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Validated HPTLC Method for Estimation of Atorvastatin Calcium and Fenofibrate in Bulk Drug and In Tablets According To ICH Guidelines

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ABSTRACT

The paper present the development and validation of an improved method for the simultaneous analysis of Atorvastatin Calcium (ATR) and Fenofibrate (FEN) as the bulk drug and in tablet dosage forms using high-performance thin-layer chromatography (HPTLC) with densitometric detection. Separation was performed on silica gel 60F254 plates. The mobile phase is comprised of dichloromethane, toluene and methanol (2:6:2, v:v:v). Densitometric evaluation of the separated zones was performed at 287 nm. The drugs were satisfactorily resolved with RF values of 0.23±0.03 and 0.83±0.03 for ATR and FEN, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (100-600 ng per spot for ATR and 200-1400 ng per spot for FEN), precision intra-day and inter-day RSD values were always less than 1.51 for the titled drugs, accuracy (96.4% ±5% for ATR and 100.2% ±5% for FEN) and specificity, in accordance with ICH guidelines.

Keywords: Atorvastatin Calcium; Fenofibrate; HPTLC; validation.

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INTRODUCTION

Atorvastatin Calcium (ATR) is chemically, [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4-[phenyl1amino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (Fig.1).

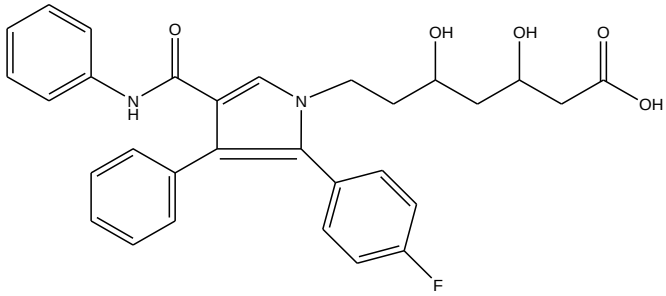

Fig. 1: Structure of Atorvastatin Calcium

ATR is a synthetic HMG –CoA reductase inhibitor [1]. It has been demonstrated to be efficacious in reducing both cholesterol and triglycerides [2]. The typical dose of ATR is 10-80 mg per day and it reduces 40-60% LDL [3]. It is used alone or in combination with statins in the treatment of hypercholesterolemia and hypertriglyceridemia [4]. Fenofibrate (FEN) is chemically propan-2-yl 2{4-[(4-chlorophenyl)-carbonyl] phenoxy}-2-methylpropanoate (Fig.2).

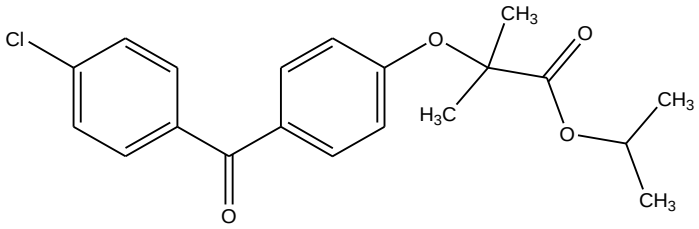

Fig. 2: Structure of Fenofibrate.

FEN is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as reducing triglycerides (TG) level. It also increases high density lipoprotein (HDL) levels [5].

Literature survey revealed that various analytical methods like spectrophotometric [6-14], HPLC [15-21] and HPTLC have been reported for the determination of ATR and FEN either individually or combination with some other drugs. Atorvastatin Calcium and Fenofibrate are available in combined dosage forms. Most methods reported in the literature for the simultaneous determination of ATR and FEN in formulations by using HPLC. However, there is lack of such equipment in many resource limited countries. In poor countries, where such equipment is available, the high costs of HPLC grade solvents and columns and the lack of the possibility to analyze many samples simultaneously, significantly affect timely release of laboratory results for action. Therefore, alternative methods are needed to facilitate and increase the speed of analysis, with relatively few costs. Cheap and quick methods using high

performance thin layer chromatography (HPTLC) have been reported in the literature [22–26]. To the best of author's knowledge, there are few methods for the determination of ATR and FEN simultaneously as the bulk drug and in tablet dosage forms using high-performance thin-layer chromatography (HPTLC). Herewith a new, simple, precise and accurate HPTLC method was developed and validated for the simultaneous determination of ATR and FEN in bulk drugs and tablet dosage form.

MATERIALS AND METHODS

EXPERIMENTAL

Materials, chemicals and equipment:

ATR and FEN reference standards were obtained from BICON Pharma Pvt. Ltd., Hyderabad (A.P., India). Fixed dose combination tablets of the two compounds (ATR & FEN) from ATROLIP-F manufacturers were bought from retail pharmacies in Aurangabad (Maharashtra, India). Dichloromethane, toluene and methanol were obtained from Merck and were of analytical grade.

A Camag HPTLC system equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland). Pre-coated silica gel 60 F_{254} TLC aluminium plates (0.2 mm thick) were obtained from E. Merck Ltd., Mumbai (India).

Method development and validation

Preparation of standard solutions

Weigh accurately 10 mg reference standard ATR and FEN individually and was dissolved in methanol and made up to 10ml in a volumetric flask separately to get the strength of 1 mg/ml. These solutions were used as Working Standard solutions for the analysis.

Method development

ATR and FEN Reference Standard solutions were prepared using methanol as solvent. The TLC plates were pre washed with methanol and activated by keeping at 115^0 C for about 30 min. Solutions of 2.0µl were applied on the TLC plates as spot bands of 8 mm using Camag Linomat V. Application positions were at least 15mm from the sides and 15mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left to saturate with mobile phase vapour for 20 min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7 cm. Then the plates were dried by hair dryer.

Densitometric scanning was done in absorbance mode at 287 nm using a deuterium lamp. The slit dimensions were set at 6mm×0.30mm, the scanning speed at 20 mm/s and the

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data resolution at 100 m/step. Single wavelength detection was performed because we are dealing with main components analyses and not impurity determinations where scanning at the individual λ max values would be preferred.

These conditions were transferred to the HPTLC system and the results were evaluated with the aim of achieving an optimum separation between spots (Rs ≥2.0) and a migration of spots with Rf values between 0.23 and 0.83 in order to ensure separation reproducibility (Fig. 3 and Fig. 4).

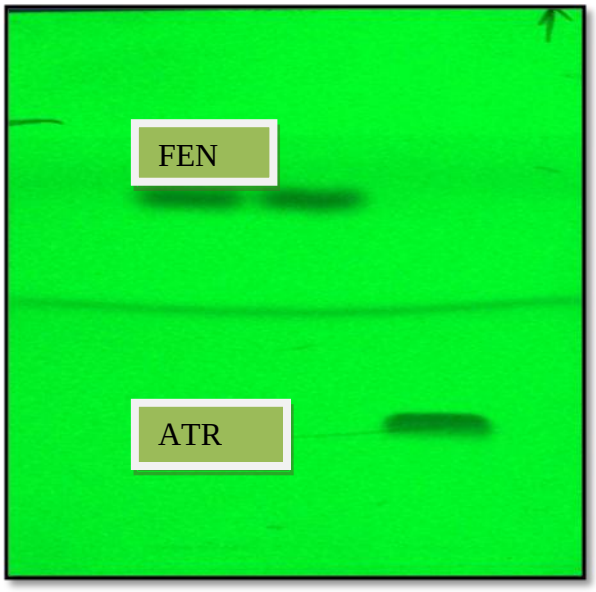

Fig. 3: Chromatogram showing resolution of Atorvastatin calcium (R_f = 0.23±0.03) and Fenofibrate (R_f = 0.83±0.02)

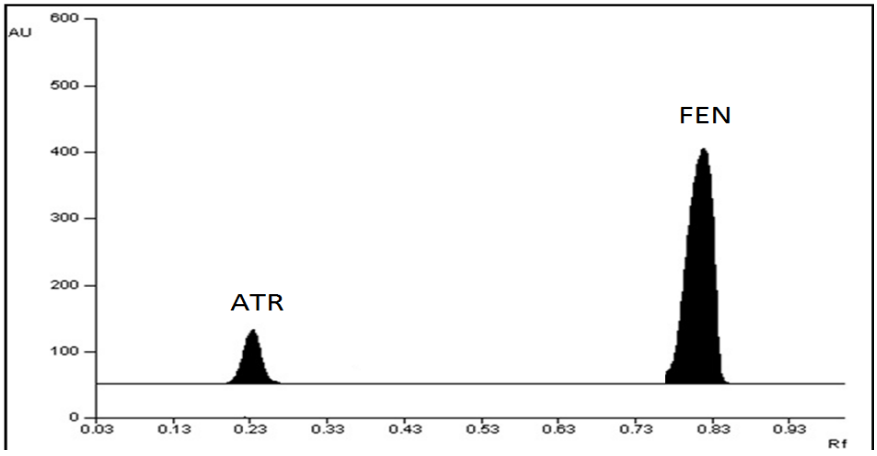

Fig. 4: Chromatogram showing resolution of Atorvastatin calcium and Fenofibrate

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Method validation

Linearity of the calibration line

A stock standard solution with 1mg/ml of each ATR and FEN were prepared in methanol. A volume of 2µl of each solution was applied on the HPTLC plate to deliver 2, 4, 6, 8, 10, 12 and 14 µl ATR per spot and 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl FEN per spot. This was done in triplicate and repeated for three days. For each concentration, the applied spot bands were evenly distributed across the plate to minimize possible variation along the silica layer (Table 1 and Table 2). The linearity was evaluated visually by looking at the calibration curves of ATR and FEN in Fig. 5 and Fig. 6 respectively.

Table 1: Observation table for calibration curve of Atorvastatin Calcium

Amount (ng / spot)	Area (cm^2)
200	1695
400	3449
600	4789
800	6148
1000	7968
1200	9413
1400	10731

Table 2: Observation table for calibration curve of Fenofibrate

Amount (ng / spot)	Area ($cm^{2)}$
100	549
200	792
300	978
400	1203
500	1452
600	1586

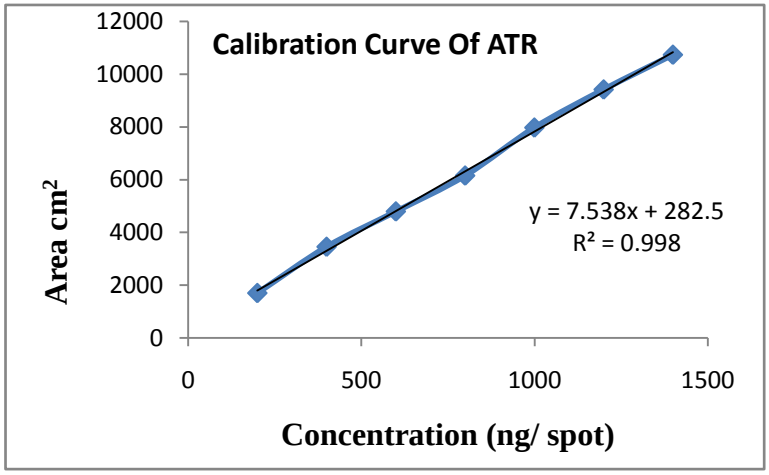

Fig. 5 : Calibration curve of Atorvastatin

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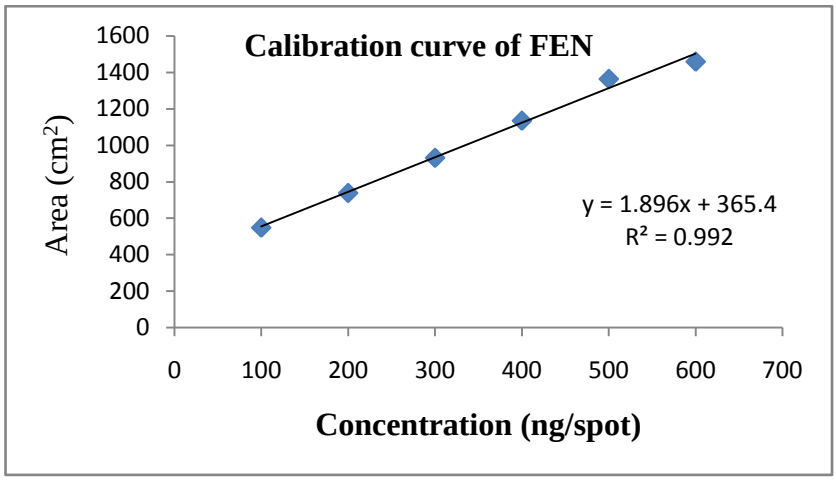

Fig. 6: Calibration curve of Fenofibrate

Precision

The repeatability and time-different intermediate precision were determined simultaneously. Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be less than 2 for both intra-day and inter-day precision. Repeatability of sample application was assessed by spotting 5 µl of drug solution, six times. From the peak areas, the percentage RSD was determined. The intra-day and inter-day accuracy and precision of ATR and FEN were shown in Table 3 and 4 respectively.

Table 3. Evaluation of intra-day and inter-day accuracy and precision of Atorvastatin Calcium

ATR taken (ng/spot)	Intraday accuracy and precision			Interaday accuracy and precision		
	ATR found (ng/spot)	RE %	RSD %	ATR found (ng/spot)	RE %	RSD %
400	412	2.5	0.18	384	2.8	0.20
600	599	1.3	0.06	585	1.7	0.09
800	805	1.8	0.07	788	0.6	0.02

Table 4. Evaluation of intra-day and inter-day accuracy and precision of Fenofibrate

FEN taken (ng/spot)	Intraday accuracy and precision			Interaday accuracy and precision		
	FEN found (ng/spot)	RE %	RSD %	FEN found (ng/spot)	RE %	RSD %
100	104	1.4	0.61	97	1.3	0.67
200	199	1.5	0.44	202	1.4	0.38
300	302	1.3	0.31	308	1.5	0.27

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Accuracy

The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (80%, 100% and 120% concentration) by addition of known amount of standard to the placebo. Solutions were prepared in triplicate and analyzed. This procedure was repeated for three consecutive days. Calibration curves to estimate the concentration of drug per spot were measured daily on the same plates as the samples. The accuracy was determined and expressed as percentage recovery (Table 5).

Table 5. Recovery Data

Level	Amount added (ng)		Amount found (ng)		% Recovery	
	ATR	FEN	ATR	FEN	ATR	FEN
80 %	320	80	307.20	80.50	96.00	100.62
100 %	400	100	388.80	102.30	97.20	102.30
120 %	480	120	460.00	117.12	95.70	97.60

Analysis of tablets samples

The method was used for quantitation of Atorvastatin calcium and Fenofibrate in tablet samples procured from local pharmacy. For sample preparation, methanol was used as solvent for extraction and dilution. Twenty tablets were ground into fine powder. Portions of powder equivalent to 10 mg of ATR were accurately weighed into a 10 ml volumetric flask. About 10 ml of methanol were added and the mixture was sonicated for 10 min. The mixture was diluted to volume with methanol, mixed well and filtered through Whattman filter paper no 41 to obtain the sample stock solution. Further dilute 1 ml of the stock solution with 10 ml of methanol to get the concentration of 0.1 mg/ml ATR and 1.450 mg /ml FEN, used as test solution for quantitative analysis of Atorvastatin from Atrolip-F tablet. 2µl of the test solution was applied on the pre-coated silica gel $60F_{254}$ plate and from the peak area obtained; the amount of Atorvastatin Calcium and Fenofibrate in formulation was simultaneously calculated using the respective calibration graph. The amount obtained per tablet and percentage label claim are shown in Table 6. Chromatogram showing ATR (peak 1) and FEN (peak 2) from the solution of spiked tablet matrix (Fig. 4) Separation was performed on silica gel $60F_{254}$ plates. The mobile phase is comprised of dichloromethane, toluene and methanol (2:6:2, v:v:v). Densitometric evaluation of the separated zones was performed at 287 nm. Chromatogram showing resolution of Atorvastatin (Rf = 0.23±0.03) and Fenofibrate (Rf = 0.83±0.02) as shown in Fig.3 and Fig.4.

Table 6: Assay Results of Tablet Dosage Form

Formulation	Actual amount (mg)		Amount Found ± SD (mg)		% of Drug Found ± SD	
Tablet	ATR	FEN	ATR	FEN	ATR	FEN
	0.20	2.90	0.20	2.88	100 ± 1.6	99.31 ± 1.5

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For the determination of Atorvastatin Calcium and Fenofibrate, sample solutions were prepared in triplicate and analyzed according to the method procedure. Sample and standard solutions were spotted on the same plate.

RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of dichloromethane, toluene and methanol (2:6:2, v:v:v) was confirmed. A good linear relationship was obtained over the concentration range 200 - 1400 ng/spot of and 100-600 ng/spot for Fenofibrate respectively. The linear regression data showed a regression coefficient of 0.996 for Atorvastatin Calcium (Fig. 5) and 0.995 for Fenofibrate (Fig. 6). The LOD with signal/ noise ratio were found to be 3.99 and 10.02 ng /spot for Atorvastatin and Fenofibrate respectively. The LOQ with signal/ noise ratio was found to be 6.99 ng and 2.51 ng /spot for Atorvastatin and Fenofibrate respectively. The repeatability showed excellent % RSD less than 2 % after six applications (Table 3 & 4). The recovery was 96.4, 97.2 and 95.7% for Atorvastatin Calcium and 100.62, 102.30 and 97.60% for Fenofibrate at 80% 100% and 120% levels (Table 5). Assay results show excellent label claim of 100 % for Atorvastatin and 99.31 % for Fenofibrate (Table 5). In conclusion, the method was considered to have an acceptable sensitivity, recovery and accuracy (Table 6).

CONCLUSION

A quick, precise and accurate method based on HPTLC has been developed. Assay results show excellent label claim of 100 % for Atorvastatin and 99.31 % for Fenofibrate (Table 6) developed for routine analysis of Atorvastatin and Fenofibrate in fixed-dose combination tablets. The method was successfully validated for linearity, precision, and accuracy. It has the advantage over HPLC methods in general. It consumes less than 35 ml of mobile phase per run (18 samples per plate), whereas HPLC methods would consume not less than 100 ml per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method takes an average of 1 hr, whereas HPLC methods would generally take more than 2 hr for the same number of samples. It is cheap and quick, therefore suitable for routine analysis of Atorvastatin and Fenofibrate in fixed-dose combination tablets.

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