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Spectrophotometric Determination of Fingolimod in Pharmaceutical Dosage Forms

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Two rapid and simple spectrophotometric methods were proposed for the determination of fingolimod in pharmaceutical dosage forms. In the direct spectrophotometric method, the absorbance of fingolimod was measured at 217 nm. The method was linear over the concentration range of 0.25-30 µg/mL. The second procedure depends on the absorption measurement of an ion-pair complex of fingolimod and fluorescein formed in acidic medium. The second method was also linear over the concentration range of 3-150 µg/mL. Both methods showed acceptable within-day and between-day precision and accuracy (CV < 2 % and error < 2 %). The validated methods were applied successfully for the determination of fingolimod in capsules without any special pre-treatment.

Keywords: Fingolimod, Spectrophotometric, Fluorescein, Ion-pair complex.

INTRODUCTION

Fingolimod, 2-amino-2-[2-(4-octylpheny)ethyl]propane-1,3-diol (Fig. 1), is a synthetic immunosuppressive compound. Fingolimod is used for the rejection of organ transplantation prophylaxis and also for the treatment of multiple sclerosis [1,2]. The phosphorylated metabolite of fingolimod reacts with sphingosine-1-phosphate receptors on lymphocytes, which lead to the reduction of peripheral lymphocytes [3,4].

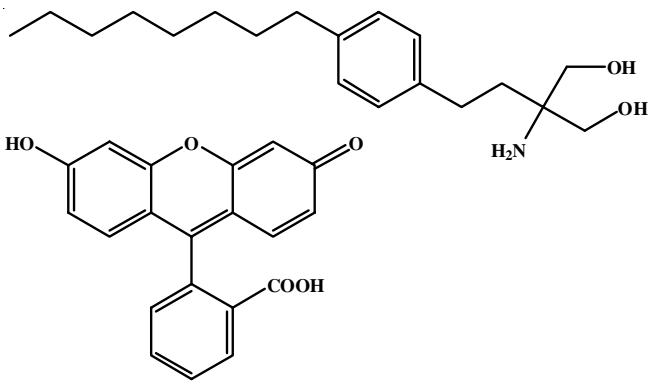

Fig. 1. Chemical structure of fingolimod and fluorescein

Literature review showed few LC-MS methods available for the determination of fingolimod and its metabolite in biological fluids [5-8]. Also, high-performance liquid chromatographic methods are reported for the determination of fingolimod in bulk drug or pharmaceutical dosage forms [9-11].

Spectrophotometric methods are convenient alternative techniques over HPLC methods for determination of drugs in pharmaceutical dosage forms due to their simplicity and significant economic advantage. Based on our literature search there is only one report for spectrophotometric determination of fingolimod with a linearity range of 4-12 µg/mL [12]. The present study describes two simple, reliable and extraction free spectrophotometric methods for the determination of fingolimod in dosage forms. The two proposed methods also showed a very wide linearity range.

EXPERIMENTAL

A double beam UV-visible spectrophotometer (UV-160, Shimadzu, Japan) at a fixed bandwidth of 2 nm and quartz cells (1 cm diameter) were used for absorption measurements.

Fingolimod bulk powder and also fingolimod 0.5 mg capsules were obtained from Osvah Pharmaceutical Company, Tehran, Iran. Analytical grade methanol, fluorescein and other chemicals were purchased from Merck (Darmstadt, Germany).

Standard solutions

Method A (direct spectrophotometric method): By dissolving fingolimod in methanol, a stock standard solution at a concentration level of 200 μg/mL was prepared. Calibration solutions of fingolimod in the range of 0.25-30 μg/mL (0.25, 0.5, 1, 2.5, 5, 10, 15 and 30 μg/mL) were prepared after appropriate dilution of the stock standard solution in methanol.

Fluorescein solution (1.3 mg/mL) was prepared in distilled water.

McLiavain buffer in the pH range of 2.2-4.2 was prepared by mixing different volumes of 0.1 M citric acid solution and 0.2 M disodium hydrogen phosphate.

Method B (ion-pair complexation spectrophotometric method): For method B, stock standard solution of fingolimod (150 μg/mL) was prepared in a mixture of methanol and water (40:60, v/v). Calibration solutions in the range of 3-150 μg/mL (3, 7.5, 15, 30, 60 and 150 μg/mL) were also prepared by appropriate dilution of stock standard solution by the same solvent.

General procedure for sample preparation

Method A: The absorbance of standard and calibration solutions of fingolimod in methanol was measured against methanol as blank solution at 217 nm.

Method B: 1 mL of standard solution of fingolimod in methanol-water (40:60, v/v) was pipetted into a 10 mL volumetric flask. Then, 6 mL of water, 1 mL of fluorescein solution (1.3 mg/mL in water) and 2 mL of buffer solution (pH 3.0) were added. After mixing, the absorbance of the solution was measured at 233 nm against a reagent blank solution treated similarly.

Optimization of ion-pair complex formation conditions

Selection of suitable pH: The effect of the pH value of McLiavain buffer in the range of 2.2-4.2 on the ion-pair complex formation was studied by using a standard solution of fingolimod treated by the general procedure (Method B).

Selection of reagent amount: Different volumes of fluorescein (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL) was used for the ion-pair complex formation according to the general procedure (Method B) and the absorbance was measured at 233 nm.

Determination of fingolimod in dosage forms: The content of 10 capsules of fingolimod 0.5 mg were mixed well. An accurately weighed quantity of the powder equal to one capsule transferred into a 10 mL volumetric flask. After addition of methanol to the flask, the mixture was sonicated for 15 min. After filtration to remove insoluble materials, the absorbance of the solution was measured at 217 nm according to the general procedure (Method A) after 3 times dilution. The content of fingolimod in each capsule was determined by comparing the absorbance of the solution with a standard solution at the same concentration value.

The same procedure for sample preparation was performed using a mixture of methanol and water (40:60, v/v) for method B. The absorbance was measured based on the general procedure for ion-pair complexation spectrophotometric method.

Relative recovery: Fingolimod relative recovery for both methods was evaluated by standard addition method. After addition of a standard concentration of fingolimod to the assay solution prepared from fingolimod capsules, the relative recovery was determined by comparing the absorbance of this solution with a standard solution at the same concentration value after subtraction of the absorbance of the assay solution.

Linearity: Six series of calibration solutions of fingolimod were treated according to the general procedure. After construction of calibration curves, the statistical data calculated. The linearity was evaluated at a concentration level of 0.25-30 μg/mL and 3-150 μg/mL for the method A and method B, respectively.

Precision and accuracy: Both spectrophotometric methods were validated for precision and accuracy. The within-day and between-day accuracy and precision were evaluated by using fingolimod standard solutions at three different concentration levels measured based on general procedure of both method.

RESULTS AND DISCUSSION

Absorption spectra

Direct spectrophotometric method (Method A): Direct spectrophotometric method was used for the determination of fingolimod. The maximum absorbance wavelength was 217 nm, which was used for spectrophotometric measurements.

Ion-pair complexation spectrophotometric method (Method B): Fluorescein is an orange red dye with yellow fluorescence, which could be used as an ion-pair reagent for spectrophotometric determinations. The absorbance of the reagent increased after the electrostatic interaction between an amino group of drug and carboxylic group of fluorescein. The maximum absorbance of the formed ion-pair complex at 233 nm was used for spectrophotometric measurements.

Selection of diluting solvent: Different diluting solvents were tested to find out the more suitable one for the ion-pair complex formation. The highest absorption was achieved by using water. Forty percent of methanol was used as co-solvent to dissolve fingolimod for the preparation of standard solutions. Increasing the amount of methanol decreased the complex formation and absorption of the solution.

Selection of suitable pH: The pH of the reaction medium affects the complex formation. The influence of different pH values were studied on the ion-pair complex formation using McLiavain buffer solution. It was observed that the absorbance reached a maximum at pH 3.0 (Fig. 2). This pH was used as the optimum pH value for ion-pair complex formation.

Selection of the reagent amount: The effect of the reagent volume was also studied on the complex formation. Maximum intensities were obtained by using 1 mL of fluorescein solution (1.3 mg/mL) (Fig. 3). Higher amounts of the reagent did not show significant influence on the absorbance of the formed complex.

Effect of reaction time: The ion-pair complex formation of fingolimod and fluorescein was followed over the time intervals of 0, 5, 10, 20, 30 and 60 min. The maximum absorbance was observed immediately after addition of the reagent and remained constant at least for 60 min. Also, the formed complex was shown to be stable at least for 8 h (recovery > 98 % at room temperature). The absorbance of the solution of the formed complex decreased after 24 h (recovery about 80 %) and the precipitation of the complex was observed in the solution.

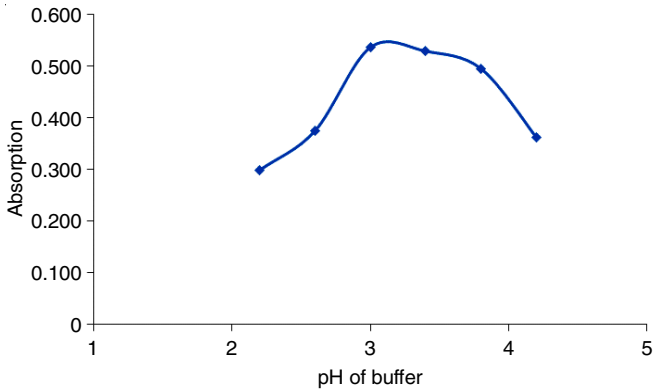

Fig. 2. Effect of pH of the buffer on the ion-pair complex formation

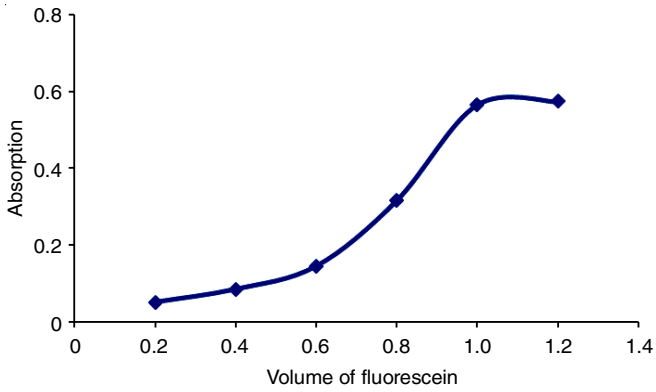

Fig. 3. Effect of fluorescein amount on the ion-pair complex formation

Method validation

Linearity: Six series of calibration curves of fingolimod were constructed by both methods and the statistical data were calculated which are shown in Table-1.

Precision and accuracy: The within-day and between-day accuracy and precision of both methods were studied by using three different concentration levels. The results are shown in Table-2.

Relative recovery: The calculated relative recovery of fingolimod from capsules was about 100.12 ± 0.46 and 101.03 ± 1.05 percent for method A and method B, respectively. These data showed no significant interferences from the excipients.

Application of the method: The proposed methods were used for determination of fingolimod in pharmaceutical dosage forms. The obtained results were statistically comparable with a standard HPLC method. The proposed spectrophotometric procedure was compared with a previously published HPLC method [11], which showed no significant differences ($p < 0.05$) (Table-3).

TABLE-1
STATISTICAL DATA OF CALIBRATION CURVES OF FINGOLIMOD IN STANDARD SOLUTIONS (n = 6)

Parameters	Method A	Method B
Linearity range (µg/mL)	0.25-30	3-150
Regression equation	y = 0.0347x + 0.0163	y = 0.0035x + 0.0617
Standard deviation of slope	5.2×10^{-5}	5.2×10^{-5}
RSD of slope (%)	0.15	1.49
Standard deviation of intercept	0.0013	0.0031
Correlation coefficient (r^2)	0.9991	0.9996

TABLE-3
COMPARISON OF THE DEVELOPED METHODS WITH THE REFERENCE METHOD FOR THE DETERMINATION OF FINGOLIMOD CAPSULES

Method	Label claimed	Found (mean ± SD*)
Method A	0.50	0.49 ± 0.02
Method B	0.50	0.51 ± 0.01
Reference method	0.50	0.49 ± 0.03

*Standard Deviation

Conclusion

The validated spectrophotometric methods with acceptable sensitivity and accuracy are relatively rapid, simple and time-saving. Both methods were applied for the determination of fingolimod in dosage forms without any special sample preparation and no significant interferences from capsule excipients. These methods are applicable for routine measurement of fingolimod in bulk powder or dosage forms in quality control laboratories.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-2
PRECISION AND ACCURACY OF THE METHOD FOR DETERMINATION OF FINGOLIMOD IN STANDARD SOLUTIONS (n = 9; 3 SETS FOR 3 days)

Concentration added (µg/mL)	Within-day (n = 3)			Between-day (n = 9)		
	Found (µg/mL)	CV (%)	Error (%)	Found (µg/mL)	CV (%)	Error (%)
Method A						
0.50	0.49 ± 0.01	2.04	-2.00	0.50 ± 0.01	2.00	0.00
5.00	4.98 ± 0.06	1.20	-0.40	4.99 ± 0.08	1.60	-0.20
30.00	30.13 ± 0.21	0.70	-0.43	30.10 ± 0.19	0.63	0.33
Method B						
3.00	3.02 ± 0.05	1.67	0.67	3.00 ± 0.04	1.33	0.00
30.00	29.82 ± 0.29	0.97	-0.60	29.69 ± 0.33	1.11	-1.03
150.00	150.41 ± 2.04	1.36	-0.27	150.74 ± 1.50	1.00	0.49

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