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Development and validation of HPTLC method for estimation of fluvastatin sodium in bulk drug and dosage form

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ABSTRACT

A simple, accurate and precise high performance thin layer chromatographic method has been developed for the estimation of fluvastatin sodium in bulk drug and dosage form. The method employed silica gel 60 F_{254} precoated plates as stationary phase and mixture of chloroform: toluene: methanol (6:2:2) as mobile phase. Densitometric scanning was performed at 305 nm using Camag TLC scanner 3 with WINCAT software of version 1.4.3 Camag. Beer's law was obeyed in the concentration range of 300ng/spot-800ng/spot. The Retention factor for fluvastatin was found to be 0.20. The limit of detection and limit of quantitation were found to be 65 ng/spot & 200 ng/spot respectively. The % RSD of intra-day variation and inter day variation were found to be 0.66-0.89 and 0.54-75 respectively. As per ICH guidelines the results of the analysis were validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification, and were found to be satisfactory. The proposed method can also be used for routine quality control to accurately determine fluvastatin sodium in bulk and capsule dosage form.

Keywords: Fluvastatin, HPTLC, densitometric estimation, method development, and validation.

INTRODUCTION

Fluvastatin sodium is designated chemically as 7-[-3(4-fluorophenyl)-1-(1- methyl ethyl)-1H –indol-2-yl]-3, 5 dihydroxy 6-heptenoic acid monosodium salt [1] (Fig. 1). Fluvastatin sodium is official in USP [2]. Fluvastatin Sodium, a fully synthetic cholesterol-lowering agent, is a competitive inhibitor of HMG-CoA reductase, which is responsible for the conversion of HMG-CoA to mevalonate, a precursor of sterols, including cholesterol [3]. .Several analytical methods have been reported for the analysis of fluvastatin such as few chromatographic [4,5] spectrophotometric [6,7], capillary electrophoresis (CE)[8,9] and electrochemical as differential pulse voltammetry (DPV)[10]' methods have been reported for the estimation fluvastatin sodium. Literature survey revealed that no HPTLC method is reported for the analysis of fluvastatin. The purpose of this investigation was to develop and validate a simple, rapid, sensitive, precise, accurate and specific HPTLC method for the estimation of fluvastatin sodium in bulk and formulation.

MATERIALS AND METHODS

Drugs, Reagents and Chemicals used

Authenticated standard of fluvastatin sodium was kindly gift samples from Sandoz Private Limited Mumbai. AR grade Methanol, Toluene and Chloroform were purchased from Sisco Research Laboratories Ltd; Mumbai. The commercial formulation of fluvastatin sodium (Lescol 20mg) procured from local market.

Instrumentation

Chromatographic separation was performed on a Merck TLC plates, precoated with silica gel 60 F_{254} (20 cm × 10 cm, layer thickness 0.2 mm thickness, E. Merck, Darmstadt, Germany, purchased by Anchrom Technologies,

Mumbai, India). The samples were applied on the plates using Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe as a band with 6 mm width using a Camag Linomat 5 applicator (Camag, Muttenz, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm).

Chromatographic conditions

The experiment was performed on a aluminum packed silica gel 60 F_{254} TLC plates, (20 cm × 10 cm, layer thickness 0.2 mm) prewashed with methanol and mobile phase comprising of chloroform: methanol: toluene (6:2:2 v/v). The developing solvent was run upto 80 mm in Camag chamber previously saturated with 10.0 ml of solvent mixture for 20 min. Samples were applied at a distance of 8 mm from lower edge the distance between two bands was 7 mm . The developing solvent was run upto 80 mm, and the development was performed at 25 ± 2°C. The average development time was 15 minutes. After development, the plate was air dried and scanned densitometrically at 305 nm with slit dimensions 6.00 x 0.30 mm, using CAMAG TLC scanner 3.A typical HPTLC chromatogram is shown in Fig. 2.

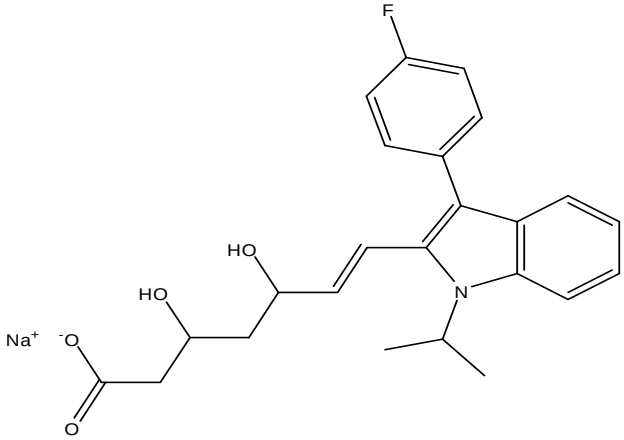

Fig. 1: Structure of Fluvastatin

Preparation of Standard Stock Solution

An accurately weighed sample (10 mg) of Fluvastatin was transferred to a 10 ml volumetric flask and dissolved in methanol to obtain a solution of strength 1000 µg/ml. Working standard solutions was prepared by serial dilution of stock solutions with methanol.

Analysis of capsules formulation

Twenty Capsules, each containing 20 mg fluvastatin sodium were weighed. Empty the content of fluvastatin capsule, a quantity of powder equivalent to 20 mg was weighed and transferred to 10 ml volumetric flask containing about 5 ml methanol, ultrasonicated for 10 min. Finally the volume was made up to mark with methanol. The solution was filtered using whatmann filter paper No.41.

Table 1: Analysis data of fluvastatin sodium

Sr.no	Amount present in (mg/capsule)	Amount found in (mg/capsule)	% of Label claim*
1	20	20.015	100.075
2	20	20.46	101.80
Mean	20	20.23	100.93

VALIDATION

The proposed method was validated according to ICH (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines [11] the method validation parameters checked were Specificity , limit of detection, limit of quantitation , linearity precision and accuracy .

Specificity:

Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of fluvastatin sodium and was demonstrated by assaying samples of fluvastatin sodium capsules.

Limit of Detection and Limit of Quantification:
The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

LOD = 3.3 σ /S

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

LOQ = 10 σ/S

Where, σ is standard deviation of the response and S is the slope of the calibration curve.

Linearity:
In linearity study the standard solutions were prepared by diluting standard stock solution with methanol in concentration range of 300, 400, 500, 600, 700, and 800 ng/spot .The peak area was plotted against corresponding concentrations to obtain the calibration graph.

Precision:
Precision of analytical methods were expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

Accuracy:
The accuracy of the method was determined by recovery experiments. A known amount of standard fluvastatin sodium corresponding to 80, 100 and 120% of the label claim (standard addition method) was added to preanalysed sample of capsule. The recovery studies were carried out in triplicate at each level.

RESULTS AND DISCUSSION

Optimization of Solvent System and Chromatographic Conditions:
Several mobile phases were tried to achieve good separation of fluvastatin sodium. Ultimately mobile phase consisting of chloroform: methanol: toluene (6:2:2 v/v) observe good resolution with R_f values 0.20

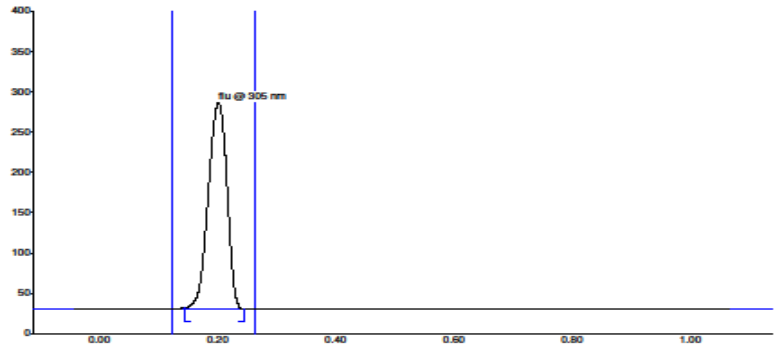

Fig. 2: HPTLC densitogram of Fluvastatin (R_f 0.20)

The method showed good linear response in concentration range of 300-800 ng /spot (r^2 =0.998) for fluvastatin sodium (Table 2). A typical calibration curve is shown in Fig.3 The method was found to be precise after quantification of six replicates of fluvastatin sodium and RSD was found to be less than 2.0% (Table 3). The recovery values were 99.81-101.35 % with R.S.D. of <2 (Table 4).

Table 2: Linear Regression data of fluvastatin sodium

Parameters	Results
Wavelength (nm)	305
Calibration range	300-800ng/spot
Correlation coefficient (r^2) $_2$	0.998
Linear regression Equation (y = mx + c)	y=6.978x+1285
Slope (m)	6.978
Intercept (c)	1285
Limit of detection (ng/spot)	65ng/spot
Limit of quantitation (ng/spot)	200ng/spot
Precision indicated by %RSD	< 2 %

Fig.3 Calibration curve of fluvastatin sodium

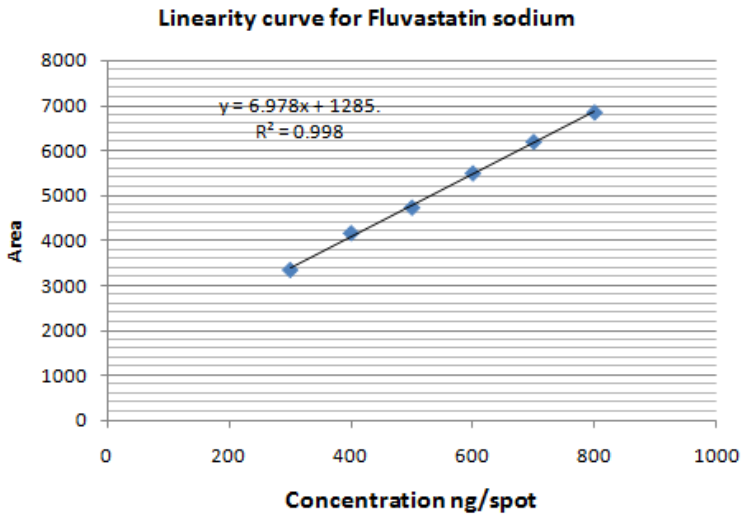

Table 3: Precision data of fluvastatin sodium

Drug	ng/spot	Intraday Precision %RSD	Interday Precision %RSD
Fluvastatin	500	0.66	0.54
	600	0.71	0.61
	700	0.89	0.75

Table 4: Recovery data of fluvastatin sodium

Level	% Recovery	Mean	% RSD
80	101.40 101.77 100.65	101.27	0.56
100	101.88 101.30 100.88	101.353	0.49
120	99.89 100.75 98.81	99.81	0.97

CONCLUSION

The proposed HPTLC method for the quantification of fluvastatin sodium in capsule was simple, precise, accurate, rapid and selective. The methods were found to be linear in wide range of concentration. The developed method was free from interference due to the excipients present in capsule and can be used for routine simultaneous quantitative estimation of fluvastatin sodium.

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REFERENCES

[1] Merck & Co. Inc., The Merck Index, Whitehouse station New Jersey, **2001**, 13th ed.,pp 747–748.
[2] The United States Pharmacopoeia **2007**, 30th Revision, MD**,** 961-964.
[3] H Rang H.P., Dale M.M. Pharmacology Elsevier a division of Reed Elsevier India Pvt.Ltd., **2003**, 5th ed., pp 309-311.
[4] Saminathan J., Sankar A.S.K., Anandakumar K. *J. Pharm. Sci. & Res*. **2009**, 1 (3), 90-96.
[5] Marianne A. Mahrouse et al */Int.J. PharmTech Res.***2010**, 2(1), 875-898.
[6] J. Saminathan *E-Journal of Chemistry* **2009**, 6 (4), 1233-1239.
[7] Safwan Ashour1, *Int. J. Biomed. Sci*. March **2010**, 6(1) 19-26.
[8] Ozkan S. A., and Uslu B.,*Anal. Bioanal.Chem*. **2002**, 372(4), 582 – 586.
[9] Ak D. D., Kircali K., Tuncel M. and Aboul-Enein H. Y.,*Biomed. Chromatogr*, **2001**, 15(6), 389 – 392.
[10] Yan J. L.,*Pak. J. Biol. Sci.*, **2006**, 9(11), 2156 – 2158.
[11] Validation of Analytical Procedure: Text and Methodology Q2 (R1), ICH Harmonized Tripartite Guideline **2005**, 1-13.