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Simultaneous Estimation of Atorvastatin and Fenofibrate by HPTLC in Pharmaceutical Mixture

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Abstract: ***The paper present the development and validation of an improved method for the simultaneous analysis of Atorvastatin (ATO) 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 60 F 254 plates. The mobile phase is composed of Toluene: chloroform: methanol: glacial acetic acid (4.6: 3: 1.4: 0.1). Densitometric evaluation of the separated zones was performed at 254 nm. The drugs were satisfactorily resolved with RF values of 0.25±0.03 and 0.89±0.03 for ATO and FEN, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity 200 - 1000 ng per spot for ATO and 200 - 1000 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 ATO and 100.2% ±5% for FEN) and specificity, based on ICH guidelines.***

Keywords: Atorvastain, Fenofibrate, HPTLC

1. Introduction

1.1. Atorvastatin

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

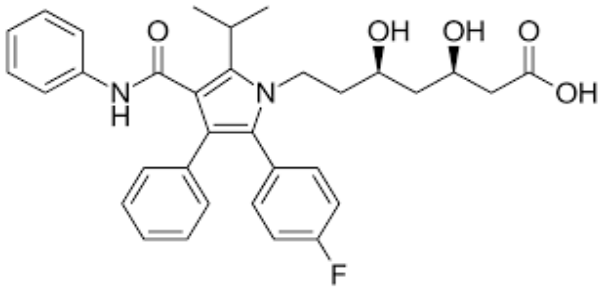

Figure 1: Chemical structure of Atorvastain

Mechanism of action: ATO is a synthetic HMG –CoA reductase inhibitor [1]. It has been determined to be efficacious in reducing both cholesterol and triglycerides [2]. The typical dose of ATO 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].

1.2 Fenofibrate

Fenofibrate is chemically propan - 2 - yl 2{4 - [(4 - chlorophenyl) - carbonyl] phenoxy} - 2 - methylpropanoate (Fig.2).

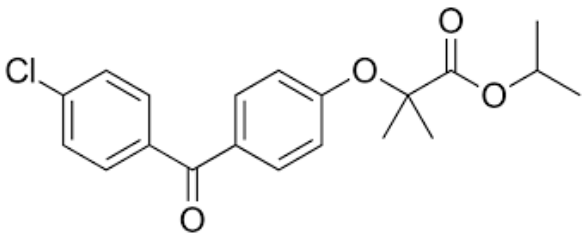

Figure 2: Chemical structure of Fenofibrate

Mechanism of action: 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 and HPTLC have been reported for the determination of ATO and FEN either individually or in combination with some other drugs. Atorvastatin and Fenofibrate are available in combined dosage forms. Many methods reported in literature for the simultaneous estimation of ATO and FEN in formulations done by HPLC. But, there is a lack of such equipment is limited in some countries. In poor countries [15 - 21], 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. Hence, alternative methods is required to facilitate and increase the speed of analysis, with relatively low costs. Cheap and quick methods using high performance thin layer chromatography (HPTLC) have been reported in the literature [22–26]. To the best of the author's knowledge, there are few methods for the determination of ATO 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 estimation of ATO and FEN in bulk drugs and tablet dosage form

2. Materials and Methods

2.1. Materials, chemicals and equipment:

ATO and FEN reference standards were obtained from Verax Lifesciences PVT. LTD., Himachal Pradesh Fixed dose combination tablets of the two compounds (ATO & FEN) from LORLIP LS - TAB manufacturers were bought from retail pharmacies in coimbatore (Tamilnadu, India). Toluene. chloroform, methanol, glacial acetic acid were obtained from Technico and were of analytical grade. Camag Linomat 5 semiautomatic sample applicator equipped with a 100µl Hamilton syringe (Camag, Switzerland) and winCATS software (CAMAG Ver.1.4.1), Camag TLC Scanner 3, Twin trough chamber instrument used. Pre - coated silica gel 60 F_{254} TLC aluminium plates (0.2 mm thick) were obtained from E. Merck Ltd., Mumbai (India).

2.2 Method development and validation

Preparation of working standard solution: - Weigh accurately 10 mg reference standard ATO 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.

2.3 Method development

Chromatographic separation was achieved on HPTLC plates (10×10 cm) pre - coated with silica gel 60 F254 of 0.2 mm thickness with aluminium sheet support. Standard solutions of markers and extracts were applied to the plates as bands of the same chromatographic plate by using of a Camag (Muttenz, Switzerland) Linomat 5 sample applicator equipped with a 100µl Hamilton syringe. Ascending development to a distance of 50 mm was performed at room temperature (24 ± 2°C) with mobile phase, in a Camag glass twin - trough chamber previously saturated with mobilephase vapour for 5 min. After development, the plates were dried and then scanned at 254 nm with a Camag TLC Scanner 3 using the deuterium lamp with win CATS software

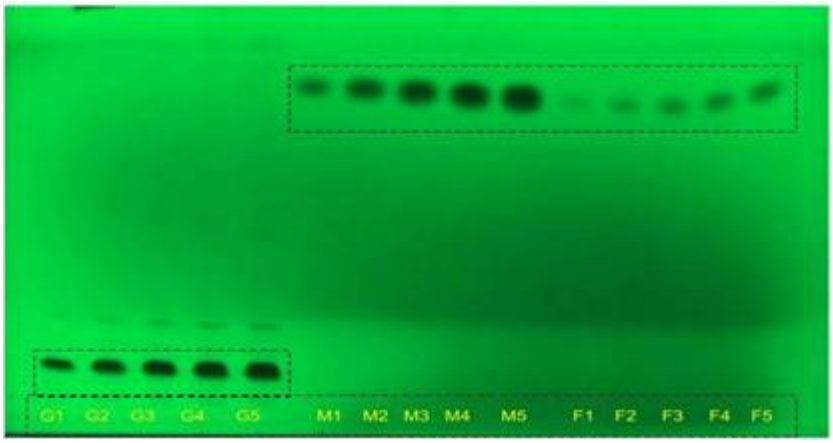

Figure 3: Chromatogram showing resolution of Atorvastatin (R_f =0.25±0.03) and Fenofibrate (R_f =0.89±0.03)

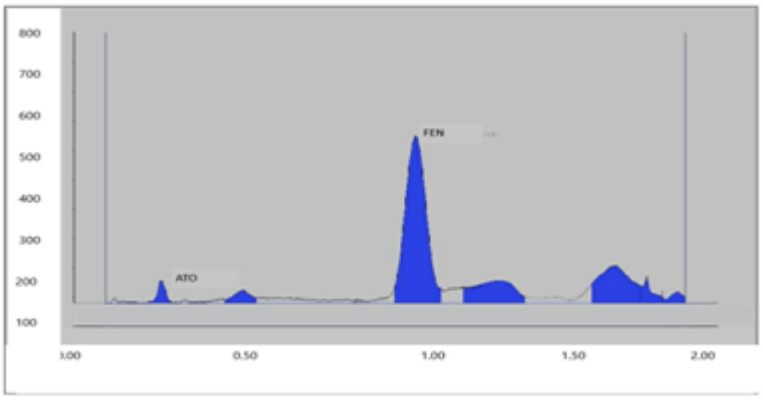

Figure 4: Chromatogram showing resolution of Atorvastatin and Fenofibrate

3. Method Validation

3.1 Linearity of the calibration line

A standard solution with 1mg/ml of each ATO 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, and10 µl ATO per spot and 2, 4, 6, 8 and 10 µl FEN per spot. This was done three times 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 calculated visually by looking at the calibration curves of ATO and FEN in Fig.5 and Fig.6 respectively.

Table 1: Observation table for calibration curve of Atorvastatin

Amount (ng / spot)	Area (cm^2)
200	19505.3
400	22175.5
600	25807.4
800	30589.2
1000	32590.6

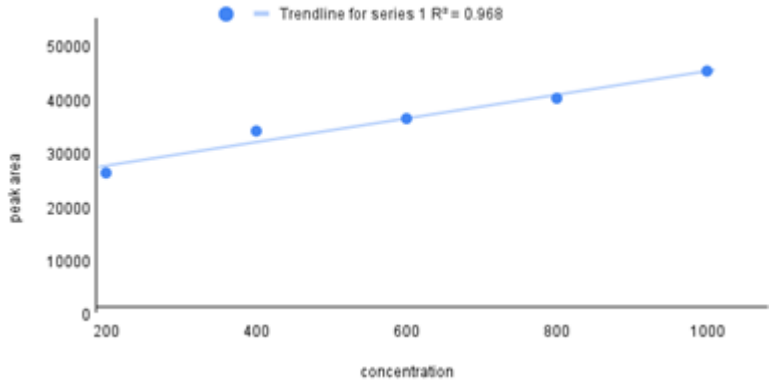

Figure 5: Calibration curve of Atorvastatin

Table 2: Observation table for calibration curve of Fenofibrate

Amount (ng / spot)	Area ($cm^{2)}$
200	26604.2
400	34416.6
600	36736.9
800	40516.9
1000	45599.3

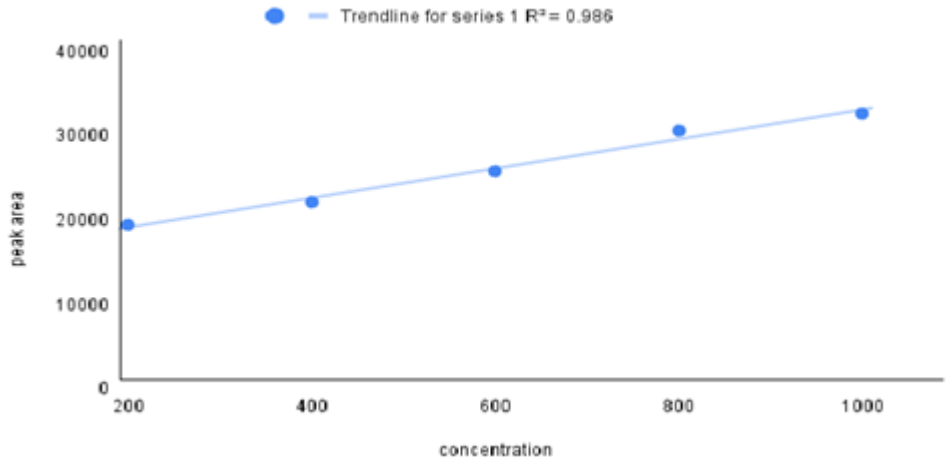

Figure 6: Calibration curve of Fenofibrate

3.2 Precision

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

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

ATO taken (ng/spot)	Intraday accuracy and precision			Interday accuracy and precision		
	ATO found (ng/spot)	RE %	RSD %	ATO 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			Interday 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

3.3 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	
	ATO	FEN	ATO	FEN	ATO	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

3.4 Analysis of tablets samples

The method was used for quantitation of Atorvastatin calcium and Fenofibrate in tablet samples procured from local pharmacy. . The weight of 10 tablets were taken and grinded with the help of pestle & mortar and the homogeneous fine powder was weighed in an Electronic balance (Afcoset). weight equivalent to 1mg was weighed for formulation. This fine powder were added into a 10ml standard flask and diluted with Methanol to get a concentration 100µg/1µl. They were further diluted with the Mobile phase to get a concentration of 1µg/1µl. These solutions were centrifuged and the supernatant liquid was taken for the HPTLC studies. The amount obtained per tablet and percentage label claim are shown in Table 6. Chromatogram showing ATO (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 Toluene: chloroform: methanol: glacial acetic acid (4.6: 3: 1.4: 0.1). Densitometric evaluation of the separated zones was performed at 254 nm. Chromatogram showing resolution of Atorvastatin (Rf = 0.25±0.03) and Fenofibrate (Rf = 0.89±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	ATO	FEN	ATO	FEN	ATO	FEN
	0.20	2.90	0.20	2.88	100 ± 1.6	99.31 ± 1.5

4. Results and Discussion

Different mobile phases were tried during the stage of method development and the mobile phase comprising Toluene: chloroform: methanol: glacial acetic acid (4.6: 3: 1.4: 0.1) of as confirmed. A good linear relationship was obtained over the concentration range 200 - 1000 ng/spot of and 200 - 1000 ng/spot for Fenofibrate respectively. The linear regression data showed a regression coefficient of 0.996 for Atorvastatin (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 and 100.62, 102.30 and 97.60% for Fenofibrate at 80% 100% and 120% levels (Table 5). Assay results show an 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).

5. Conclusion

HPTLC has been developed is very quick, precise and accurate method 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 many advantages over HPLC methods in general. It utilises less than 50 ml of mobile phase per run (15 samples per plate), whereas HPLC methods would consume not less than 100 ml per run of similar number of samples The time from sample preparation to densitometric evolution for one plate, this 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.

6. Acknowledgement

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7. Conflict of Interest

Authors declare no conflict of interest

References

[1] Remington's. The Science & Practice of Pharmacy. Wolters Kluwer Health Pvt. Ltd.2005, 21st Ed, Vol.2, pp.1369.
[2] Indian Pharmacopoeia. The Indian Pharmacopoeia Commission 2007, volume 3, pp.1676 - 1678.

[3] Goodman & Gillman's. (11th Edition). The Pharmacological Basis of Therapeutics.2006, pp.933 - 966.
[4] Dhabale PN and Gharge DS. Simultaneous Spectrophotometric Estimation of Atorvastatin and Fenofibrate in Bulk Drug and Dosage Form by using Simultaneous Equation Method International Journal of ChemTech Research 2010; 2 (1): 325 - 328.
[5] Vijayamirtharaj R, Ramesh J, Jayalakshmi B and Hashim HB. Development and validation of RP - HPLC method for the simultaneous estimation of telmisartan and atorvastatin calcium in tablet dosage forms International Journal Of Comprehensive Pharmacy 2010; 1 (4).
[6] Thamake SL, Jadhav SD, Pishawikar SA. Development and Validation of Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril from Capsule Dosage Form by First Order Derivative Spectroscopy. Asian Journal of Research In Chemistry 2009; 2 (1): 52 - 53.
[7] Shirkhedkar AA and Surana SJ. Simultaneous Densitometric TLC Analysis of Atorvastatin Calcium and Fenofibrate in the Bulk Drug and in Pharmaceutical Formulations Journal of Planar Chromatography Morden TLC; 2009; 22: 355–358.
[8] Jain N, Raghuwanshi R and Jain D. Development and Validation of RP - HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Tablet Dosage Forms Indian J Pharma Sci 2008; 70 (2): 263 - 265.
[9] Mishra P, Gupta A and Shah KSimultaneous estimation of atorvastatin calcium and amlodipine besylate from tablets Indian J Pharm Sci 2007; 69 (6): 831 - 833.
[10] Wysocki J, Belowski D, Kalina M, Kochanski L, Okopien B and Kalina Z. Effects of micronized fenofibrate on insulin resistance in patients with metabolic syndrome Int J Clin Pharma Th 2004; 42 (4): 212–217.
[11] Sonawane SS, Shirkhedkar AA, Fursule RA and Surana SJ. Simultaneous spectrophotometric estimation of atorvastatin calcium and ezetimibe in tablets Indian J Pharma Sci 2007; 69 (5): 683 - 684.
[12] Khan MR and Jain DSimultaneous spectrophotometric determination of atorvastatin calcium and amlodipine besylate in tablets Indian J Pharma Sci 2006; 68 (4): 546 - 548.
[13] Mishra P, Gupta A and Shah K. Simultaneous estimation of atorvastatin calcium and amlodipine besylate from tablets Indian J Pharma Sci 2007; 69 (6): 831 - 833.
[14] Wang L and Asgharnejad M. Second - derivative UV spectrometric determination of simvastatin in its tablet dosage form Journal of Pharmaceutical and Biomedical Anaysis 2000; 21: 1243 - 1248.
[15] Shah DA, Bhatt KK, Mehta RS, Shankar MB, Baldania SL and Gandhi TR. Development and Validation of a RP - HPLC Method for
[16] Determination of Atorvastatin Calcium and Aspirin in a
[17] Capsule Dosage Form Indian J Pharma Sci 2007; 69 (4): 546 - 549.
[18] Rajeswari RK, Sankar GG, Rao AL and Seshagirirao JV. Analytical and Bioanalytical Techniques for the Quantification of the Calcium Channel Blocker – Amlodipine: A Critical Review Indian J Pharma Sci 2006; 68 (2): 275 - 277.
[19] Yadav SS, Mhaske DV, Kakad AB, Patil BD, Kadam SS and Dhaneshwar SR. A Simple And Sensitive HPTLC For The Determination Of Content Uniformity Of Atorvastatin Calcium Tablets Indian J Pharma Sci 2005; 67 (2): 182.
[20] Chaudhari BG, Patel NM, Shah PB and Modi KP. Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe Indian J Pharma Sci 2006; 68 (6): 793 - 796.

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