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A Validated High Performance Thin Layer Chromatographic Determination of Fenofibrate

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The present work describes a validated high performance thin layer chromatographic method for estimation of fenofibrate in capsules. Aluminum plates precoated with Silica gel 60 F_{254} was used as stationary phase and toluene:chloroform (7:3 v/v) as mobile phase. Quantification was carried out by the use of densitometric absorbance mode at 296 nm. The amount of fenofibrate estimated as percentage of labeled claimed was found to be 101.43/100.68 (as per the peak height and peak area) in capsules. The proposed high performance thin layer chromatographic method was quantitatively evaluated in terms of stability, precision, repeatability and accuracy. The calibration correlation proving, it's utility for routine analysis of fenofibrate capsule.

Fenofibrate, chemically is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propionic acid,1-methyl ethyl ester. It is a lipid-lowering agent. Fenofibrate is official in BP[1]. Literature survey revealed HPLC and GC-MS methods for the estimation of fenofibrate aquatic environmental samples[2,3] and in human plasma[4-6]. No methods have been reported in the literature for its estimation in pharmaceutical dosage form. The present study describes the development and validation of a simple, specific, accurate and precise HPTLC method for determination of fenofibrate in capsules.

Fenofibrate working standard was a gift sample from US Vitamin India limited, Mumbai. Silica Gel 60 F_{254} TLC plates (10x10 cm, E. Merck) were used as stationary phase. Twenty fenofibrate capsules (Lipicard-200 mg, USV Ltd.) were purchased from a local pharmacy. Chloroform and toluene of GR grade (E. Merck) purity were procured from local supplier. A Camag HPTLC system (Switzerland) comprising of Camag Linomat IV semiautomatic sample applicator, Camag TLC scanner 3, Camag twin trough chamber (10x10 cm), Camag Cats 4 Software, Hamilton syringe (100 μl) were used during the study.

An accurately weighed quantity of fenofibrate (50 mg) was transferred in to a 50 ml volumetric flask. It was dissolved and diluted up to the mark with methanol to give a standard stock solution of 1 mg/ml. Five milliliters of this stock solution was further diluted to 25 ml with methanol to give a working standard solution of 200 μg/ml.

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TABLE 1: ESTIMATION OF FENOFIBRATE IN CAPSULES

Labeled Claim (mg)	Amount Found (mg)		% Labeled Claim±SD* (n=5)	
	Peak Height	Peak Area	Peak Height	Peak Area
200.0	202.86	201.36	101.43±0.65	100.68±0.96

*Percent labeled claim ±standard deviation of five observations

The chromatographic estimation was performed using the following conditions, stationary phase, aluminum sheets precoated with silica gel 60 F_{254} (10x10 cm). Spotting parameters used were, 4 mm bandwidth, 4 mm space between two bands and a spraying rate of 6 s/μl. Mobile phase used was toluene: chloroform in the ratio of 7:3 v/v. The chamber saturation time employed was 10 min and the plates were developed using ascending technique to a distance of 7-cm. Scanning wavelength of 296 nm with a slit dimension of 3.0 x 0.45 mm.

Aliquots of 6-18μl of working standard of fenofibrate were spotted as sharp bands on the precoated TLC plate, using Camag linomat IV semiautomatic applicator under nitrogen stream. The plate was developed as described under chromatographic condition. The plate was removed from the chamber and dried using hot air dryer. Densitometric measurements were performed at 296 nm in absorbance mode. Peak height and peak area of each band was recorded. The calibration curve was prepared by plotting peak height and peak area versus concentration corresponding to each spot.

The contents of twenty capsules were ground to fine powder. An accurately weighed quantity equivalent to about 20 mg of fenofibrate was transferred to a 50 ml volumetric flask, dissolved in 25 ml methanol, shaken for 15 min and volume was made up to mark with methanol. The solution was filtered through Whatman filter paper No. 1. The filtrate has concentration of fenofibrate equivalent to about 400 μg/ml (on labeled claim basis). A fixed volume of the working standard solution and sample solution were spotted as sharp bands on the TLC plate. After development, the bands of the drugs were scanned at 296 nm. The amount of fenofibrate per capsule was calculated by applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions.

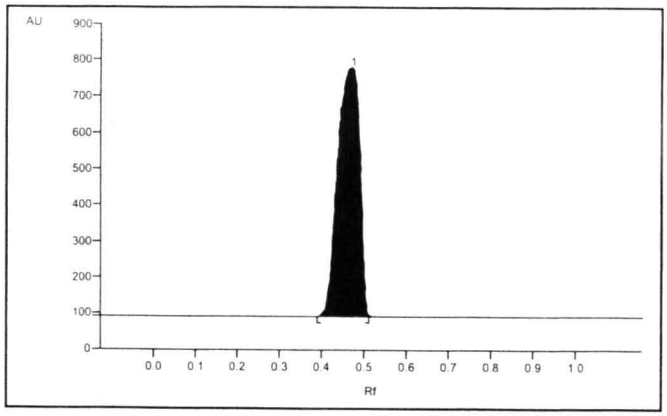

Fig. 1: Typical chromatogram of fenofibrate from marketed formulation

Analytical method validation was performed as per the ICH guidelines. The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, reproducibility of sample application and specificity/ stability. Accuracy of the method was ascertained by performing recovery studies using standard addition method. To a fixed amount of preanalysed powdered tablet sample, pure drug was added at four different levels respectively. The total amount of drug was determined by above proposed method and the amount of pure drug recovered was calculated using the formula, percent recovery=(T-A/S)x100. Precision of the analytical method is expressed as SD or RSD of series of measurement by replicate estimation of the drugs by proposed method. Repeatability of sample application was assessed by spotting fenofibrate solution six times (5μl) on a TLC plate by semiautomatic spotter, followed by

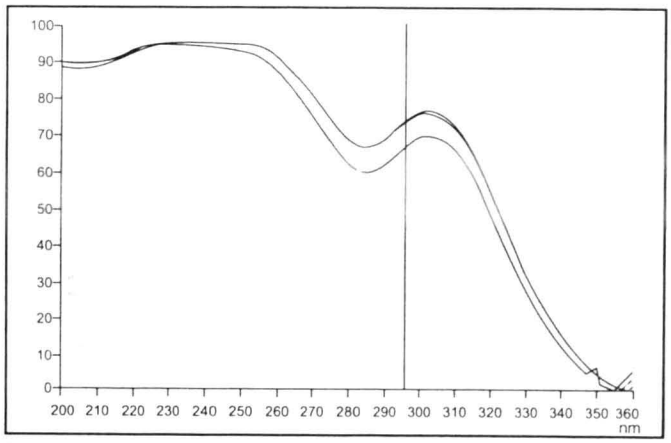

Fig. 2: Peak Purity spectra of fenofibrate.

Peak purity spectra of standard fenofibrate and sample at peak start, peak apex and peak end.

development of plate and recording the peak area and height of spots.

The intra-day precision was determined by analyzing fenofibrate sample solution 3 times on the same day, while inter-day precision was determined by analyzing corresponding solution for a period of 3 days. The specificity of the method was ascertained by spotting a sample solution of fenofibrate and developing and scanning the plate as described earlier. Purity of sample peak was ascertained by overlaying the spectra of standard fenofibrate with the spectra of sample recorded on a TLC scanner in UV range.

Literature survey indicated HPLC and GC-MS methods for analysis of fenofibrate in environmental samples and in biological fluids but not in any pharmaceutical dosage form. HPLC method is sophisticated but costly and time consuming. Therefore, it was decided to develop HPTLC method for analysis of fenofibrate as it is simple, speedy and cost effective. Fenofibrate was completely extracted from tablet matrix with methanol. Combination of toluene and chloroform offered optimum migration (Rf=0.46) and resolution of fenofibrate from other components of formulation matrix (fig. 1) The amount of fenofibrate in capsule formulation was calculated on applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. The content of fenofibrate in capsule formulation was calculated using peak area and peak height and was found to be 101.43±0.65 % and 100.7±0.96 %, respectively (Table 1). The linearity of response for fenofibrate was found in the range of 1.2 μg to 3.8 μg with a correlation coefficient 0.9994. The percent recovery was calculated using suitable dilution factor. The average recovery value was found to be 100.09 %/99.98 %. The intra-day and inter-day RSD (n=3) was found to be in the range of 0.189/1.467 and 2.77/2.96, respectively according to peak height/area. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. SD for measurement of peak area for repeatability of sample application (n=6) was found to be 0.062, ensuring proper functioning of HPTLC applicator.

TABLE 2: METHOD VALIDATION PARAMETERS

Parameters	Results
Linearity Range	1.2– 3.8μg
Correlation Coefficient	0.9994
Precision	
Repeatability SD (n=6) Height of sample application	0.046
Area	0.062
Intra-day RSD (n=3) Height	0.189
Area	1.467
Inter-day RSD (n=3) Height	2.77
Area	2.96
Specificity	Specific
Accuracy (%) Height	99.47 - 101.09
Area	98.05 – 101.07

The purity of fenofibrate peak was determined by comparing the spectra at three different levels i.e. at peak start (S), peak apex (M and peak end (E). Good correlation between these three spectra indicated the purity of fenofibrate peak (correlation, r(S, M)=0.9946, r(M, E)=0.9980, (fig. 2). The spectrum of fenofibrate extracted from capsule was also confirmed by overlaying the spectra of standard fenofibrate, which showed good correlation of 0.9994. Different validation parameters for the proposed HPTLC method for determination of fenofibrate have been summarized in Table 2. The proposed HPTLC method was found to be simple, specific, precise and accurate. Thus it can be employed for routine analysis of fenofibrate from capsules.

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