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Research Article

NOVEL HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD FOR SIMPLE, ECONOMICAL, AND RAPID DETERMINATION OF FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

RANI S POTAWALE[1]*, TABASSUM I HANGAD[1]

Department of Pharmaceutical Chemistry, Allana College of Pharmacy, Pune, Maharashtra, India. Email: deshmukh_rani83@yahoo.co.in

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ABSTRACT

Objective: A simple, novel, sensitive, and rapid high-performance thin-layer chromatographic (HPTLC) method has been developed and validated for quantitative determination of fenofibrate in bulk and formulations.

Methods: The chromatographic development was carried out on HPTLC plates precoated with silica gel 60 F_{254} using a single solvent dichloromethane as a simple mobile phase. Densitometric detection was carried out at 292 nm.

Results: R_f value of drug was found to be 0.33±0.02. The method was validated as per International Conference on Harmonization Guideline with respect to linearity, accuracy, precision, and robustness. The calibration curve was found to be linear over a range of 20–400° ng band^{-1}° with a regression coefficient of 0.999. The method has proved high sensitivity and specificity.

Conclusion: Proposed densitometric method was found to be new, simple, and economic for routine quantification of fenofibrate in bulk and pharmaceutical formulation.

Keywords: Fenofibrate, High-performance thin-layer chromatographic, Single solvent analysis, Validation.

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INTRODUCTION

Fenofibrate is the lipid regulating drug. Chemically, it is propan-2-yl2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propionate. Fenofibrate increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III (an inhibitor of lipoprotein lipase activity) [1,2]. It is official in British pharmacopoeia [3]. The literature survey showed that there are some analytical methods reported for fenofibrate (Fig. 1) like spectrophotometric [4-8], high-performance liquid chromatographic [9-16], high-performance thin-layer chromatographic (HPTLC) [17,18], and polarography analysis [19] either individually or in combination with other drug/s.

The present research manuscript describes a novel, simple, economical, accurate, and rapid HPTLC method developed in single solvent and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1) [20], for quantification of fenofibrate as a bulk drug and in its tablet dosage form.

METHODS

The fenofibrate pure standard was supplied by Emcure Pharmaceuticals Pvt. Ltd., Pune. Faint 160 mg tablets (Franco-Indian Pharmaceuticals Pvt. Ltd.); labeled to contain 160 mg fenofibrate, were obtained from the market. Analytical reagent grade chemicals were procured from Company Merck Specialties Pvt. Ltd. (Mumbai, India). Precoated silica gel HPTLC plates 60 F_{254} (E. Merck, Darmstadt, Germany) were used in the research.

Instrumentation, chromatographic conditions

The HPTLC instrument comprised a Linomat V sample applicator with a 100 µl Camag syringe with a TLC III scanner having WinCats Software Version 1.4.4. (Camag, Muttenz, Switzerland). The slit dimension was retained at 5×0.45 mm and a scanning speed of 10 mm/s was maintained. Prewashed HPTLC plates were activated at 120°C for 15 min before analysis.

HPTLC plates were then developed in a Camag 20×10 cm twin trough chamber (Camag, Muttenz, Switzerland) with 20 ml mobile phase comprising only dichloromethane. The optimized chamber saturation time for solvent system was 15 min at room temperature (25±2°C). The length of chromatographic run was 80 mm. After chromatographic development, plates were dried in an air current. Densitometric scanning was performed in the reflectance-absorbance mode at 292 nm by Camag TLC Scanner III using WinCats Software Version 1.4.4.

Preparation of standard stock solutions

Standard stock solution of fenofibrate was prepared individually by dissolving 10 mg of standard drug in 10 ml methanol to get concentration of 1000 µg/ml and from this 1 ml further diluted to get 100 µg/ml concentrations.

Selection of detection wavelength

Fenofibrate showed significant absorbance at 292 nm and thus was selected for densitometric analysis (Fig. 2).

Preparation of sample solutions

For the study of marketed tablet dosage form, 20 tablets were accurately weighed. The average weight was assessed and tablets were crushed to get fine powder. Powder equivalent to 5 mg of fenofibrate was weighed and shifted to the volumetric flask (50 ml) containing 20 ml methanol. The solution was sonicated for 15 min, diluted up to the mark with solvent methanol. The solution was filtered through Whatman filter paper (No. 41). The resulting solution is used for further study.

Assay validation

The proposed HPTLC method was validated as per the guidelines of the ([ICH] Q2 [Rl]) for various parameters.

Linearity and range

On the HPTLC plate, a stock solution was applied in the concentration of 20–400 ng band^{-1} of fenofibrate to evaluate linearity. The graph of peak area versus concentration was plotted. Least square linear regression analysis was done. The correlation coefficient, intercept, and slope were calculated.

Sensitivity

Limit of detection (LOD) and limit of quantitation (LOQ) was calculated using formula 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot.

Specificity

The peak purity for fenofibrate was assessed by comparing ultraviolet (UV) spectrum acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of the band.

Precision studies

Precision was calculated by intra- and inter-day precision studies. 100 ng band^{-1} fenofibrate sample was analyzed 6 times on the similar day to find out any differences in the results. Interday precision study was done on 3 successive days.

Accuracy studies

By estimating recovery of fenofibrate by the standard addition method, the accuracy was determined. The samples were spiked at three levels with 80, 100, and 120% of 100 ng band^{-1} of the fenofibrate standard solution. Recovery was assessed from the following equation:

([Spiked concentration-mean concentration]/spiked concentration)×100

Robustness studies

The effect of small but deliberate variations in method parameters such as the volume and composition of the mobile phase, time from spotting to development, and development to scanning were evaluated in this study. Only one parameter was varied at a time. 100 ng band^{-1} concentration of fenofibrate was used in six replicates to study robustness of the method. The standard deviation of peak areas and % relative standard deviation (RSD) was determined.

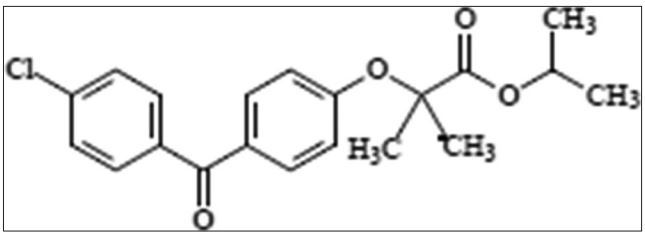

Fig. 1: Chemical structure of fenofibrate

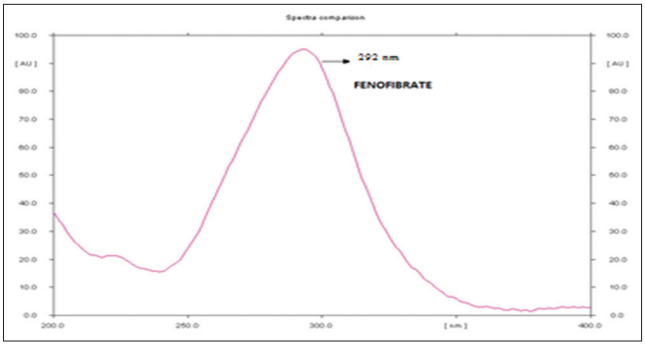

Fig. 2: Spectrum obtained from standard solution of fenofibrate

RESULTS AND DISCUSSION

Chromatographic development

Different solvent systems were tried, to accomplish the R_f value in the range 0.2–0.8, and minimum resolution Rs ≥1.5. Finally, the mobile phase consisting of only dichloromethane was selected for obtaining sharp peak and promising results. The retention factors were found to be 0.33±0.02 for fenofibrate (Fig. 3).

Validation of the method

Linearity

The analyte response was linear (r^2=0.999) for fenofibrate over the concentration range between 20 and 400 ng band^{-1}. The results were shown in Table 1. Calibration curve was constructed as described and showed acceptable accuracy and precision over a wide concentration range. Results demonstrate that an excellent correlation between the absorbance and concentration of fenofibrate drug substances. To ascertain linearity, residual analysis was performed (Fig. 4).

Table 1: Linear regression data for the calibration curves (n=6)

Parameters	
Linearity range (ng/band)	20–400
r^2	0.999
Slope	21.24
Intercept	492.55
Confidence limit of slope[a]	20.84–21.65
Confidence limit of intercept[a]	409.19–575.90
Sy.x	33.75

a: 95% confidence limit, Sy.x: Standard deviation of residuals from line

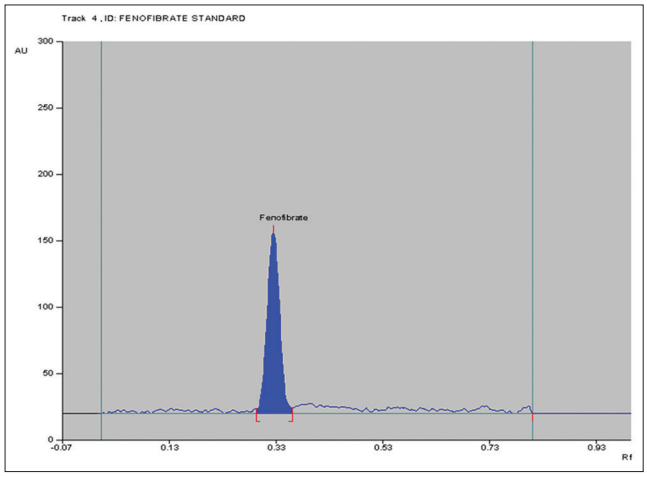

Fig. 3: Densitogram obtained from standard solution of fenofibrate scanned at 292 nm

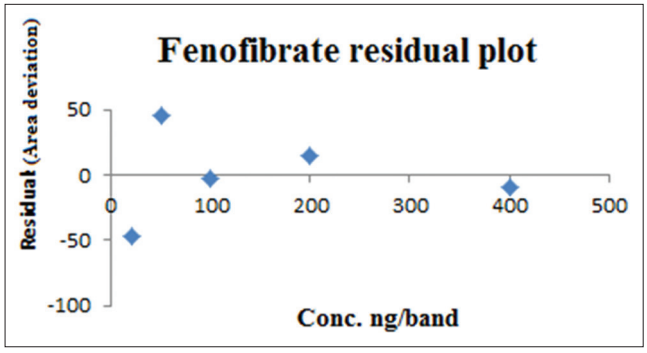

Fig. 4: Concentration residual plot of fenofibrate

Sensitivity

The LOD was found to be 5.24 ng band^{-1} for fenofibrate. The LOQ for fenofibrate was found to be 15.89 ng band^{-1}, representing good sensitivity of the method.

Specificity

The peak purity for fenofibrate was assessed by comparing UV spectrum acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of band, that is, r (S, M)=0.998, 0.998 and r (M, E)=0.998, 0.998, respectively. Peak purity data showed that peak obtained for fenofibrate was pure and method is specific.

Precision

Intra- and inter-day variation in estimation of fenofibrate (Table 2) showed that the % RSD was <2% during the analysis. These low values of RSD show that the precision of the method is good.

Accuracy

The study of accuracy reveals influences of additives that are usually present in the dosage forms on the quantitative parameters. The recovery study data presented in Table 3 indicates that the accuracy of the quantification of fenofibrate was more than 98%, which indicate that the proposed simultaneous densitometric method is reliable for the estimation of fenofibrate in the marketed formulation used in the study.

Robustness studies

The % RSD of peak areas was calculated for each parameter and was found to be <2% (Table 4).

Analysis of a marketed preparation

The results obtained for the amount of fenofibrate in tablets as against the label claims were in good agreement signifying that there is no interference from any of the excipients presents in tablets. The percent assay was found to be 98.47%, for fenofibrate, in marketed formulation in six replicate determinations.

Table 2: Intra- and inter-day precision of the HPTLC method (n=6)

Drug	Actual concentration intra-/inter-day[a]	Intra-/inter-day	(%) RSD
Fenofibrate	100	98.52/99.34	0.69/1.00

n: Number of determinations, a: ng band^{-1}, RSD: Relative standard deviation. HPTLC: High-performance thin-layer chromatographic

Table 3: Results of recovery studies (n=6)

Amount taken[a]	Amount added[a]	Amount found[a]	Recovery±% RSD
100	80	179.06	99.48±0.93
100	100	200.43	100.21±1.09
100	120	218.96	99.53±0.86

n: Number of determinations, a: ng band^{-1}, RSD: Relative standard deviation

Table 4: Robustness testing (n=6, 100 ng band^{-1})

Parameter varied	% RSD
Mobile phase (ethyl acetate) composition (±0.1 ml)	0.50
Amount of mobile phase (±5%)	1.01
Time from band application to chromatography (±10 min)	0.47
Time from chromatography to scanning (±15 min)	0.83

n: Number of determinations, RSD: Relative standard deviation

CONCLUSION

In the present research work, an attempt has been made to develop and validate a quick, precise, and accurate method based on normal-phase HPTLC has been developed for routine analysis of fenofibrate in fixed-dose combination tablets. The method was validated for linearity, precision, accuracy, and specificity. It is cheap, quick and does not use chloroform and combination of solvents, therefore, suitable for routine analysis of fenofibrate in fixed-dose combination tablets. When compared with the reported HPTLC method, the developed HPTLC method is both time and cost-effective for the determination of fenofibrate.

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CONFLICT OF INTERESTS

The authors have no conflict of interest.

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