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VALIDATED UF-HPLC METHOD FOR THE ESTIMATION OF EZETIMIBE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible Ultra Fast high performance liquid chromatography (UF-HPLC) method has been developed for the quantitative analysis of Ezetimibe in pharmaceutical dosage form. Chromatographic separation of Ezetimibe was achieved on Prominence LC-20A Quaternary Gradient HPLC system, by using Shimpack C-18 5µm4.6×250mm column and the mobile phase containing Acetonitrile and 25Mm potassium hydrogen orthophosphate with pH 4.0 in the ratio 70:30 v/v. The flow rate was 1.0ml/min; detection was carried out by absorption at 232nm using a UV detector at ambient temperature. LOD and LOQ were found to be 0.0004148µg/ml and 0.0004298µg/ml respectively and retention time was found to be 5.4mins. The % Recovery was found to be 100.93%. The number of theoretical plates and tailing factor for Ezetimibe were NLT 2000 and not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Ezetimibe.

KEYWORDS

Ezetimibe, RP- HPLC, Validation and Pharmaceutical formulations.

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INTRODUCTION

Ezetimibe is a medication used to treat high blood cholesterol and certain other lipid abnormalities. Generally, it is used together with dietary changes and a statin. Alone, it is less preferred than a statin and it is also available in the fixed combo of Ezetimibe and simvastatin, Atorvastatin and bempedioc acid. It is used in the management and treatment of hypercholesterolemia. Ezetimibe belongs to selective cholesterol-absorption

inhibitors. Ezetimibe was discovered and approved by Harry Davis from USFDA and is currently owned in India by Anant Pharmaceuticals Pvt. Ltd. Ezetimibe was commercially manufactured by Healing Pharma under the brand name of Zetiheal in India.

A literature survey revealed that there were few analytical methods have been reported for the determination of Ezetimibe in pure drug and pharmaceutical dosage forms by using UV spectrophotometric[1-5], HPLC[6-18] and HPTLC[19] so far. The present work aims to develop and validate a novel, rapid, simple, precise and specific UF-HPLC (Ultra fast liquid chromatography method for the estimation of Ezetimibe in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Ezetimibe was obtained as a gift sample from the pharmaceutical industry and Zetia tablet was obtained from Pharmacy store. Acetonitrile and 25mM Potassium Hydrogen Ortho Phosphate (70:30 v/v) were obtained Bharathi College of Pharmacy, Bharathinagara, KM Doddi, Maddur, Mandya, India. All chemicals used are of HPLC grade was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a UFLC, DGU-20AD, SIL-20AC and SPD-20AUV/VIS

Detector, Shimadzu by using Shimpack C-$_{18}$ 5µm4.6 × 250mm columns is used.

Preparation of solutions Mobile phase preparation

Preparation of 25mM Potassium hydrogen ortho-Phosphate Buffer solution

Weigh accurately 3.40g of potassium dihydrogen phosphate dissolved in 1000ml Millipore water to produce 50mM potassium dihydrogen phosphate buffer. PH is maintained to 4.0 using ortho-phosphoric acid.

Preparation of Mobile Phase

Mobile phase was prepared by mixing Potassium hydrogen ortho phosphate pH-4.0 and Acetonitrile taken in the ratio 30:70. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of sample Solution

The stock solution was prepared by dissolving 0.1g of Ezetimibe in 100ml of Methanol (1000µg/ml). A series of standard solutions were prepared by the appropriate dilution of the stock standard solution of with methanol to prepare the working standard solution (10µg/ml) to a concentration of 10-30µg/ml.

Preparation of Standard solution

The stock solution was prepared by dissolving 0.1g of Ezetimibe in 100ml of Methanol (1000µg/ml). A series of standard solutions were prepared by the appropriate dilution of the stock standard solution of Ezetimibe with methanol to prepare the working standard solution (10µg/ml) to a concentration of 10-30µg/ml.

Tailing factor: NMT 2.0

Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines[20-23]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose, blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific.

Linearity

Preparation of stock solution

Accurately weigh and transfer 100mg of Ezetimibe working standard into a 100ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Preparation of Level – I (10ppm of Ezetimibe)
1.0ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (15ppm of Ezetimibe)
1.5ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Ezetimibe)
2.0ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level –IV (25ppm of Ezetimibe)
2.5ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (30ppm of Ezetimibe)
3.0ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent**.**

The linearity of the response of the drug was verified at six concentration levels, ranging from 10-30µg/ml of Ezetimibe in each linearity level were prepared. 10µl of each concentration was injected into the HPLC system. The response was read at 232nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, Three replicate standard solutions (15, 20 and 25µg/ml) of Ezetimibe was injected. % RSD was calculated and it was found to be 0.0515, 0519 and 0.209. Interday precision done same as intraday, Three replicate standard solutions (15, 20 and 25µg/ml) of Ezetimibe was injected. % RSD was calculated and it was found to be 0.036, 0.455 and 0.093 which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table No.4 and Table No.5.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (10mg of Ezetimibe). The recovery values for Ezetimibe ranged from 98.58 to 100.93%. The results are shown in the Table No.6.

Limit of detection and Limit of quantification

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

LOD for Ezetimibe was found to be 0.0004148µg/ml And LOQ for Ezetimibe was found to be 0.0004298 µg/ml. Results were shown in Table No.6.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (0.8 ml/min to 1.2ml/min.), and organic phase change shown in Table No.7.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	Shim pack C-$_{18}$ 5µm4.6 × 250mm
2	Flow rate	1.0ml /min
3	Wavelength	232nm
4	Column temperature	28°C
5	Injection volume	10µL
6	Run time	10 minutes
7	Diluents	Mobile phase
8	Elution	Isocratic mode

Table No.2: Specificity of Ezetimibe

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Ezetimibe (Standard)	5.4

Table No.3: Linearity of Ezetimibe

S.No	Concentration (µg/ml)	Peak area* (mv)
1	10	240905
2	15	407868
3	20	542069
4	25	706869
5	30	826422

Table No.4: Intraday and interday precision result of ezetimibe

S.No	Conc	Area	Mean	%SD	%RSD
1	15	409620	409384.33	210.988	0.0515
	15	409320			
	15	409213			
2	20	545472	548511.66	2850.129	0.5196
	20	551124			
	20	548939			
3	25	703496	705178	1474.390	0.2090
	25	705791			
	25	706247			

S.No	Conc	Area	Mean	%SD	%RSD
1	15	414650	414817.33	152.04	0.0366
	15	414855			
	15	414947			
2	20	555774	552973.33	2516.82	0.4551
	20	550901			
	20	552245			
3	25	709301	708953.66	663.14	0.0935
	25	708189			
	25	709371			

Table No.5: Results of recovery studies

S.No	% of drug added	% of drug added	Amount of drug added (ng/ml) (sample)	Total amount of drug (n=3)	Total amount of drug found	% Recovery	Mean	%SD	%RSD
1	50%	20	10	30	30.25	100.85	100.93	0.00105	0.001041
					30.25	100.85			
					30.33	101.11			
2	100%	20	20	40	39.58	98.95	98.58	0.00227	0.002310
					39.36	98.4			
					39.36	98.4			
3	150%	20	30	50	50.37	100.74	100.56	0.00109	0.001092
					50.23	100.46			
					50.24	100.48			

*Average of three determinations

Table No.6: System suitability parameters

S.No	Parameters	Ezetimibe
1	Linearity	10-30µg/ml
2	Regression equation	y = 28167x - 15424
3	Correlation coefficient	R^2 = 0.9979
4	Retention time	5.4min
5	Run time	10min
6	Limit of detection(LOD)	0.0004148µg/ml
7	Limit of quantification(LOQ)	0.0004298µg/ml
8	Tailing factor	0.89
9	Theoretical Plate	12344

Table No.7: Robustness results for Ezetimibe

S.No	Condition	Variation	%SD	%RSD
1	Flow rate of Mobile Phase (1mL min-1 ±2)	1.2	1748.9	0.714
		0.8	4004.64	1.631
2	Methanol Ratio in mobile phase (65%±2)	67	965.56	0.402
		63	688.6	0.285
3	Wavelength (280nm±2)	282	4285.92	1.738
		278	770.18	0.312
4	Temperature (25°C)	27	1748.9	0.714
		23	1170.976	0.484

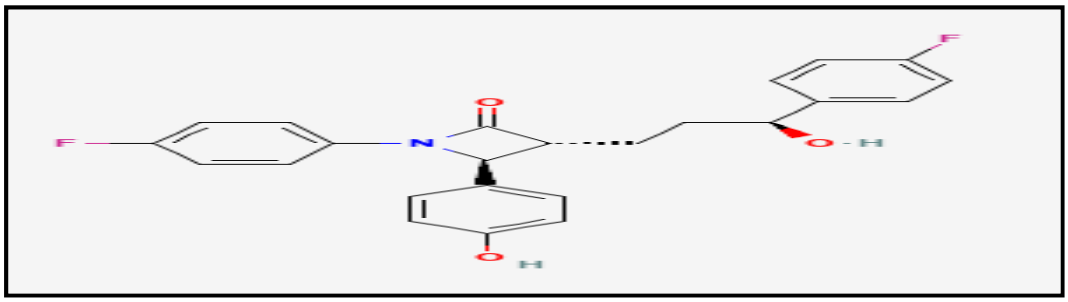


Figure No.1: Chemical structure of Ezetimibe

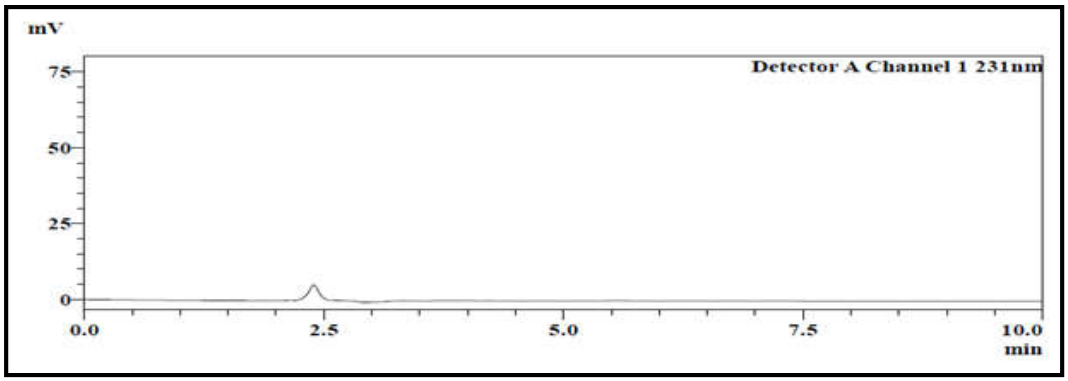


Figure No.2: Chromatogram of blank solution of Ezetimibe

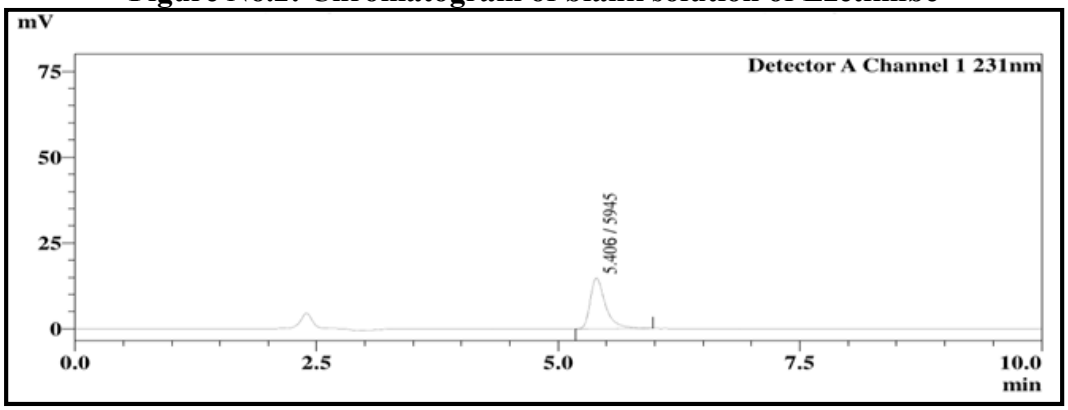


Figure No.3: Typical Chromatogram of standard solution of Ezetimibe (10µg/ml)

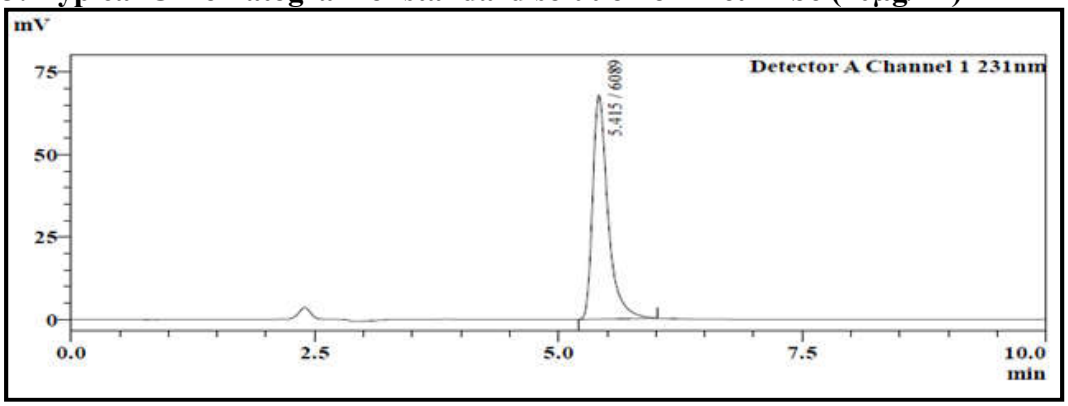
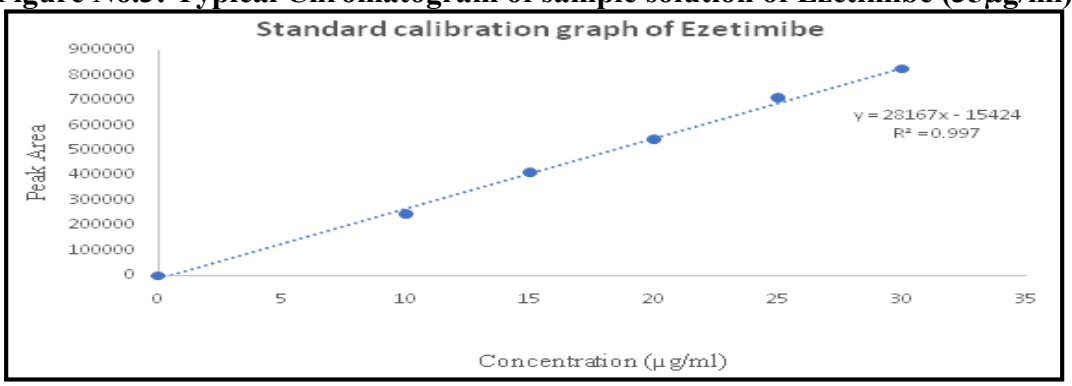


Figure No.3: Typical Chromatogram of sample solution of Ezetimibe (35µg/ml)



*Average of six determinations

Figure No.4: linearity of Ezetimibe

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive and can be used for routine analysis of Ezetimibe bulk drug and pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Gupta K H, Singhvi I N. Development of UV spectrophotometric method for the estimation of ezetimibe from tablet formulation, *Int. J of Chem Sci,* 13(2), 2015, 1051-1056.
2. Seema M D, Manjusha P Y. First derivative UV-spectrophotometric method for simultaneous determination of simvastatin and ezetimibe in tablet dosage form, *Pharmaceutical Leterature,* 7(3), 2015, 124-128.
3. Sharma M, Mhaske D V, Mahadik M, Kadam S S, Dhaneshwar S R. UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation, *Ind J. of Pharma. Sci,* 70(2), 2008, 258.
4. Lakshmi P, Ramchandran D, Rambabu C. Spectrophotometric determination of ezetimibe, *E.J. of Chem,* 7(1), 2010, 101-104.
5. Imran M, Singh R S, Chandran S. Stability indicating ultraviolet spectroscopic method for the estimation of ezetimibe and carvedilol, *Die Pharmazie,* 61(9), 2006, 766-769.
6. Beludari M I, Prakash K V, Mohan G K. RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe from their combination tablet dosage form, *İnt. J of Chemi and Analy Science,* 4(4), 2013, 205-209.
7. Jain N, Jain R, Swami H, Jain D K. RP-HPLC method for simultaneous estimation of simvastatin and ezetimibe in bulk drug and its combined dosage form, *Asian J. Research Chem,* 1(1), 2008, 29-31.
8. Kumar P, Ahmad Y, Ghosh A. A stability indicating RP-HPLC method development for determination of ezetimibe in tablet dosage form, *Der Pharma Chemica,* 4(3), 2012, 1296-1304.
9. Shrivastava P K, Basniwal P K, Shrivastava S K, Jain D. Validated RP-HPLC method for estimation of ezetimibe in different tablet dosage form, *Int. J. Ph. Sci,* 1(1), 2009, 174-181.
10. Sonawane S S, Shirkhedkar A A, Fursule R A, Surana S J. Application of UV-spectrophotometry and RP-HPLC for simultaneous determination of atorvastatin calcium and ezetimibe in pharmaceutical dosage form, *Eurasian J of A. Chem,* 1(1), 2006.
11. Rahman, Mujeeb Ur, *et al*. Simultaneous estimation of simvastatin and ezetimibe in pharmaceutical tablet dosage forms by RP-HPLC: A review, *Int J Pharma. Res Dev,* 2, 2010, 56-62.
12. Baokar Shrikrishna B, Erande R S, Shaikh S G. Analytical method development and validation for estimation of ezetimibe from tablet dosage form by using RP-HPLC, *Int. J. of Res. Pharma and Biomed. Sci,* 2(2), 2011, 833-841.
13. Kumar S A, Debnath M, Rao J S, Sankar D G. New validated stability-indicating RP-HPLC method for simultaneous estimation of atorvastatin and ezetimibe in human plasma by using PDA detector, *Advanced Pharmaceutical Bulletin,* 5(3), 2015, 385.
14. Gajjar A K, Shah V D. Development and validation of a stability-indicating reversed-phase HPLC method for simultaneous estimation of rosuvastatin and ezetimibe from their combination dosage forms, *Eurasian J. A. Chem,* 5(3), 2010, 265-283.

15. Mukthinuthalapati M A, Bukkapatnam V, Bandaru S P, Grandhi N S. Simultaneous determination of rosuvastatin and ezetimibe in pharmaceutical formulations by stability indicating liquid chromatographic method, *J of BE and BA,* 6(6), 2014, 174.
16. Neelima B, Kumar P R, Krishna M M, Bindu V H. Simultaneous estimation of simvastatin and ezetimibe by RP-HPLC in pure and pharmaceutical dosage form, *Oriental J of Chem,* 24(1), 2008, 195.
17. Khile A S, Devi N G, Rao M S, Ramachandran D. Development and validation of RP-LC method for simultaneous estimation of rosuvastatin and ezetimibe in bulk and its pharmaceutical formulations, *Am J Pharma. Tech. Res,* 7(2), 2017.
18. Sistla R, Tata V S, Kashyap Y V, Chandrasekar D, Diwan P V. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms, *J of Pharma and Biomedical Analysis,* 39(3-4), 2005, 517-522.
19. Dixit R P, Barhate C R, Nagarsenker M S. Stability-indicating HPTLC method for simultaneous determination of ezetimibe and simvastatin, *Chromatographia,* 67(1), 2008, 101-107.
20. ICH, Q2A Text on Validation of Analytical Procedures, 1994.
21. ICH, Q2B Validation of Analytical Methodology, 1996.
22. ICH, Q2 (R1) Validation of Analytical Procedures: Text and methodology, 2005.

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