

## Simultaneous RP-HPLC Method Development and Validation of Atorvastatin, Ezetimibe and Fenofibrate

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### Abstract

A simple, accurate, precise and robust reverse phase high performance liquid chromatographic method has been developed and subsequently validated for the simultaneous estimation of atorvastatin (AT), ezetimibe (EZ) and fenofibrate (FE) in commercial formulation. The method has shown an adequate separation for AT, EZ and FE. The drugs were resolved on an enable C-18 Column (25 mm x 4.6 mm i.d, 5 µm particle size) using Shimadzu SPD-20A prominence UV-Visible detector with the mobile phase composed of acetonitrile and phosphate buffer (pH 3.3) in the ratio of 90:10% V/V as mobile phase at a flow rate of 1 mL/min and the detection was carried out at 254 nm. The retention time of AT, EZ and FE were found to be 3.155, 5.299 and 6.215 min respectively. The linearity of the proposed method was investigated in the range of 10-100 µg/mL, 10-100 µg/mL, and 160-1600 µg/mL for AT, EZ and FE, respectively. The limit of detection (LOD) was 2.18, 0.87, and 20.9 for AT, EZ and FE, respectively. The limit of quantification (LOQ) was 6.8, 2.6 and 63.6 for AT, EZ and FE, respectively. The % RSD from the precision and accuracy studies was found to be below 2%. The proposed method was statistically evaluated and can be applied in regular quality control of AT, EZ and FE in pharmaceutical dosage forms.

**Keywords:** Atorvastatin; Ezetimibe; Fenofibrate; RP-HPLC; Method validation

### Introduction

Lowering low-density lipoprotein cholesterol (LDL-C) with the use of the most potent statins improves mortality and morbidity related to cardiovascular events in patients with hypercholesterolemia. Statins, which are known as “3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors”, act to block the synthesis of cholesterol in the liver. Statin therapy has been shown to reduce the rate of major vascular events in patients with established vascular disease [1] and is considered the first line therapy for the management of dyslipidemia in such individuals [2]. These medications are generally well tolerated by the vast majority of patients, but a small number experience side effects, most seriously those of myopathies, rhabdomyolysis and elevated liver enzymes - recognition of this fact, that statins are not universally without problems, highlights the need for viable alternatives. EZ is a novel cholesterol absorption inhibitor that prevents cholesterol absorption by inhibiting the transport of cholesterol across the intestinal wall. It is an intestinal cholesterol binder that is known to have a modest effect (approximately 18%) [3] in the lowering of LDL-C. FE is a medication that also works through the liver and has long been used to control blood lipid levels in patients with mixed lipid problems. It is a fibric acid derivative that binds to peroxisome proliferator-activated receptor alpha and alters lipoprotein synthesis [4]. Treatment with FE monotherapy has also been proven to

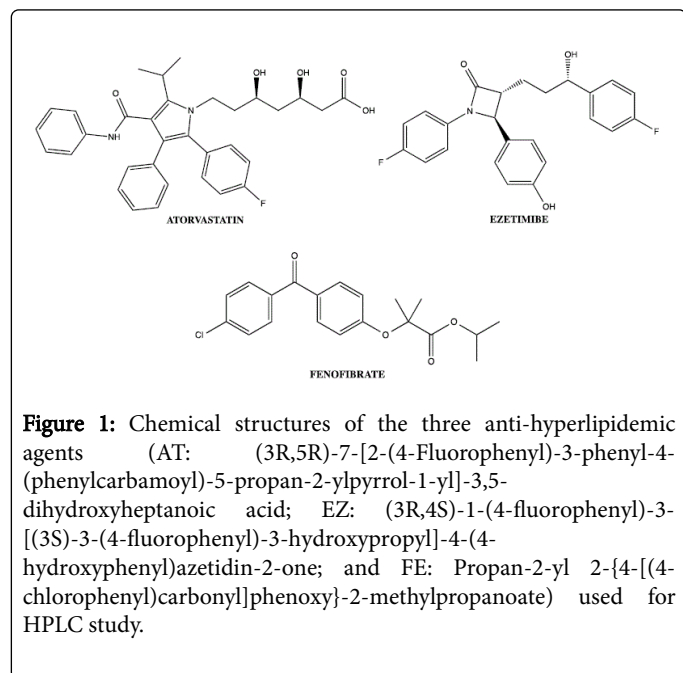
provide modest reductions in LDL-C, and may also be an effective therapeutic option for patients who are intolerant of statins [5].

In contrast to monotherapy where both EZ and FE each provide only modest effect, the combined therapy produces significantly greater reductions in LDL-C. This combination of EZ and FE in most of the cases is effective as AT and may be an effective second-line therapeutic option in patients who are intolerant to statins, but still require medication for elevated cholesterol. Recently many studies have been successfully demonstrated the effectiveness and tolerability of the co-administration of EZ and FE with statin therapy in patients with hypercholesterolemia with complex vascular disease [6]. Hence, a suitable analytical method is highly desirable for simultaneous determination of these drugs in bulk and pharmaceutical formulations.

Figure 1 presents the chemical structures of the three antihyperlipidemic agents used for HPLC study. From the literature search, it was evidenced that few analytical methods such as: RP-HPLC [7-10]. High-Performance Thin-Layer Chromatography (HPTLC) [11,12] were reported for simultaneous determination of AT, EZ, and FE. Pathak et al., reported RP-HPLC and chemometric assisted UV-spectrophotometric methods for simultaneous analysis of the three drugs in combined dosage form [13]. A gradient HPLC and a HPTLC method have been demonstrated by Varghese et al., for quantitative simultaneous determination of the said drugs. AT have been determined by multicomponent analysis of HPLC along with EZ [14-17] and FE [18]. Ashutosh et al., reported a stability-indicating RP-HPLC method for simultaneous estimation of AT and EZ in human plasma [19]. Few quantitative methods for determination of AT and

EZ using HPTLC [20-22], stability-indicating UPLC [23] methods were also reported. A spectrophotometric method for simultaneous estimation of AT and FE in binary mixture is also available.

In spite of so many methods dealt with quantification of analytes of interest available, the lack of sensitivity, complex mobile phase and gradient elution mode seem to be responsible for some drawbacks of these analytical tools. Therefore, a sensitive RP-HPLC method has been proposed for multicomponent analysis of AT, EZ, and FE in its bulk and dosage form.



**Figure 1:** Chemical structures of the three anti-hyperlipidemic agents (AT: (3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid; EZ: (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one; and FE: Propan-2-yl 2-[4-[(4-chlorophenyl)carbonyl]phenoxy]-2-methylpropanoate) used for HPLC study.

## Experimental

### Chemicals and reagents

Standard AT, EZ and FE samples were obtained as gift samples from Lee Pharma Ltd., Hyderabad, Telangana. Acetonitrile (HPLC grade) and Potassium dihydrogen phosphate of AR grade were obtained from Merck, Mumbai. High purity deionised water was obtained by double distillation and purification through milli-Q water purification system. The tablet formulation (FibatorR EZ Sun pharma.) containing 10 mg of EZ, 10 mg of AT and 160 mg of FE was procured from local market and used for analysis of marketed formulation.

### Instrumentation

The liquid chromatographic system was a Binary Shimadzu prominence HPLC System in gradient mode with a 20  $\mu$ L sample injection loop (manual) and SPD 20A Photo diode array UV-Visible detector. The output signal was monitored and integrated using LC solutions software on an enable C18G column (250 mmX4.6 mm i.d, 5  $\mu$ m particle size). High purity deionized water was obtained by double distillation and purification through milli-Q water purification system. The 0.45  $\mu$ m membrane filter was purchased from Advanced Microdevice Pvt Ltd., Chandigarh. In addition, an Analytical balance (Shimadzu uniblock ATX 124, 0.1 mg sensitivity), a sonicator (SONICA, Spincotech Pvt Ltd.) were used in this study.

### Preparation of buffer solution

Potassium dihydrogen orthophosphate (100 g) was dissolved in 800 mL of HPLC grade water and pH 3.3 was adjusted with Hydrochloric acid and finally makes up to 1000 mL with HPLC grade water.

### The mobile phase

A mixture of potassium dihydrogen phosphate buffer pH 3.3 and acetonitrile in the ratio of 90:10 v/v was prepared and used as mobile phase.

### The diluent

Acetonitrile was used as diluent.

Preparation of stock and standard solutions.

Stock solution of AT calcium (equivalent to 1000  $\mu$ g/mL), EZ (equivalent to 1000  $\mu$ g/mL) and FE (equivalent to 16000  $\mu$ g/mL) were prepared in acetonitrile. Aliquots of standard stock solutions of AT, EZ and FE were transferred using A-grade bulb pipettes into 100 mL volumetric flask and the solution was made up to volume with acetonitrile to yield final concentration of 10,20,40,80 and 100  $\mu$ g/mL for AT; 10,20,40,80 and 100  $\mu$ g/mL for EZ and 160,320,640,1280 and 1600  $\mu$ g/mL for FE.

### Chromatographic separation

An Enable C-18 (250x4.6 mm; 5  $\mu$ m) analytical column (25°C column temperature) was used with a mobile phase comprised of phosphate buffer (pH 3.3) and acetonitrile (10:90) pumped at a flow rate of 1.00 mL/min for the HPLC separation of the three analytes in mixture. The detection of the column effluents was realized on a photo diode array detector set at 265 nm. The sample injection volume was 20  $\mu$ L.

### Method Validation

At the optimal condition, the proposed HPLC method has been validated with respect to the following parameters outlined by ICH [ICH Q2 (R1), 2005]:

#### Linearity

Suitable dilutions from the working standard solution were prepared to yield a series of solutions in the concentration range of 10-100  $\mu$ g/mL, 10-100  $\mu$ g/mL and 160-1280  $\mu$ g/mL for AT, EZ and FE, respectively. The resultant solutions were chronologically injected in triplicate into the HPLC column. A calibration curve was constructed thereby plotting the corresponding peak areas against the concentrations to obtain regression equation and correlation coefficient, which is used to indicate the linearity of the method.

#### Detection and quantitation limits

The limit of detection (LOD) and limit of quantification (LOQ) represent the concentration of the analyte that would yield a signal-to-noise ratio of 3 and 10, respectively.

#### Precision

Intra-day precision of the method was determined using quality control samples (40, 40 and 640  $\mu$ g/mL of AT, EZ and FE, respectively)

each six injections on the same day and percentage relative standard deviation (% RSD) were calculated. Furthermore, these experiments were repeated on three consecutive days to assess inter-day precision.

### Accuracy

Accuracy was determined by analyzing a known concentration of drug, viz., 80%, 100%, and 120% spiked with formulation in triplicate and then determining the percent recovery.

### Robustness

The robustness study was carried out to assess the influence of minor variations in the optimal chromatographic factors. A deliberate variations in the separation parameters i.e., flow rate ( $\pm 0.1$  mL/min), percent acetonitrile in mobile phase ( $\pm 2\%$ ) were experimented. The resultant responses to the variations were statistically compared with the proposed method.

### Assay of commercial formulation

For assay of formulation (fibator, Sun Pharmaceutical Industries Ltd.) was procured from the local market. For sample preparation, 20 tablets containing AT, EZ and FE from the same batch were weighed and finely powdered. Thereafter, tablet powder equivalent to 5 mg of AT, 5 mg of EZ and 80 mg of FE, was transferred into a 100 mL volumetric flask and dissolved in acetonitrile. After the immediate dissolution, the volume was made up to the mark with same solvent and filtered with 0.45  $\mu$ M filter to get the stock solution. 5 mL of stock solution was further diluted up to 50 mL and filtered to get the working standard solution. The filtrate was then injected onto the HPLC under the optimal chromatographic condition. The % assay of the formulation by the method was determined by using equation:

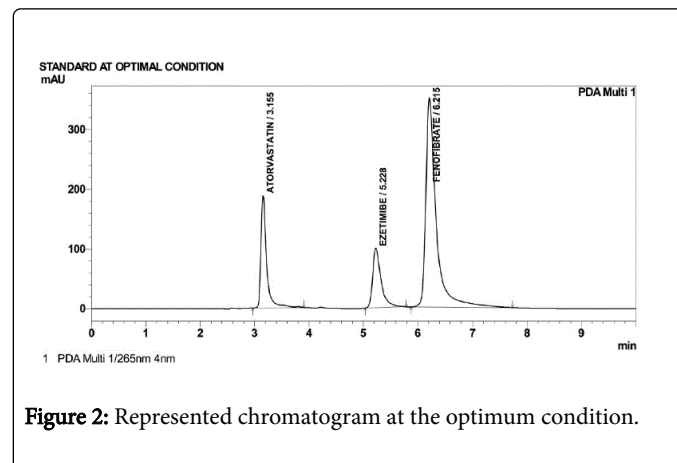
$$\% \text{Assay} = \frac{\text{Sample area} \times \text{Standard weight} \times \text{Average weight of 20 tablets}}{\text{Standard area} \times \text{Sample weight} \times \text{Label claim}}$$

## Results and Discussion

### Development and optimization of the method

Column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. Separation with good resolutions was studied on different type's columns (C8 and C18). The mobile phase conditions were optimized so that the components were not interfered from the solvent and excipients. Several buffer systems at different pH values were trailed in various ratios with MeOH, and MeCN as mobile phase. Mobile phase and flow rate selection was based on peak parameters (height, area, tailing, theoretical plates, capacity factor and resolution) and run time. Decisively after several experimental trials, the best result was obtained by use of 90:10 (v/v) ratios of acetonitrile and buffer with 1.0 mL/min flow rate. Among different buffer pH values, better selectivity and good peak parameters were observed at phosphate buffer pH 3.3. The suitable wavelength considered for monitoring the drugs was 265 nm. Solutions of AT, EZ and FE in diluent were also injected directly for HPLC analysis and the responses (peak area) were recorded. It was observed that there was no interference from the mobile phase or baseline disturbances and all the analytes absorbed well at 265 nm. The obtained chromatograms of the mixture sample indicate that separation of all the analytes was successful. The typical chromatogram

at the optimum condition is depicted in Figure 2. Under the optimum chromatographic conditions, there retention time obtained for AT, EZ and FE was 3.155, 5.299 and 6.215 min, respectively. The system suitability parameters (retention time, tailing factor, Number of theoretical plates and resolution) obtained is found to be within the acceptance criteria indicating the fitness of the method for separation and determination of the compounds.



**Figure 2:** Represented chromatogram at the optimum condition.

### Validation of the method

The analytical method was validated with respect to the following parameters:

#### Linearity

The peak areas for drug samples were linear in the concentration range between 10 to 100  $\mu$ g/mL, 10 to 100  $\mu$ g/mL and 160 to 1600  $\mu$ g/mL for AT, EZ and FE, respectively. The data were analyzed with least squares linear regression indicating good linearity for all the analytes ( $R^2=0.999$ ). Typically, the regression equations were:  $y=5016.8x + 2534.5$  ( $R=0.9998$ ),  $y=5043x - 118.57$  ( $R^2=1$ ),  $y=5098x - 572.39$  ( $R^2=0.9994$ ) for AT, EZ and FE, respectively.

#### LOQ and LOD

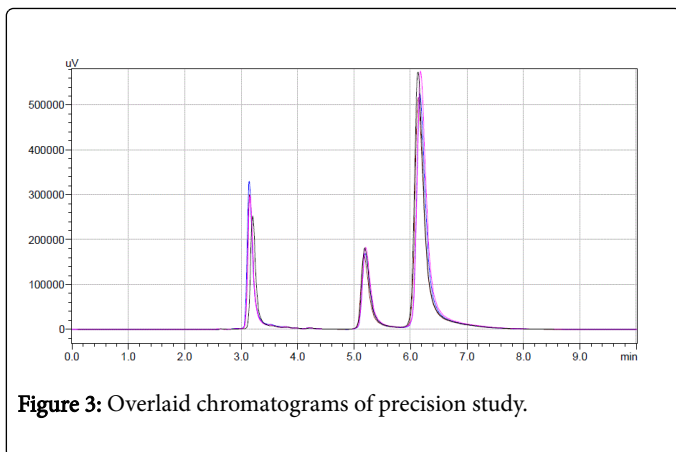
The method sensitiveness was explained by establishing the LOD and LOQ of 2.186  $\mu$ g/mL and 6.893  $\mu$ g/mL for AT, 0.877  $\mu$ g/mL and 2.65  $\mu$ g/mL for EZ and 20.99  $\mu$ g/mL and 63.61  $\mu$ g/mL for FE, respectively. The obtained LOD and LOQ results demonstrate that the method is highly sensitive as compared to the reported methods.

#### Precision

The data attained from precision study is presented in Table 1 for intra- and inter-day precision experiments. The calculated %R.S.D values for intra-day precision study were  $<0.27\%$  and for inter-day study were  $<0.20\%$ , proving that the method was suitably precise. The overlaid chromatograms of the study are depicted in Figure 3.

#### Accuracy

The mean percentage recovery from the accuracy study was calculated for fortified and unfortified solutions. Excellent recoveries were obtained ( $>98.8\%$ ) at each added concentration. The represented data were shown in Table 2.



**Figure 3:** Overlaid chromatograms of precision study.

Drug	Intra-day (n=6)	Inter-day (n=6)
	Peak area (mean $\pm$ SD; %RSD)	Peak area (mean $\pm$ SD; %RSD)
AT	201932.33 $\pm$ 47.42; 0.023	201872.5 $\pm$ 43.41; 0.021
EZ	199084.5 $\pm$ 544.20; 0.27	199252 $\pm$ 406.47; 0.20
FE	3224740.3 $\pm$ 2658.38; 0.08	3225015.2 $\pm$ 2017.5; 0.06

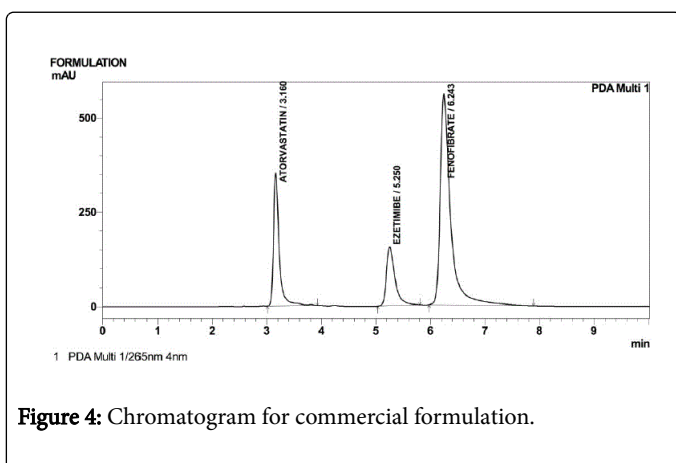
**Table 1:** Intra and Inter-day precision data.

### Assay of formulation

The amounts of drugs present in the tablet dosage forms were calculated to be >99.21 % for all the three analytes. The represented chromatogram for formulation was shown in Figure 4.

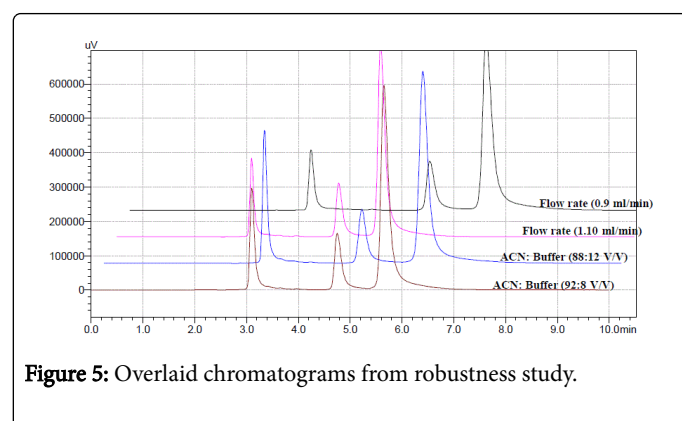
Compound	Level	Mean peak area $\pm$ S.D; %RSD (n=3)		% Recovery	% Accuracy
		Standard	Spiking		
AT	80%	161515 $\pm$ 69.081; 0.04	163562.33 $\pm$ 43.09; 0.02	99.09	101.26
	100%	201935 $\pm$ 45.791; 0.02	204187.66 $\pm$ 2036.70; 0.99	99.35	101.11
	120%	242228.6 $\pm$ 211.09; 0.08	245539 $\pm$ 3095; 1.26	99.5	101.3
EZ	80%	159496.6 $\pm$ 79.538; 0.05	161317 $\pm$ 1761.82; 1.09	98.9	101.1
	100%	199390 $\pm$ 45.981; 0.02	201715.66 $\pm$ 2698.21; 1.33	98.9	101.16
	120%	239347.3 $\pm$ 48.80; 0.02	246679.3 $\pm$ 2079.10; 0.84	98.9	103.06
FE	80%	2580694.6 $\pm$ 351.923; 0.01	2541360.93 $\pm$ 20033; 0.788	98.8	98.4
	100%	3225502.6 $\pm$ 786.7; 0.02	3258835.46 $\pm$ 20816.72; 0.63	98.8	101.03
	120%	3870750.8 $\pm$ 837.23; 0.02	3881417.46 $\pm$ 10016.65; 0.25	98.87	100.27

**Table 2:** Accuracy study of AT, EZ and FE.



**Figure 4:** Chromatogram for commercial formulation.

resulted overlaid chromatograms from the robustness study are shown in Figure 5.



**Figure 5:** Overlaid chromatograms from robustness study.

### Robustness

The method was found to be sufficiently robust under the tested conditions (Table 3). There was no significant change in the total analysis time, resolution and tailing factor of AT, EZ and FE. The

Parameter	Level	Rs (EZ-AT)	Rs (FE-EZ)	Asf (FE)	Rt (FE)
Nominal	0	8.988	3.39	1.438	6.215



Flow rate (mL/min) <sup>a</sup>	-1 (0.9)	9.054 ± 0.004; 0.052	3.347 ± 0.007; 0.225	1.651 ± 0.002; 0.121	6.885 ± 0.016; 0.234
	+1 (1.1)	8.095 ± 0.006; 0.074	3.002 ± 0.013; 0.433	1.474 ± 0.011; 0.780	5.695 ± 0.007; 0.132
% Acetonitrile in mobile phase <sup>a</sup>	-2 (08)	7.974 ± 0.008; 0.111	3.853 ± 0.006; 0.172	1.444 ± 0.024; 1.666	6.174 ± 0.024; 0.402
	+2 (12)	7.622 ± 0.053; 0.706	3.311 ± 0.005; 0.168	1.572 ± 0.010; 0.667	5.665 ± 0.009; 0.159
aMean peak area ± SD; %RSD (n=3)					
Where, Rs (EZ-AT) is resolution between EZ and AT; Rs (FE-EZ) is resolution between FE and EZ; Asf (FE) is tailing factor of FE; and Rt (FE) is retention time of FE (total analysis time).					

**Table 3:** Robustness study.

### System suitability study

System suitability is an integral part of the method development and is used to ensure adequate performance of chromatographic system. The system suitability test (SST) parameters are presented in Table 4. From the SST results, it has been confirmed that the system was deemed to be suitable as it complies with the limits of peak parameters. Resolution (>3.33), peak asymmetry (<2.0) and total analysis time of 6.215 min confirms the good selectivity of the method.

Parameter	Values obtained			Preferable values
	AT	EZ	FE	
Retention time (min)	3.155	5.229	6.215	<10min
Theoretical plates (N)	30628	38459	41036	>2500
Resolution (R)	-	8.988	3.33	>1.5
Tailing factor	1.565	1.601	1.684	<2.0

**Table 4:** System suitability parameters.

### Conclusion

A simple and efficient reversed-phase HPLC method was developed for simultaneous determination of AT, EZ and FE in bulk and tablet formulation and the method was validated according to ICH guideline requirements. The method was found to be precise, accurate, linear, sensitive & robust during validation. Satisfactory results were obtained from the validation of the method. The method can be recommended for routine analysis.

### References

- Collins R, Armitage J, Parish S (2002) MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet* 360: 7-22.
- Genest J, McPherson R, Frolich J, Anderson T, Campbell N, et al. (2009) Canadian Cardiovascular Society/Canadian guidelines for the diagnosis

- and treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. *Can J Cardiol* 25: 567-579.
- Knopp RH, Gitter H, Truitt T, Bays H, Manion CV, et al. (2003) Ezetimibe study group. Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur Heart J* 24: 729-741.
- Kosoglou T, Statkevich P, Fruchart JC, Pember LC, Reyderman L, et al. (2004) Pharmacodynamic and pharmacokinetic interaction between fenofibrate and ezetimibe. *Curr Med Res Opin* 20: 1197-207.
- Ramjattan BR, Callaghan DJG, Theiss U (2002) Efficacy and tolerability of a "suprabioavailable" formulation of fenofibrate in patients with dyslipidemia: A pooled analysis of two open-label trials. *Clin Ther* 24: 1105-1116.
- Shoba SK, Lahey KA, Day A, LaHaye SA (2009) Comparison of the efficacy of administering a combination of ezetimibe plus fenofibrate versus atorvastatin monotherapy in the treatment of dyslipidemia. *Lipids Health Dis* 8: 56.
- Patel A, Macwana C, Parmar V, Patel S (2012) Simultaneous determination of atorvastatin calcium, ezetimibe, and fenofibrate in a tablet formulation by HPLC. *J AOAC Int* 95: 419-23.
- Choudhari VP, Nikalje AP (2010) Simultaneous Estimation of Atorvastatin, Ezetimibe and Fenofibrate in Pharmaceutical Formulation by RP-LC-PDA. *Pharm Anal Acta* 1: 111.
- Abhijit D, Deshpande PB, Gandhi SV, Nair S, Vanjari S (2013) A Simple and Sensitive RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium, Ezetimibe and Fenofibrate in Combined Tablet Dosage Form. *Res J Pharm Technol* 6: 1085-1088.
- Pavankumar KLNNSVK, Rao AS, Satyanarayana P, Sastry GS (2015) Determination of Atorvastatin, Ezetimibe and Fenofibrate in Combined Pharmaceutical Dosage Form Using High-Performance Liquid Chromatography. *Int J Clin Pharm* 4: 1504-1514.
- Macwana CR, Patela AJ, Parmar VM, Patel SG (2012) Simultaneous HPTLC analysis of Atorvastatin calcium, Ezetimibe, and Fenofibrate in tablet dosage forms. *J Liq Chromatogr Relat Technol* 35: 524-532.
- Nikalje AG, Choudhari VP (2011) Validated TLC method for simultaneous quantitation of atorvastatin, ezetimibe, and fenofibrate in bulk drug and formulations. *Acta Chromatographica* 23: 267-280.
- Pathak A, Rajput SJ, Gamit RS (2011) RP-HPLC and chemometric assisted UV-spectrophotometric methods for simultaneous in vitro analysis of atorvastatin calcium, ezetimibe and fenofibrate in their pharmaceutical formulation. *Indo Am J Pharm Res* 1: 61-77.
- Reddy SJ, Rao RK, Narayana S, Rao PR (2010) Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Formulations by RP-HPLC Method. *Der Pharmacia Lettre* 2: 427-436.
- Seshachalam U, Kothapally CB (2008) HPLC Analysis for Simultaneous Determination of Atorvastatin and Ezetimibe in Pharmaceutical Formulations. *J Liq Chromatogr Relat Technol* 31: 714-721.
- Mani G, Pushparaj H, Kavimani S, Mei MP, Gil LS (2012) Simultaneous Estimation of Atorvastatin and Ezetimibe in Combined Formulation by RP-HPLC. *Asian J Chem* 24: 1867-1871.
- Ashutosh KS, Debnath M, Seshagiri Rao JVLN (2014) New Validated RP-HPLC Analytical Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in Bulk Samples as Well in Tablet Dosage Forms by Using PDA Detector. *Curr Drug Discov Technol* 11: 259-270.
- Patil K, Narkhede S, Navade V, Sapkale P, Deshmukh T (2015) Development of RP-HPLC Method for Simultaneous Estimation and Validation for Atorvastatin and Fenofibrate in Bulk and Tablet Dosage Form. *Indo Am J Pharm Res* 5: 3776-3784.
- Ashutosh SK, Debnath M, Seshagiri Rao JVLN, Sankar DG (2015) New Validated Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in Human Plasma by Using PDA Detector. *Adv Pharm Bull* 5: 385-391.
- Chaudhari BG, Patel NM, Shah PB, Modi KP (2006) Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe. *Indian J Pharm Sci* 68: 793-796.

- 
21. Baghdady YZ, Al-Ghobashy MA, Abdel-Aleem AE, Weshahy SA (2013) Spectrophotometric and TLC-densitometric methods for the simultaneous determination of Ezetimibe and Atorvastatin calcium. *J Adv Res* 4: 51-59.
  22. Dhaneshwar SS, Dhaneshwar SR, Deshpande P, Patil M (2007) Development and validation of a method for simultaneous densitometric estimation of atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms. *Acta Chromatographica* 19: 141-148.
  23. Goel A, Baboota S, Sahni JK, Srinivas KS, Gupta RS, et al. (2013) Development and Validation of Stability-Indicating Assay Method by UPLC for a Fixed Dose Combination of Atorvastatin and Ezetimibe. *J Chromatogr Sci* 51: 222-228.