingolimod Hydrochloride in bulk drug and dosage form. LC separation was achieved gradient mode on a XBridge C18 (4.6x150) mm, 5 µm column using mobile phase containing solution A (0.1% perchloric acid) solution B(acetonitrile) at flow rate 0.8 ml/min. The detection wavelength was 220 nm and temperature was 40˚c. The retention time was 9.30 min and linearity was observed in the concentration range of 20-150 µg/ml with correlation coefficient of 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The method was successfully validated as per ICH guidelines. Fingolimod undergoes degradation under acidic, basic, oxidation, dry heat and photolytic conditions, degradation impurities did not interfere with the retention time of fingolimod, and assay method is thus stability indicating.

Key words: Fingolimod,validation,HPLC,Stability indicating.

INTRODUCTION

Fingolimod is an immunomodulating drug, approved for treating multiple sclerosis. It has reduced the rate of relapses in relapsing-remitting multiple sclerosis by over half, but has serious adverse effects. Fingolimod is a sphingosine 1-phosphate receptor modulator, which sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction (10-11). Multiple sclerosis is the most common chronic inflammatory disease of the central nervous system and it is the leading cause of neurological disability in young adults with the prevalence rate of about 2.5 million people worldwide. The latest treatment option for multiple sclerosis is Fingolimod Hydrochloride which is the first oral drug approved by the USFDA. Fingolimod is an immunomodulator which is derived from myriocin, a metabolite isolated from the ascomycete *Isaria sinclairii*, which is a fungus used in traditional Chinese herbal medicine. Chemically, Fingolimod Hydrochloride is 2-amino-2-[2-(4-octylphenyl) ethyl] propane-1, 3-diol hydrochloride with molecular formula $C_{19}H_{33}NO_2$ HCl and chemical structure as shown below figure 1.

Fingolimod Hydrochloride is available as capsule at the dose of 0.5 mg in the market under the brand name of Gilenya.

Literature survey reveals that formulation evolution of fingolimod capsules and drug metabolism and deposition. No reports were found for the stability indicating HPLC assay method for fingolimod in bulk drug and pharmaceutical dosage form.

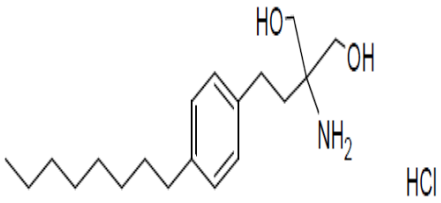


Fig.1.Chemical structure of Fingolimod Hydrochloride

EXPERIMENTAL

Chemicals & Reagents

Fingolimod is available as tablets with brand name Gilenya was purchased from local market, containing Fingolimod 0.5mg. HPLC acetonitrile, methanol, Phosphoric acid and perchloric acid were purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic Conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 0.1% perchloric acid in water (1 ml of perchloric acid in 1000 ml of water). A Waters XBridge C18 (4.6x150) mm, 5 µm column and gradient mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The gradient program (T/%B) was fixed as 0/50, 10/80, 15/80,15.2/50,20/50. Water: methanol: Phosphoric acid (30:70:0.1) (%v/v) used as diluent. The column oven maintained at 40˚c with 0.8ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 220 nm.

Preparation of Stock and standard solutions

Accurately 10mg of Fingolimod standard dissolved in 50ml diluent to get a concentration of 200µg/ml. Further 10ml of stock solution was taken in 20ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml.

Preparation of Tablets for assay

20 tablets of Gilenya were powdered and an amount of powder equivalent to 10mg of drug was weighed and transferred to the 50ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 10ml of this solution was taken into 20 ml flask and diluted volume with diluent to get concentration 100µg/ml.

Forced Degradation studies/Specificity

Forced degradation studies were performed to evaluate the stability indicating properties. All solutions for used in stress studies were prepared at an initial concentration of 200µg/ml of Fingolimod Hydrochloride.

Acid Degradation studies

Acid decomposition was carried out in 1N HCL at concentration of 200µg/ml fingolimod hydrochloride and after refluxation for 7days at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (g). The results are tabulated in table 3.

Alkali Degradation studies

Base decomposition was carried out in 0.5N NaOH at concentration of 200µg/ml fingolimod hydrochloride and after refluxation for 7days at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (i). The results are tabulated in table 3.

Oxidation

Oxidation was conducted by using 15%H2O2 solution at room temperature.

After 7days 10ml of solution was taken in 20ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml filtered and injected. The resulting chromatogram is shown in fig.3(k). The results are tabulated in table 3.

Temperature Stress studies

1g of Fingolimod Hydrochloride sample was taken into a petridish and kept in oven at 80˚c for 7 days. 10mg of sample was taken into 50 ml flask diluted volume with diluent, further 10ml to 20ml made up with diluent. The results are tabulated in table 3.

Photo stability

1g of fingolimod was taken in to a petridish and kept in photo stability chamber 200 W.hr/m^2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 10mg of sample was taken in 50ml flask, dissolved in diluent, further 10ml in 20ml flask diluted volume with diluent. The results are tabulated in table 3.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

To develop a rugged and suitable HPLC assay method for the determination of Fingolimod, the analytical condition were selected after the consideration of different parameters such as diluents, buffer, organic solvent for mobile phase, column and other chromatographic conditions [6,7,8]. Initial trails were performed with different composition of buffer (phosphate, acetate and formate) and organic phase (methanol, teterhydrofuran), but fingolimod peak shape was not good. Finally o.1% perchloric acid and acetonitrile with gradient was optimized. Different diluents were tried to dilute sample like water, acetonitrile, tetrahydrofuran and mixture of water: acetonitrile and water: teterhydrofuran fingolimod was not dissolved, finally (water: methanol: phosphoric acid) (30:70:0.1) was optimized. The detection wavelength was chosen as 220nm for fingolimod because they have better absorption and sensitivity at this wavelength (fig-2). Hence selected method was best among the all trails by many aspects.

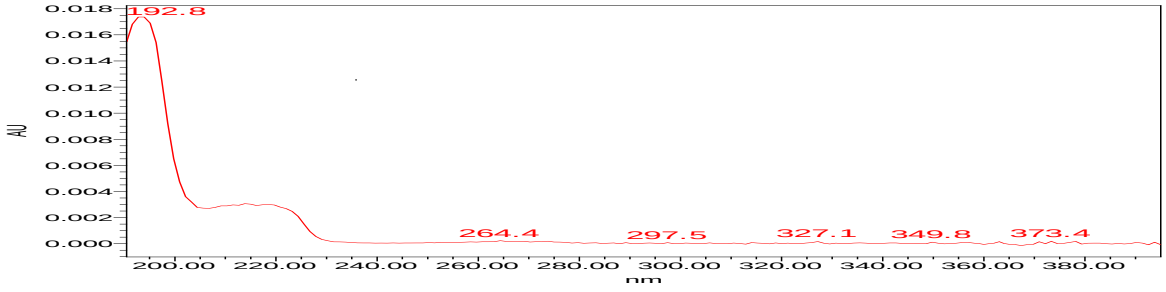


Fig.2.wavelength spectrum of Fingolimod

Method Validation

After method development, the validation of the current method was established as per the guidelines of ICH (3, 4, 9) and USP (5).

Precision

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Intermediate precision of the analytical method was determined by performing method precision on another day and another analyst under same experiment condition. The result obtained for method precision and intermediate precision are shown in table 5. The percentage of RSD was calculated. The %RSD range was obtained as 0.14 and 0.49 for method precision and intermediate precision respectively (Table 5) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was assessed by determination of recovery for three concentrations (corresponding to 50,100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Fingolimod were calculated, the results obtained are shown in table 2. The percentage recovery was found to be 99.64-100.04% with %RSD 0.09 - 0.14(<2.0%) indicating that the method is more accurate (table 2)

LOD and LOQ

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.04µg/ml and 0.11µg/ml respectively. The %RSD value was noticed to be less than 2.0% at LOQ concentration level.

Linearity

The linearity plot was prepared with six concentration levels (20, 40, 80,100,120 and 150 µg/ml of fingolimod hydrochloride). These concentration levels were respectively corresponding to 20, 40, 80,100,120 and 150 % of test solution concentration. The results obtained are shown in table 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 4)

Robustness

Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min) and column temperature (±5˚c). The results are tabulated in table 4. Under all the deliberately varied chromatographic conditions, the reproducibility of results was observed to be

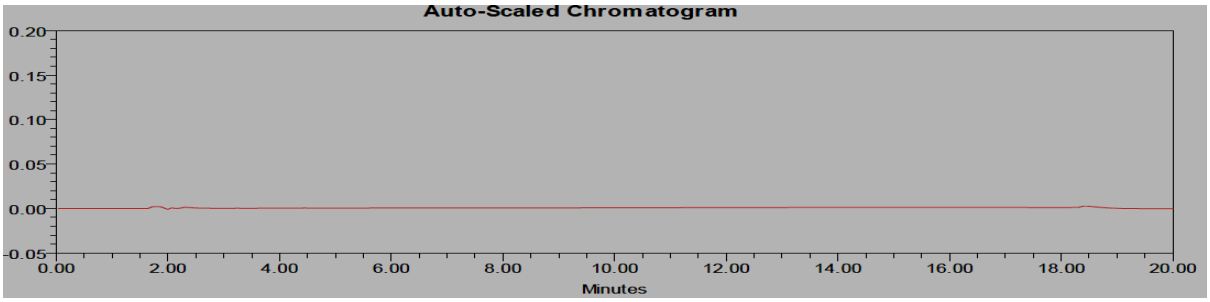
reasonably good. Hence the proposed method has good robustness for the assay of Fingolimod in bulk and dosage forms.

Solution stability

Solution stability checked for stability of standard and sample solutions. Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

Forced Degradation Studies

Stress studies on Fingolimod were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in oxidation (fig 3k) of Fingolimod. There was no significant degradation of Fingolimod upon exposure to dry heat at 80˚c for 7days, acid, base and photolysis total impurity increased to 0.10%, 0.22%, 0.23% and 0.08% which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.



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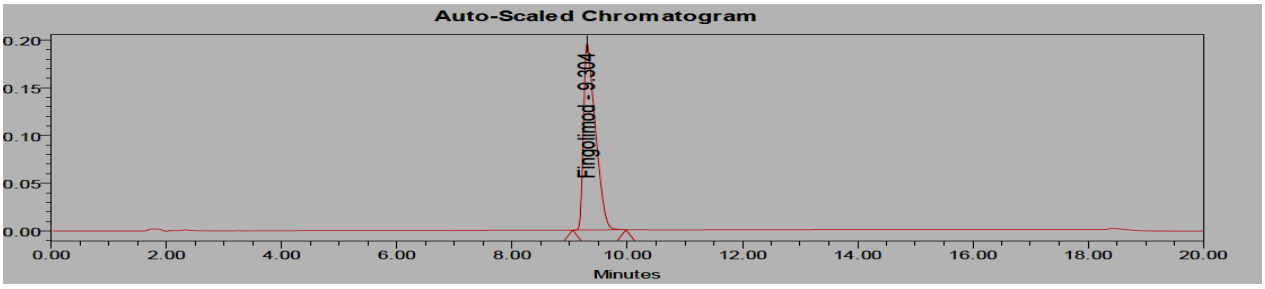
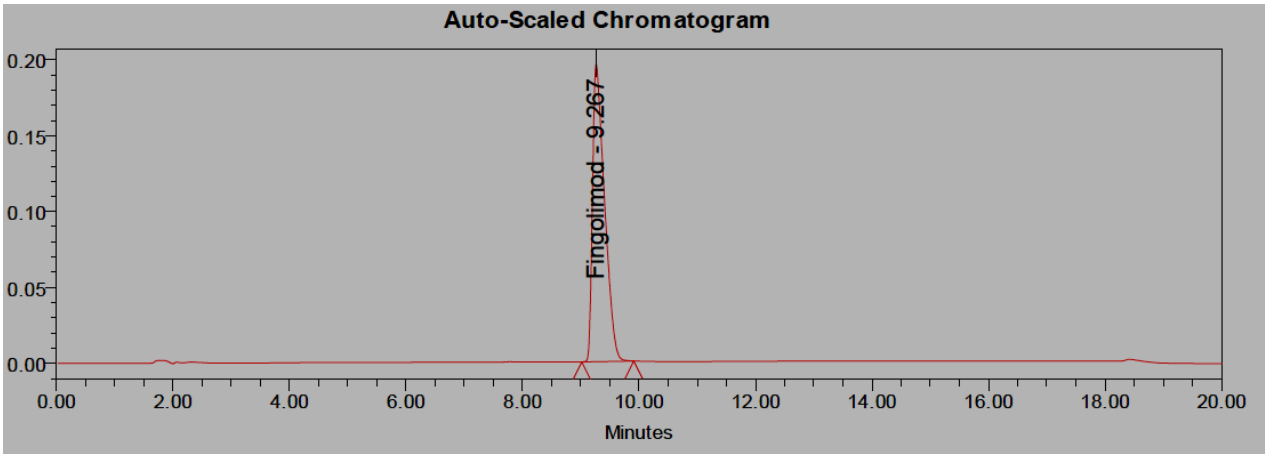
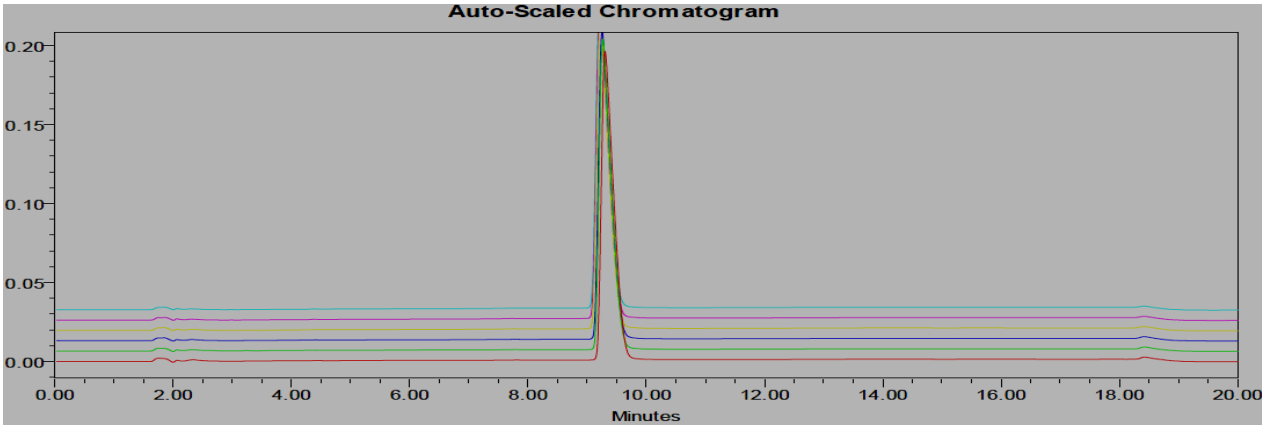


(b)


Auto-Scaled Chromatogram
Fingolimod - 9.267
0.20
0.15
0.10
0.05
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

(c)


Auto-Scaled Chromatogram
0.20
0.15
0.10
0.05
0.00
0.00
2.00
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6.00
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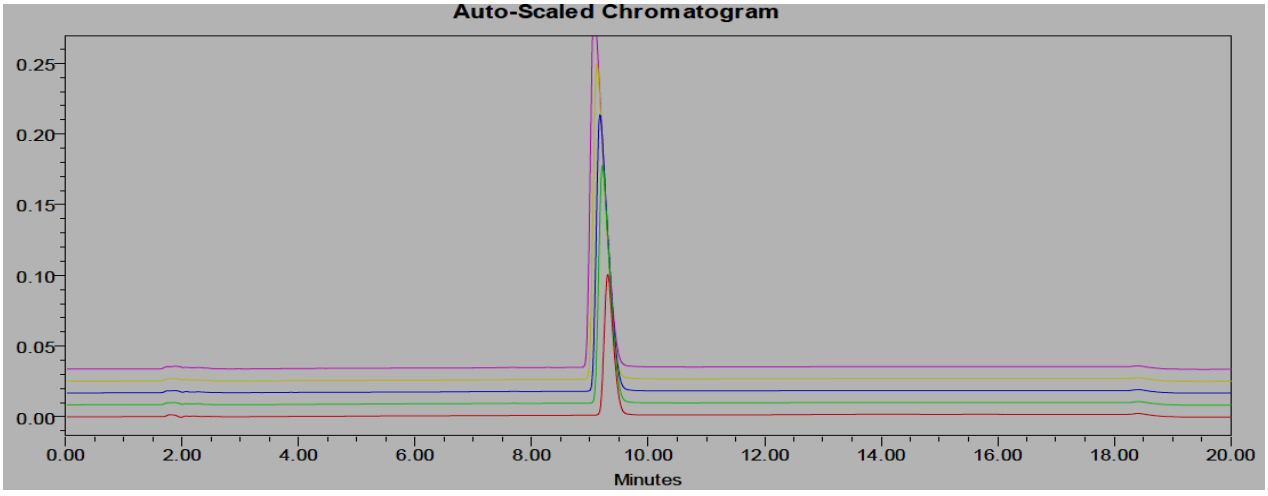
14.00
16.00
18.00
20.00
Minutes

(d)


Auto-Scaled Chromatogram
0.25
0.20
0.15
0.10
0.05
0.00
0.00
2.00
4.00
6.00
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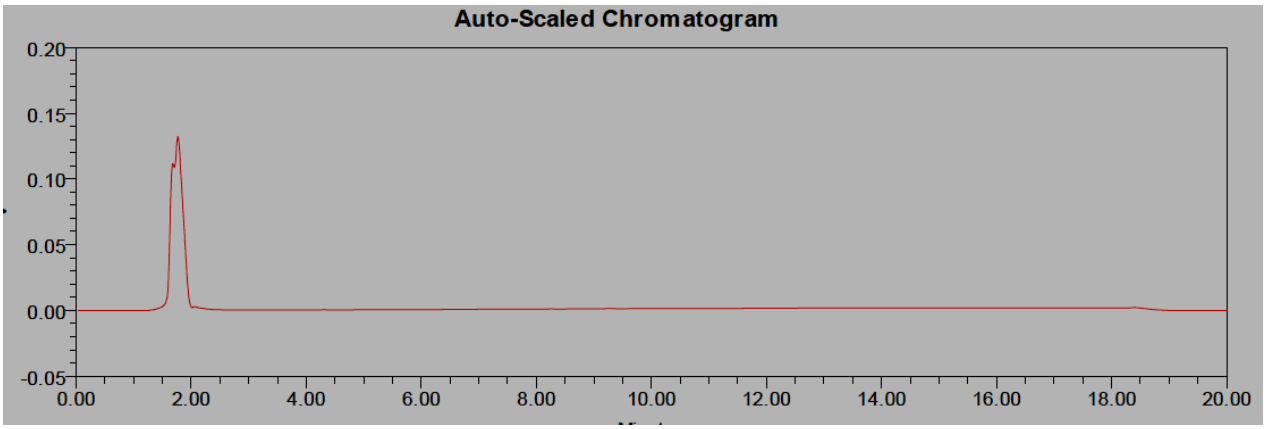
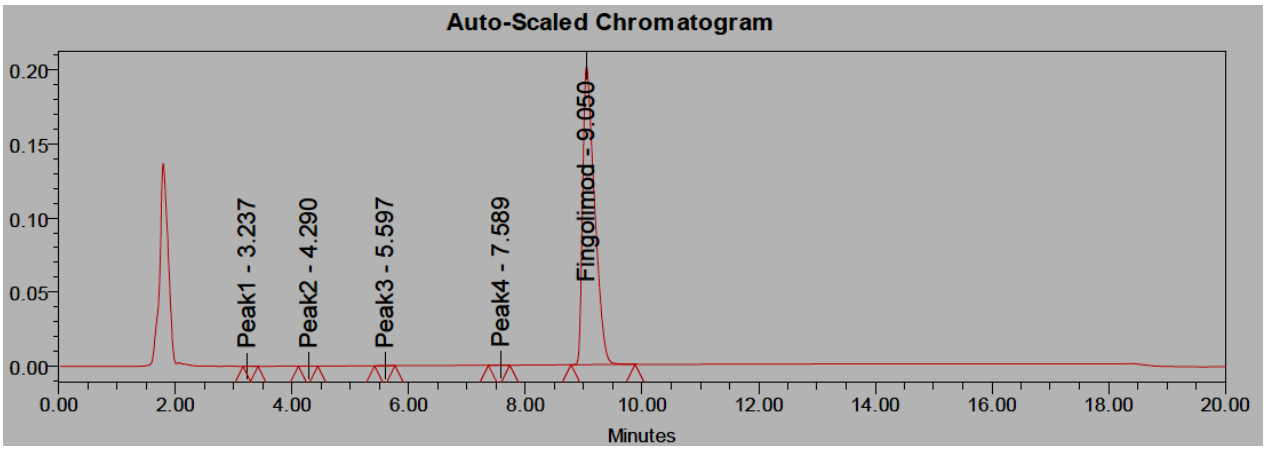
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

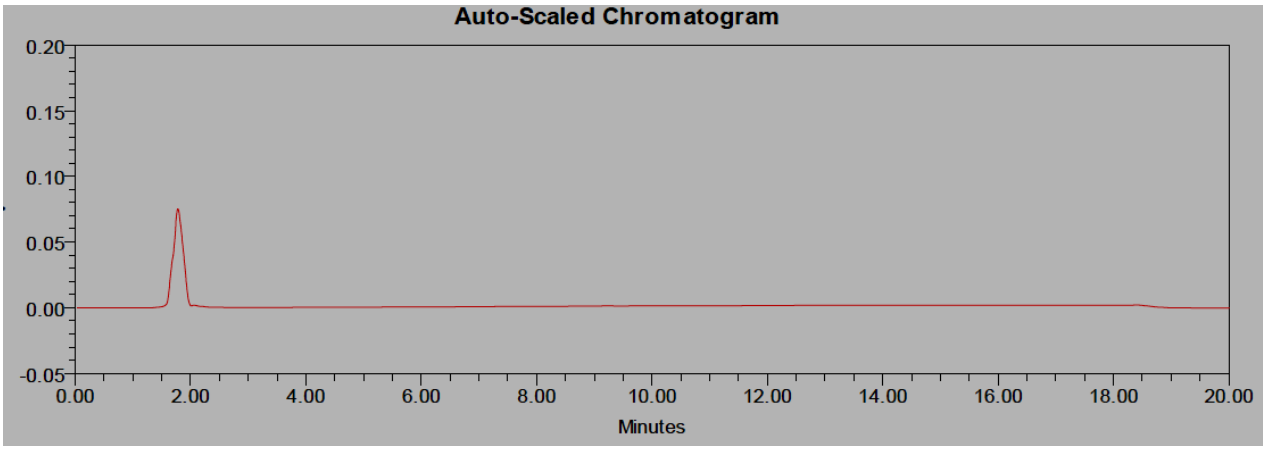
(e)


Auto-Scaled Chromatogram
0.20
0.15
0.10
0.05
0.00
-0.05
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00

(f)


Auto-Scaled Chromatogram
Peak1 - 3.237
Peak2 - 4.290
Peak3 - 5.597
Peak4 - 7.589
Fingolimod - 9.050
0.20
0.15
0.10
0.05
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

(g)


Auto-Scaled Chromatogram
0.20
0.15
0.10
0.05
0.00
-0.05
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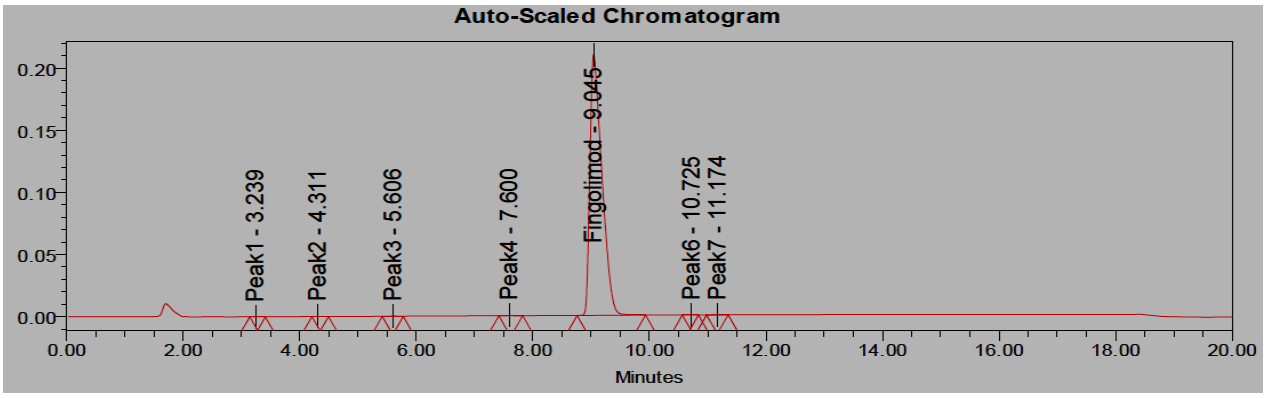
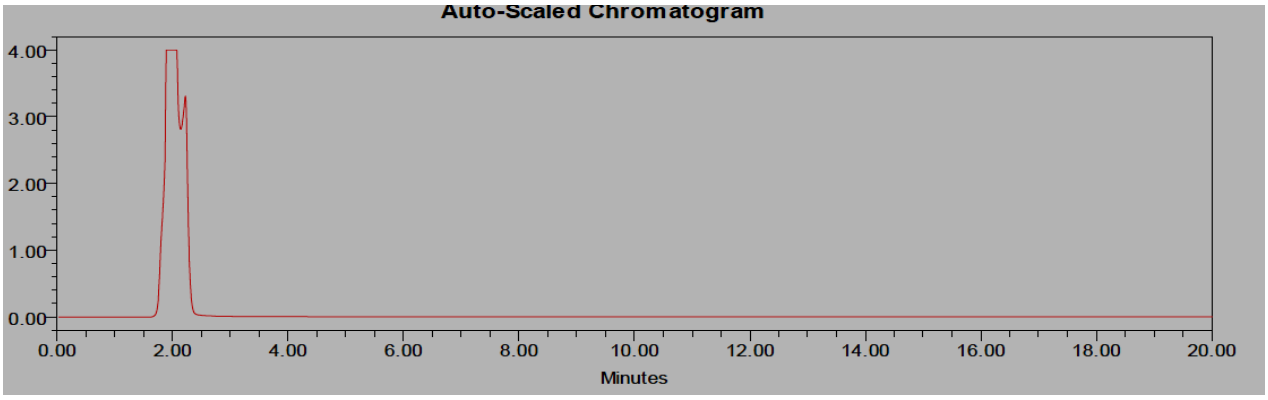
Minutes

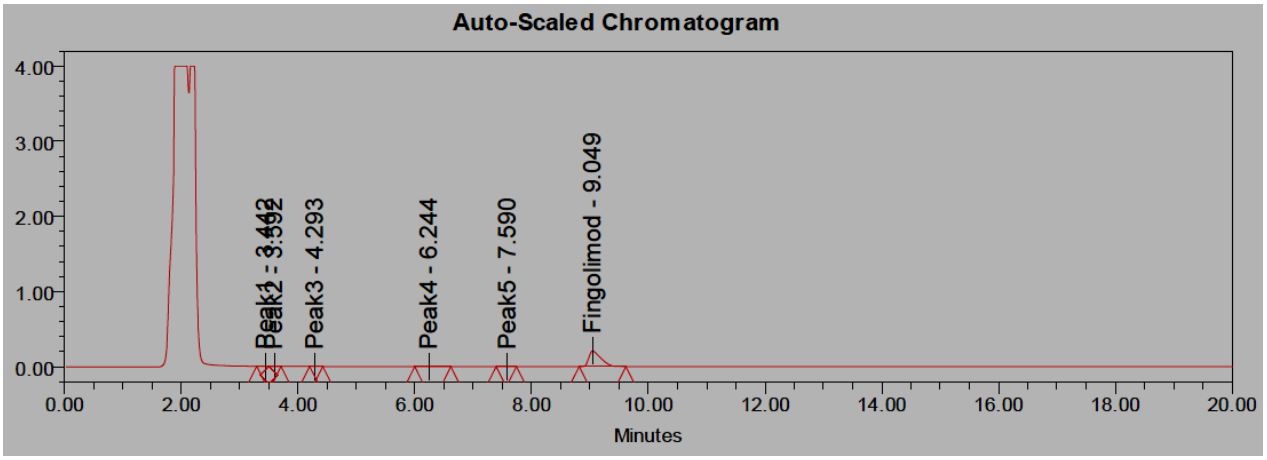
(h)



(i)



(j)



(k)

Fig.3 Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) precision injections (e) Linearity injections (f) Acid blank (g) Acid sample (h) Base blank (i) Base sample (j) Peroxide blank (k) Peroxide sample

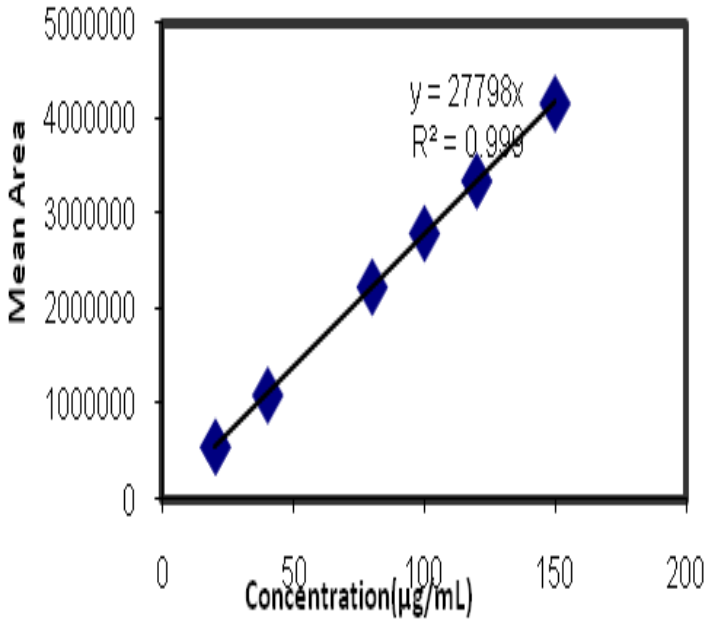


Fig.4.Linearity of Fingolimod

Table.1.Results for linearity of fingolimod

Linearity level	%Level	Area
1	20	544390
2	40	1088806
3	80	2228175
4	100	2792060
5	120	3345106
6	150	4159445
Correlation co-efficient		0.99996
	intercept	-14653.4
	slope	27933.14

Table.2.Recoveries study for Fingolimod

Accuracy (Recovery) study							
Accuracy Level	**Set No**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
50%	1	50.06	49.93	99.74	99.64	0.09	0.09
	2	50.04	49.85	99.62			
	3	50.02	49.80	99.56			
100%	1	100.08	100.20	100.12	100.08	0.14	0.14
	2	100.02	100.21	100.19			
	3	100.10	100.02	99.92			
150%	1	150.06	150.21	100.10	100.04	0.10	0.10
	2	150.12	150.02	99.93			
	3	150.15	150.25	100.10			

Table.3.Forced degradation results for Fingolimod

Stress condition	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	100	-
Acid degradation	99.87	0.23
Alkali degradation	99.88	0.22
Oxidation degradation	89.70	10.30
Thermal degradation	99.90	0.10
Photolytic degradation	99.92	0.08

Table.4.Robustness results for Fingolimod

Robust conditions	variation	Retention time(min)	USP Tailing	USP Plate count
Flow	0.7ml	10.42	1.35	7543
	0.8ml	9.30	1.25	8200
	0.9ml	8.23	1.18	8356
Temperature	35˚c	9.41	1.26	8145
	40˚c	9.30	1.25	8200
	45˚c	9.16	1.23	8424

Table.5.Precision results for Fingolimod

Study	Set no	Assay (%)	Mean assay(%)	Stdev	RSD%
Method precision	1	100.05	99.96	0.14	0.14
	2	99.85			
	3	99.90			
	4	100.15			
	5	100.05			
	6	99.78			
Intermediate precision	1	99.75	100.0	0.49	0.49
	2	100.6			
	3	99.98			
	4	100.4			
	5	100.2			
	6	99.24			

CONCLUSIONS

A validated RP-HPLC method has been developed for determination of fingolimod in bulk drug and pharmaceutical dosage form. The proposed method was found to be a new, simple, precise, linear, accurate and specific. Degradation impurities did not interfere with the retention time of fingolimod, and assay method is thus stability indicating.

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