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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF EZETIMIBE AND FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Sk. JAMEELA*, G. NAGA RAJU, M. SIVA PRASAD, P. PRACHET, N. RAMARAO

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur-522034

***Corresponding Author: Shaik Jameela: E Mail: sk.jameela0525@gmail.com**

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ABSTRACT

The primary goal of this research work was to develop a simple, precise, accurate and specific method for the estimation of Ezetimibe and Fenofibrate in bulk and pharmaceutical dosage form by RP-HPLC. Method development was carried out by HPLC autosampler Schimadzu 2030C 3D Plus with a stationary phase of YMC C_8 column(150mmx 4.6mm.ld,3µ,12nm) using mobile phase Acetonitrile: Methanol: Phosphate buffer adjusted to pH 3 with OPA in the ratio 55:15:30v/v/v at a flow rate 1.0mL/min with the injection volume of 20µL and detection is carried out at 255nm. The retention time for Ezetimibe and Fenofibrate was found to be2.458minutes and 7.488minutes. The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines with respect to system suitability, specificity, accuracy, precision, linearity range, limit of detection (LOD), limit of quantification (LOQ) and robustness. The % recovery ranged between 98-102% and %RSD was <2%. Linearity was observed in concentration range of 2-10µg/mL for Ezetimibe and 60-180µg/mL for Fenofibrate with a correlation coefficient 0.9998 for Ezetimibe and 0.999 for Fenofibrate. Hence can be used for routine analysis in industries and institutes.

Keywords: Fenofibrate, Ezetimibe, RP-HPLC

INTRODUCTION

Ezetimibe is a novel cholesterol-lowering drug that acts at the brush border of the small intestine, which works by preventing the absorption of cholesterol in the intestine **[1]**. Ezetimibe (EZM) is chemically (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl)] azetidine-2-one **[2]**. The dose of Ezetimibe is 10 mg daily, when added on to statin therapy there is an increase in the lipid lowering effect **[3]**. The chemical structure of Ezetimibe was shown in **Figure 1**.

Fenofibrate is a of derivative fibric acid used in the treatment of primary hyper cholesterolaemia, mixed dyslipidaemia and hyper triglyceridaemia **[4]**. Its lipid-modifying effects are mediated by activation of peroxisome proliferator-activated receptor-α (PPARα) **[5]**. Fenofibrate (FNB) is chemically propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2 methylpropanoate **[6]**. The dose of Fenofibrate is 200mg daily, as an adjunct to diet for the treatment of severe hypertriglyceridemia **[7]**. The chemical structure of fenofibrate was shown in **Figure 2**.

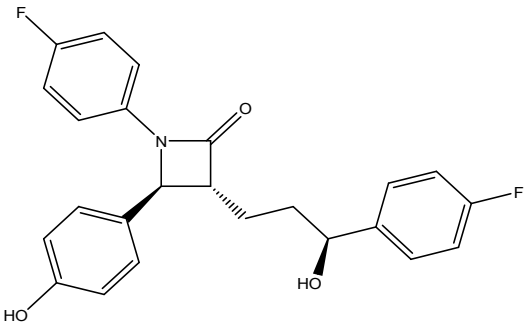

Figure 1: Chemical structure of Ezetimibe

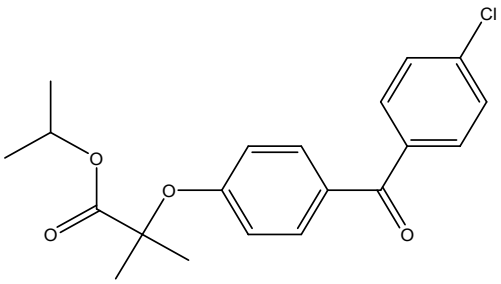

Figure 2: Chemical structure of Fenofibrate

Literature studies on antihyperlipidemic agents showed administration of EZM and FNB in combination provides effective control of LDL cholesterol and TG levels when compared to treatment alone **[8]**. Therefore a pharmaceutical dosage form containing both the drugs was prepared in the ratio of 10:200 (EZM: FNB) using the

most commonly used excipients like magnesium stearate, microcrystalline cellulose.

Various methods such as, UV Spectrophotometry **[9]**, HPLC **[10]**, HPTLC **[11]**, methods have been reported for individual drugs in formulation. An attempt has been made to develop a simple, rapid and accurate RP-HPLC method for simultaneous estimation of fenofibrate and ezetimibe from its pharmaceutical dosage form.

METHODOLOGY:

Materials and methods:

Chemicals used:

Pharmaceutical compounds (API) Ezetimibe and Fenofibrate,Acetonitrile, Methanol, Potassium dihydrogen phosphate.

Instrument:

HPLC 2030 C 3D Plus Schimadzu, prominence-i serieswith PDA detector consists of Lab solutions software with YMC C8 Column (150 x 4.6mm.ld S-3µm,12nm).

Preparation of Mobile Phase:

The mobile phase consisting of Phosphate buffer adjusted to pH3 with 0.1%OPA filtered through 0.45µ membrane filter paper and sonicated for 10min.

Acetonitrile:Methanol:Phosphate buffer in ratio of 55:15:30v/v/v.

Preparation of standard stock solution of Ezetimibe and Fenofibrate:

About 10mg of Ezetimibe and Fenofibratewas accurately weighed individually and transferred in to 10mL volumetric flask add methanol to dissolve and the solution was made up to the volume with methanol to obtain 1000µg/mL.

Preparation of sample solution:

About 10mg of tablet powder was accurately weighed which was calculated based on average weight of 20 tablets and was dissolved using methanol in 10ml volumetric flask. From the above stock solution 0.1mL was pipette out into 10mL volumetric flask and the solution was made up to the volume with methanol to obtain 10µg/mL.

Chromatographic conditions:

Mobile phase: Acetonitrile:Methanol:Potassium dihydrogen ortho phosphate buffer adjusted to pH 3 with 0.1%OPA (55:15:30v/v/v).

Flow rate: 1mL/min

Column: YMC C_8(150mm X 4.6mm, 3µm)

Detector wavelength: 255nm

Injection Volume: 20µL

Run time: 10min

Method Validation:

The following parameters were validated according to ICH guidelines include System suitability, Specificity, Linearity, Accuracy, Precision, Limit of detection, Limit of quantification and Robustness.

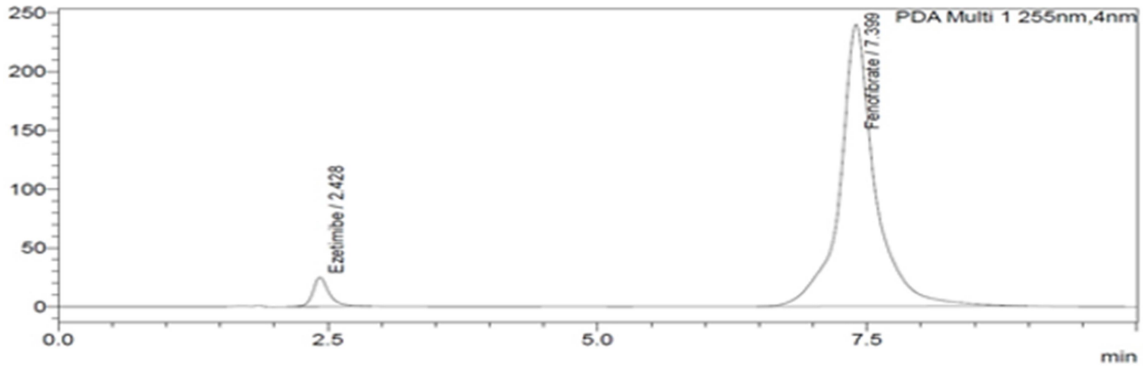

Figure 3: Standard chromatogram of Ezetimibe and Fenofibrate

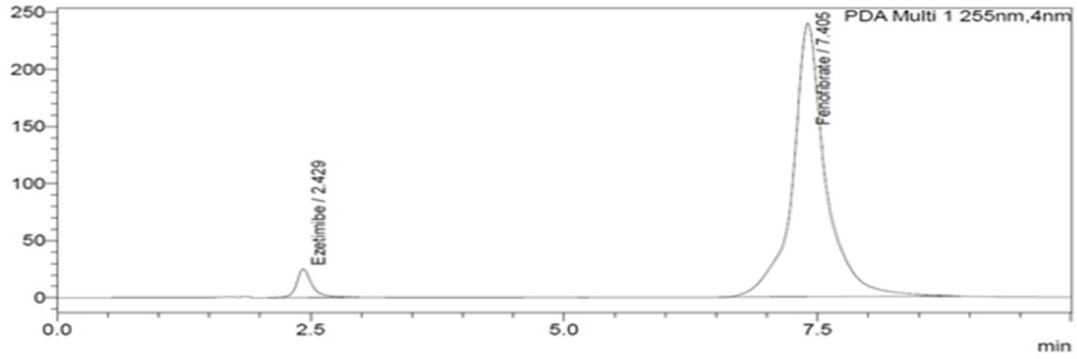

Figure 4: Sample chromatogram of Ezetimibe and Fenofibrate

RESULTS AND DISCUSSION

System Suitability:

HPLC system was optimized as per the chromatographic conditions. 20µLof standard solutions of drugs were injected six times into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, number of theoretical plates, resolution, tailing factor and %RSD were calculated and compared with standard specification of system given in **Table 1**.

Linearity:

Standard stock solutions of Ezetimibe and Fenofibrate were pipette out into a 10mL volumetric flasks and diluted up to the mark with methanol to obtain the range of 2-10µg/mL for Ezetimibe and 60-180µg/mL for Fenofibrate. Each standard solution was injected into the column at a flow rate of 1ml/min with a correlation coefficient (R^2)0.9998 and 0.999 for Ezetimibe and Fenofibrate given in **Table 2**.

Accuracy:

A known amount of samples were prepared at 50%, 100% and 150% level. They were injected in a triplicate at each level given in **Table 3**.

Precision:

System and method precision results were given in **Table 4**.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD of Ezetimibe and Fenofibrate was performed using the concentration 0.2µg/mL and 6µg/mL and LOQ of Ezetimibe and Fenofibrate was performed using the concentration 0.6 µg/mL and 18µg/mL.

Robustness:

Robustness was performed by change in the parameters like wavelength (255 $^{\pm}$5nm) and flow rate changed (1 $^{\pm}$ 0.2mL/min). The results were given in **Table 5**.

Assay:

The % Purity of Ezetimibe and Fenofibrate was found to be 100.02 and 99.84% respectively.

Table 1: Data of System suitability

Injection No	Ezetimibe		Fenofibrate	
	Retention time(min)	Peak area	Retention time(min)	Peak area
1	2.428	264231	7.399	5414219
2	2.428	255595	7.399	5465852
3	2.427	263601	7.382	5411864
4	2.429	262807	7.405	5435321
5	2.44.	260434	7.442	5450225
6	2.408	265445	7.363	5403558
Mean		262019	5430173	
Standard deviation		3564.67	24493.61	
%RSD		1.36	0.45	

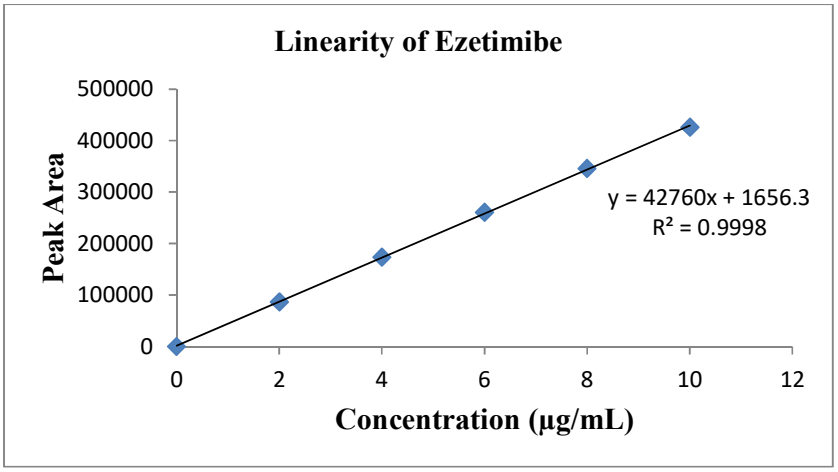

Figure 5: Calibration curve of Ezetimibe

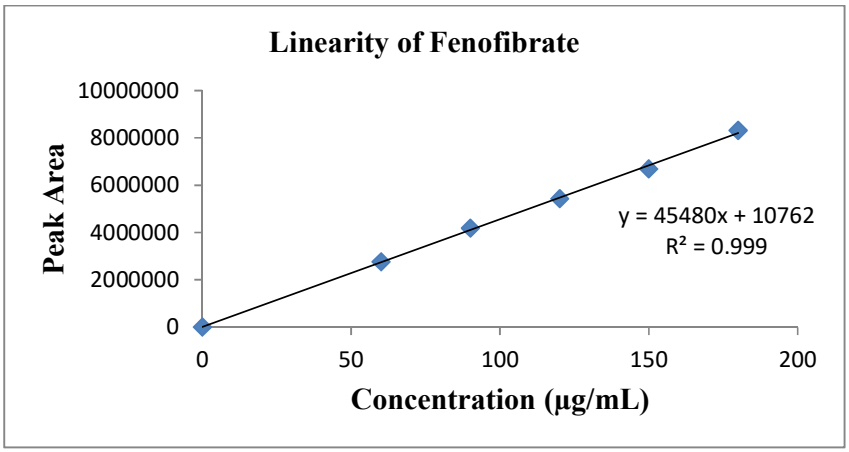

Figure 6: Calibration curve of Fenofibrate

Table 2: Data of Linearity

S. No	Ezetimibe		Fenofibrate	
	Concentration(µg/mL)	Peak Area	Concentration(µg/mL)	Peak Area
1.	2	86839	60	2758672
2.	4	173658	90	4179557
3.	6	260518	120	5425475
4.	8	345891	150	6679754
5.	10	425841	180	8309030
R^2		0.9998	R^2	0.999

Table 3: Data of Accuracy

Drug	% Level	Standard peak area	Sample Peak area	%Recovery	%Mean recovery	Average percentage recovery
Ezetimibe	50%	262019	131025	99.49	100.08	100.25%
		262019	132564	100.44		
		262019	132265	100.33		
	100%	262019	261059	99.10	99.32	
		262019	262854	99.64		
		262019	261459	99.24		
	150%	262019	396541	100.40	100.25	
		262019	395964	100.21		
		262019	395465	100.14		
Fenofibrate	50%	5430173	2714591	99.76	100.13	99.99%
		5430173	2720145	99.75		
		5430173	2726594	100.09		
	100%	5430173	5445120	100.03	99.93	
		5430173	5439126	99.79		
		5430173	5442569	99.98		
	150%	5430173	8145695	99.82	99.91	
		5430173	8156541	99.91		
		5430173	8165489	100.01		

Table 4: Data of System Precision and Method Precision

S. No	System Precision		Method Precision	
	Ezetimibe	Fenofibrate	Ezetimibe	Fenofibrate
1.	264231	5414219	264247	5414599
2.	255595	5465852	254499	5475990
3.	263601	5411864	263599	5412997
4.	262807	5435321	262835	5436325
5.	260434	5450225	260424	5451215
6.	265445	5403558	265565	5403998
Average	262019	5430173	261862	5432521
SD	3991.139	27457.01	3913.97	25621.54
% RSD	1.36	0.45	1.52	0.51

Table 5: Data of Robustness

S.No	Parameter	Ezetimibe			Fenofibrate		
		R_t (min)	Peak area	Tailing factor	R_t (min)	Peak area	Tailing factor
1.	Change in flow rate-0.8 ml/min	3.029	354065	1.291	9.297	6836333	1.010
	Change in flow rate-1.2 ml/min	2.065	220777	1.998	6.283	4633037	0.960
2.	Change in wave length-250nm	2.434	287513	1.244	7.449	4518098	0.973
	Change in wave length-260nm	2.434	196572	1.255	7.449	6149590	0.972

DISCUSSION

The developed RP-HPLC method was validated as per ICH guidelines for system suitability, specificity, linearity, precision, accuracy LOD, LOQ and robustness. The method showed the recoveries 100.25% and 99.99%which is in acceptance range of 98%-102% and system precision 1.36 and 0.45 whereas for method precision 1.52 and 0.51 which is in acceptance range of % RSD<2. Linearity was observed in the concentration range between 2-10µg/mL for Ezetimibe and 60-180µg/mL for Fenofibrate and correlation coefficient of 0.9998% for Ezetimibe and 0.999% for Fenofibrate which is with in acceptance limit of NLT 0.999.

CONCLUSION

In present research work, an attempt was made to provide a simple, precise, accurate and specific RP-HPLC method. The system with mobile phase of Acetonitrile: Methanol: Phosphate buffer pH 3 adjusted with 0.1% OPA in ratio of 55:15:30v/v/v with 1.0mL/min flowrate is quite robust. The optimum wavelength for detection was 255nm at which better detector response for drug was obtained. The retention time for ezetimibe was found to be 2.428and fenofibrate was 7.399. The linearity was observed in the range of 2-10µg/mL for Ezetimibe and 60-180µg/mL for Fenofibrate with a correlation coefficient of 0.9998 for Ezetimibe and 0.999 for Fenofibrate. Themean recoveries were found in the range of 98%-102% which are with in acceptance limit. Hence, the chromatographic method developed for Ezetimibe and Fenofibrate can be effectively applied for routine analysis in research institutions, quality control department in industries.

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