Research Article

Development and validation of HPTLC method for simultaneous estimation of Fenofibrate and Rosuvastatin in tablet dosage form

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ABSTRACT

A sensitive, specific and precise high performance thin layer chromatographic method for simultaneous estimation of Fenofibrate (FEN) and Rosuvastatin (RSV) has been developed and validated. The method employed aluminium plates pre-coated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of Toluene: Chloroform: n-butanol: Formic acid (6:2:1.5:0.5, v/v) which was found to give compact and dense spots for FEN and RSV (Rf value 0.73 ± 0.02 and 0.43 ± 0.02 respectively). Densitometric analysis of both drugs was carried out in the absorbance mode at 261 nm. The method was validated with respect to linearity, specificity, precision, limit of detection, limit of quantification and recovery. The linear regression analysis data for the calibration plots showed a good linear relationship with r^2 , 0.9983 for FEN in the concentration range of 100-500 ng/spot and 0.9973 for RSV in the concentration range of 100-500 ng /spot. The LOD and LOQ for FEN were found to be 23.52 and 71.29 ng/spot respectively and for RSV, 15.68 and 47.05 ng/spot respectively. Assay results for tablet formulation were found to be 99.27 ± 1.52 % and 100.48 ± 0.69% of label claim for FEN and RSV respectively. The proposed method was found to be accurate, precise and specific and can be used for routine analysis of FEN and RSV in their combined dosage form.

Keywords: Fenofibrate (FEN), Rosuvastatin (RSV) and method validation.

1. INTRODUCTION

Fenofibrate (FEN), chemically known as 1-Methylethyl2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate (Figure 1a), is an antihyperlipidemic drug. It is official in IP, BP, and EP, all of which describe liquid chromatographic method for its estimation [1-3].

Rosuvastatin (RSV), chemically known as (E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2 {methyl(methyl–sulphonylamino)]pyrimidin-5-yl} -3,5-dihydroxyhepten-6-oicacid calcium [5] (Figure 1b) is a selective and competitive inhibitor of HMG-CoA reductase. It is official in IP, which describes liquid chromatographic method for its estimation [1].

*Central Drugs Standard Control Organization (*CDSCO) has approved FEN (67/145/160 mg) and RSV (5/10/20 mg) tablets on 23/08/2010. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of FEN and RSV in their combined dosage forms. Literature survey [7-16] shows that numbers of analytical methods are available for estimation of both the drugs either alone or in combination with other drugs. No HPTLC method has been reported for simultaneous estimation of FEN and RSV in

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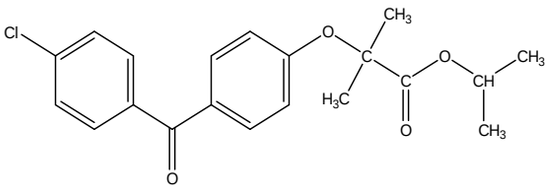
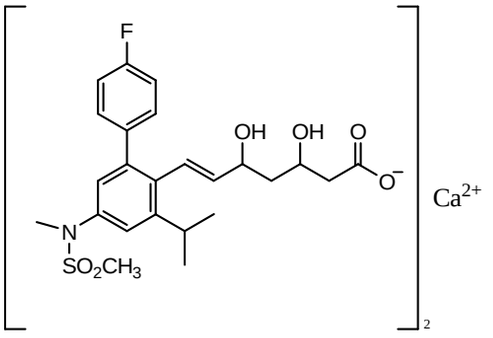
their combined dosage form. Therefore, it was thought of interest to develop and validate HPTLC method for simultaneous estimation of FEN and RSV in their combined dosage form. The present work deals with estimation of these two drugs in combined dosage form by HPTLC method.



(a)



(b)

Figure 1: Chemical structure of (a) Fenofibrate (b) Rosuvastatin Calcium

2. MATERIALS AND METHODS

2.1. Instrumentation

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV, twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, winCATS software and syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for weighing purpose.

2.2. Chemicals and reagents:

Fenofibrate was kindly supplied as a gift sample by Torrent Pharma, Ahmedabad, Gujarat, India and Rosuvastatin has been supplied by Wockhardt ltd., Aurangabad, Maharashtra, India. All chemicals and reagents used were of LR grade, purchased from S.D.Fine-Chemicals, Mumbai, India. Rolistat-F (FDC Limited/Biocon Limited) tablets containing FEN (160mg) and RSV (10mg) were procured from local pharmacy.

2.3. Chromatographic conditions:

Chromatographic separation was performed on 10 × 10 cm aluminium plates pre-coated with 250µm layer of silica gel 60 F_{254} (E. Merk, Darmstsdt, Germany). TLC plates were pre-washed with methanol and dried in oven at 70˚C for 15 min. Samples were spotted on TLC plate 15 mm from the bottom edge using Linomat V semi-automatic spotter; plate was developed and analysed using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1 µl/s; volume of mobile phase, 10 ml; temperature, 25 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. TLC plates was dried, scanned and analysed by TLC Scanner IV and winCATS software using following parameters: slit dimention,4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength 261 nm. The mobile phase consisted of toluene: chloroform: n-butanol: formic acid (6:2:1.5:0.5, v/v/v/v).

2.4. Preparation of solutions

2.4.1. Preparation of stock solution of standard FEN and RSV

Accurately weighed quantity of FEN (25mg) and RSV (25 mg) were separately dissolved and diluted with methanol up to mark in 25 ml volumetric flask, to get 1000 µg/ml of each drug. Aliquot of 1 ml of each solution was further diluted separately up to 10 ml with methanol to get solution having strength of 100 µg/ml.

2.4.2. Preparation of combined working standard solution

The combined working standard solution was prepared by mixing of 1 ml of FEN standard stock solution and 1 ml of RSV standard stock solution in to a 10 ml volumetric flask and diluted up to mark with methanol to get a solution FEN 10 µg/ml and RSV 10 µg/ml.

2.5. **Preparation of calibration curve:**

Aliquots of 10, 20, 30, 40 and 50µl of combined working standard solution were spotted on the TLC plate, plate was developed and analyzed as described under chromatographic conditions.[section 2.3] Calibration curves were obtained by plotting peak area against respective concentration of each drug.

2.6. **Method Validation** [6]

The method was validated with respect to linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ).

2.6.1. Linearity

The linearity response was determined by analyzing five independent levels of calibration curve in the concentration range of 100-500ng/spot for FEN and RSV (n=5). The calibration curves of peak area versus respective concentration were plotted and correlation coefficient and regression line equations were computed.

2.6.2. Precision

2.6.2.1. Repeatability of measurement of peak area

Combined working standard solution (30 µl) was spotted on a TLC plate, plate was developed, dried and analysed as described under chromatographic conditions [section 2.3]. The separated bands were scanned for seven times without changing plate position and RSD for measurement of peak area was calculated for each drug.

2.6.2.2. Repeatability of sample application

Combined working standard solution (30 µl) was spotted on a TLC plate seven times, plate was developed, dried and analysed as described in chromatographic conditions [section 2.3]. The peak area of each band was measured and RSD of peak area was calculated for both drugs.

2.6.2.3. Interday precision and Intraday precision

The interday precision (RSD) was determined by analysing combined working standard solution of FEN and RSV over the entire calibration range for 3 times on 3 different days and the intraday precision (RSD) was determined by analysing combined working standard solution of FEN and RSV over the entire calibration range for three times on the same day.

2.6.3. Accuracy

Accuracy of the method was determined by calculating recovery of FEN and RSV using the standard addition method. Accurately weighed quantities of standard FEN and RSV were spiked at 80, 100 and 120% level to pre-analysed sample of FEN and RSV. Percent recovery was determined in triplicate and % mean recovery was calculated.

2.6.4. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and the LOQ of the method were calculated using the following equations as per ICH guideline Q2 (R1).

LOD = 3.3 s/S and LOQ = 10 s/S

Where,
s = Standard deviation of intercept of five calibration curves
S = Mean slope of five calibration curves

2.6.5. Specificity

The specificity of the method was ascertained by analysing drugs from standard and sample.The bands of FEN and RSV from tablet formulation were confirmed by comparing their R_f and absorbance/reflectance spectrum with that from standard. The peak purity was assessed by comparing UV spectra acquired at three different positions on the band, i.e. peak start (S), peak apex (M), and peak end (E).

2.6.6. Procedure for assay of marketed formulation

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 160 mg of FEN or 10 mg RSV was accurately weighed and transferred to a 10 ml volumetric flask, 5 ml of methanol was added and the solution was sonicated for 20 minutes and diluted to 10 ml with methanol. The resulting solution was filtered through Whatman filter paper no. 41. Aliquot of 1 ml of filtrate was transferred into a 10 ml volumetric flask and diluted up to the mark with methanol. Aliquot of 1ml of resulting solution was transferred in to 10 ml volumetric flask and diluted up to mark with methanol. Two separate bands were spotted by applying 5 µl and 75 µl of resulting solution to TLC plate and the chromatogram was developed and analyzed as described under chromatographic conditions [section 2.3]. First band (5µl) was used for analysis of FEN and second band (75µl) was used for analysis of RSV. Amount of FEN and RSV from tablet formulation was calculated using calibration curve for respective drug. Analysis of sample was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Selection of wavelength

Overlay UV spectra of solutions of FEN (10 µg/ml) and RSV (10 µg/ml) in methanol over the range of 200-400 nm were recorded using methanol as blank. It was observed that both drugs showed considerable absorbance at 261 nm (Figure 2). Therefore, 261 nm was selected as wavelength of detection.

3.2. Mobile phase optimization

Different solvent systems have been tried for separation of FEN and RSV, but good separation was obtained using Toluene: Chloroform: n-butanol: formic acid (6:2:1.5:0.5, v/v/v/v). The R_f values were found to be 0.73 ± 0.02 and 0.43 ± 0.02 for FEN and RSV respectively..

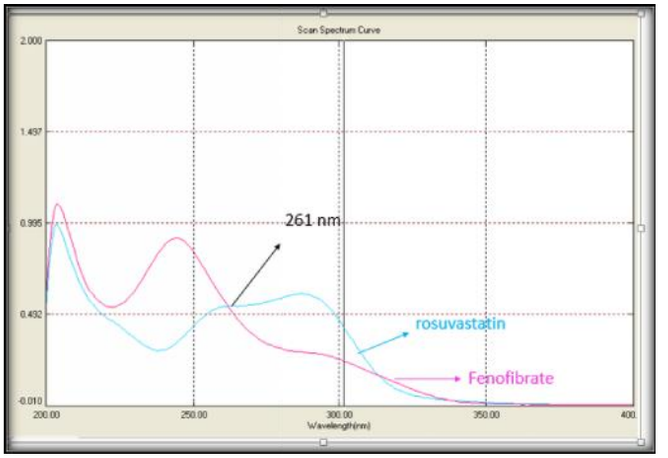


Figure 2: Overlain UV spectra of FEN (10ug/ml) and RSV (10ug/ml) in methanol

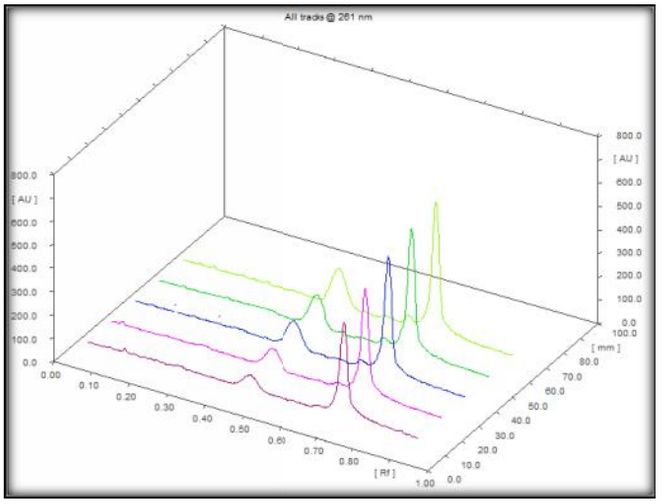


Figure 3: 3D chromatogram of calibration levels of FEN (100-500 ng/spot) and RSV (100-500 ng/spot)

3.3. Method validation

3.3.1. Linearity and range

Representative calibration curves of FEN and RSV were obtained by plotting the mean peak area of FEN and RSV against concentration over the range of concentration 100-500 ng/spot for both drugs (n=5). They were found to be linear in the above mentioned range with correlation coefficient of 0.9983 for FEN and 0.9973 for RSV. Summary of linearity data is presented in Table 1. 3D chromatogram of calibration levels is shown in Figure 3.

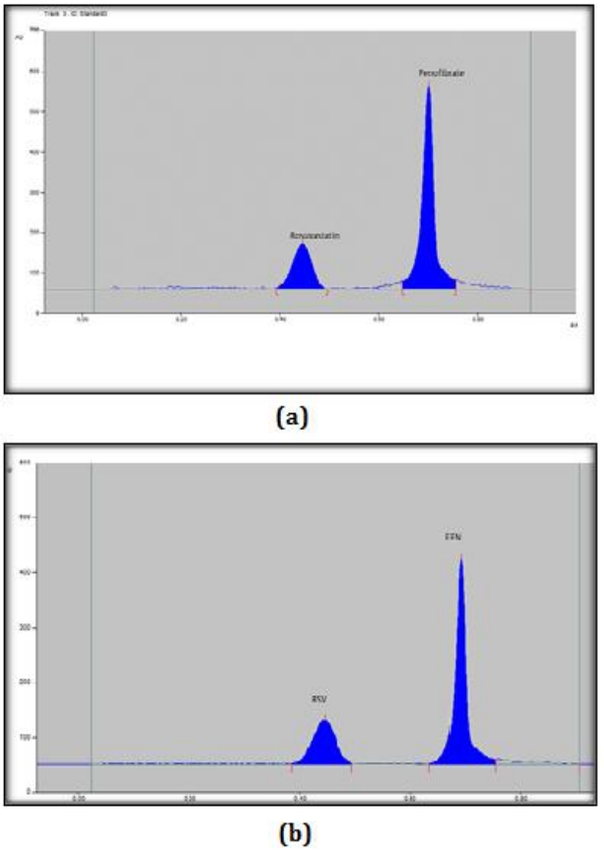


Figure 4: Densitograms of (a) FEN and RSV from standard (300 ng/spot) (b) FEN and RSV from sample measured at 261 nm.

3.3.2. Precision

Precision of instrument was checked by repeated scan of same band of respective drugs seven times without changing plate position. The RSD for repeatability of sample measurement for FEN and RSV was found to be 1.11% and 1.09%, respectively. Repeatability of sample application (RSD) for FEN and RSV was found to be 1.33% and 1.75%, respectively. Intraday precision (RSD) was found to be 1.01- 1.68% and 0.95- 1.91 % for FEN and RSV, respectively. Interday precision (RSD) was found to be 1.60-1.94% and 1.16-1.95% for FEN and RSV, respectively.

3.3.3. Accuracy

Accuracy was determined by standard addition method. The proposed method was applied for estimation of FEN and RSV in their combined pharmaceutical dosage forms after spiking with known quantity of standard drugs. The recovery of spiked sample was 98.43-101.56% and 99.20-101.20% for FEN and RSV, respectively. The results of accuracy study are presented in Table-2.

3.3.4. Limit of detection and limit of quantification

The LOD for FEN and RSV was found to be 23.52ng/spot and 15.68ng/spot respectively. The LOQ was found to be 71.29ng/spot for FEN and 47.05ng/spot for RSV.

3.3.5. Specificity

Comparison of chromatograms of FEN and RSV from standard and that from the formulation showed identical R_f values for both drugs, i.e. 0.73 ± 0.02 for FEN and 0.43 ± 0.02 for RSV (Figure 4). Excipients and other components present in tablet formulation did not interfere in separation and resolution of FEN and RSV. Apart from R_f values, the UV spectrum of individual bands from sample were also correlated with spectrum of standard drugs. Comparison of the spectra scanned at peak start(s), peak apex (m) and peak end (e) positions of individual bands of sample FEN and RSV showed a high degree of correlation [i.e., r^2 (S, M) = 0.9997 and r^2 (M, E) = 0.9999], [r^2 (S, M) = 0.9999 and r^2 (M, E) = 0.9998] respectively, confirmed the purity of the corresponding spots. Summary of the validation parameters is presented in Table 3.

3.4. **Assay of marketed formulation**

The spots at R_f 0.73 ± 0.02 for FEN and at 0.43 ± 0.02 for RSV were observed in the chromatogram of the drug sample from marketed formulation. The drug content was found to be 99.27 ± 1.52 % and 100.48 ± 0.69% of label claim for FEN and RSV respectively. There was no additional peak observed in the chromatogram of combined marketed formulations except FEN and RSV that indicate no interference of excipients in estimation of FEN and RSV in their pharmaceutical dosage form. The assay value was found in good agreement with that of the label claim. Results for assay of marketed formulation are given in Table 4.

Table 1: Linearity data for FEN and RSV

Parameters	FEN	RSV
Linearity range	100-500 (ng/spot)	100-500 (ng/spot)
Correlation coefficient	0.9983	0.9973
Slope ± S.D. (n= 5)	6.326±0.14	4.0605±0.08
Y intercept ± S.D.(n= 5)	1972.6±45.10	149.31±19.30
Regression line equation	y = 6.326x + 1972.6	y = 4.0605x + 149.31

Table 2: Recovery data for FEN and RSV

Drug	Level at which drug is spiked	Quantity of drug spiked (mg)	Quantity of drug recovered (mg)	% Recovery, Mean ± SD (n=3)
FEN	80%	128	126	98.42±7.91
(160 mg)	100%	160	162.5	101.56±7.34
	120%	192	191.3	99.63±5.44
RSV	80%	120	119.34	99.45±5.33
(150 mg)	100%	150	152.28	101.20±6.43
	120%	180	178.56	99.20±8.02

Table 3: Summary of validation parameters

Parameters	Results	
	FEN	RSV
Linearity Range	100-500 ng/spot	100-500 ng/spot
Correlation Co-efficient	0.9983	0.9973
Precision (RSD)		
o Repeatability of measurement (n=7)	1.11	1.09
o Repeatability of sample application (n=7)	1.33	1.75
o Intra-day precision (n=5)	1.01-1.68	0.95-1.91
o Inter-day precision (n=5)	1.60-1.94	1.16-1.95
Recovery	98.43-101.56	99.20-101.20
Limit of Detection (LOD)	23.52 ng/spot	15.68 ng/spot
Limit of Quantification (LOQ)	71.29 ng/spot	47.05 ng/spot

Table 4: Analysis of marketed formulation

Tablet	Label claim	Quantity of drug found(mg)	Assay value (% of label claim) (n=3)
Rolistat - F	160 mg FEN	158.83 mg	99.27 ± 15.22 %
	10 mg RSV	10.04 mg	100.48 ± 0.69%

4. CONCLUSION

HPTLC method was developed for estimation of FEN and RSV in tablet formulation. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was successfully applied for quantitative analysis of FEN and RSV in their combined tablet formulation. Results were found to be in good agreement with label claim of their combined tablet formulation. The proposed method can be applied for routine analysis of FEN and RSV in their combined dosage form.

5. ACKNOWLEDGEMENT

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