Research Article

UV Spectrophotometric Method Development and Validation of Ezetimibe and Simvastatin in Bulk and Pharmaceutical Dosage Form

Namratha Sunkara[1*], B. Uma Rajeswari[1], B. Swathi [1], Sanapala Arunkumar [2] and Nathi Ratnakar[2]

[1]Bharat Institute of Technology and Bharat school of Pharmacy, Ibrahimpatnam, Hyderabad, Telangana, India.

[2]Pulla Reddy Institute of Pharmacy, Annaram, Sangareddy District, Telangana, India.

ABSTRACT

A simple UV-Spectrophotometric method was developed for the determination of Ezetimibe and Simvastatin in bulk and its pharmaceutical formulation. Ezetimibe exhibited maximum absorption at 244nm and Simvastatin at 248nm in 0.1 N NAOH and obeyed linearity in the concentration range of 0.5 to 30 µg /ml for Ezetimibe and 1.0 to 40 µg /ml for simvastatin. The proposed method was statistically validated. From the results obtained for Precision, It was found that % RSD is less than 2% for both the drugs. It indicates that the proposed method has good reproducibility. From the results obtained for Accuracy, it was found that Percentage Recovery values of pure drug from the analyzed formulation was 99.54 for Ezetimibe and 99.72 for Simvastatin which indicates that the method is accurate and commonly used excipients and additives present in the formulation was not interfering in the proposed method.

INTRODUCTION

Ezetimibe is Anticholesteremic Agent cholesterol absorption inhibitors, chemical name is 1-(4-fluorophenyl)-3-[3-(4-fluorophenyl)-3-hydroxy- propyl]-4-(4-hydroxyphenyl)-azetidin-2-one [(1-5)]. It is soluble in methanol and 0.1N NaoH. Mechanism action of Ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. Hypercholesterolemias, Homozygous Familial Sistosterolaemia are uses of drug.

Simvastatin is a Anticholesteremic Agent and Antilipidemic Agents. Chemical name is [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate [(1-5)]. It is soluble in methanol and 0.1N NaoH. Mechanism action of Simvastatin is by inhibiting the HMG-CoA reductase enzyme, which plays a central role in production of cholesterol in liver.

EXPERIMENT

Materials

Equipment and Apparatus Used: Double beam UV-Vis spectrophotometer, Vacuum filter pump, Millipore filtration kit, 1 cm quartz cells, ER 200A electronic balance.

Reagents and Chemical: NaOH - AR grade, Ezetimibe and Simvastatin reference standard was procured from Pharmagel Pvt Ltd. (Visakhapatnam, A.P, India).

METHOD

OPTIMIZATION

Scanning and determination of maximum wavelength (λ_{max})

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drugs, different solutions of the drugs (10µg/ml and 20µg/ml) in 0.1N NaOH was scanned using spectrophotometer within the wavelength region of 200 – 380 nm against 0.1N NaOH as blank. Ezetimibe shows λ_{max} at 244nm.Simvastatin shows maximum absorption at 3 different wavelengths such as

234, 239 and 248. But the work for Simvastatin was carried out at λ_{max} 248 only because the absorption values following Beer Lambert's law at λ_{max} 248. The resulting spectrum was shown in fig.3 and fig.4 and the absorption curve showed characteristic absorption maxima at 244nm for Ezetimibe and 248nm for Simvastatin.

Preparation of stock solution

Standard stock solution was prepared by dissolving 25 mg of each drug in 25 ml of 0.1N NaOH to get concentration of 1mg/ml (1000 µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph

The prepared stock solution was further diluted with methanol to get working standard solutions of 10 µg/ml and 100 µg/ml of Ezetimibe and Simvastatin**.** To construct Beer's law plot for pure drug, different aliquots Ezetimibe (0.5-30µg/ml) and Simvastatin (1-40µg/ml) (1:1) was taken and diluted to 10 ml with 0.1N NaOH. The absorbance was measured maximum at 244 and 248nm against 0.1N NaOH as blank. The result was shown in table. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig.11 & 12. The drug has obeyed Beer's law in the concentration range of 0.5-30µg/ml (for Ezetimibe) and 1-40 µg/ml (for Simvastatin).

Estimation of Ezetimibe and Simvastatin in commercial formulation

20 tablets was weighed and powder equivalent to 10 mg of Ezetimibe and 10 mg of Simvastatin (1:1) was taken and dissolved in methanol, sonicated for 1hr and filtered. The filtrate was considered as stock solution and from this various solutions of Ezetimibe and Simvastatin combination in the ratio 1:1 was prepared and estimated at their λmax.

VALIDATION PARAMETERS

Precision: The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, Mean, Standard deviation, % RSD was calculated. The reading was shown in table-4.

Accuracy: To determine the accuracy of the proposed method, recovery studies was carried out by adding different amounts (80%, 100%, and 120%) of Ezetimibe and Simvastatin bulk samples within the linearity range was taken and added to the pre-analyzed formulation of concentration 10:10µg/ml. From that percentage recovery values was calculated. The reading was shown in table-5.

CONCLUSION

It was found that Ezetimibe and Simvastatin can effectively be analyzed by the UV method with methanol and 0.1N NaoH and detection wavelength of 244 nm and 248nm. The linearity range was found to be 0.5 to 30 µg /ml for Ezetimibe and 1.0 to 40 µg /ml for simvastatin. In the precision study, %RSD was found to be less than 2% for both the drugs which indicates that the method has good reproducibility. The accuracy studies showed percent recovery in the range 99.54 for Ezetimibe and 99.72 for Simvastatin which indicates that the method is accurate and also revealed that the commonly used excipients present in the pharmaceutical formulations do not interfere in the proposed method.

ACKNOWLEDGEMENT

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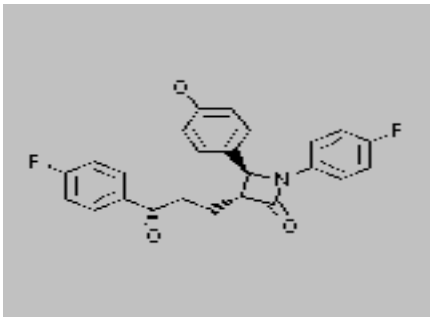

Fig, 1: Ezetimibe

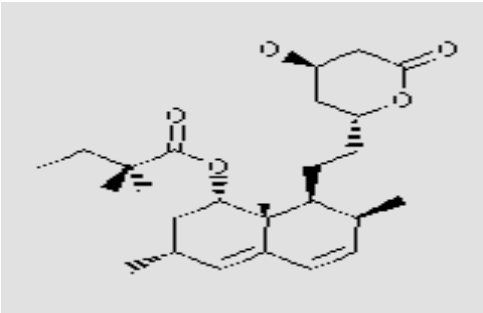

Fig. 2: Simvastatin

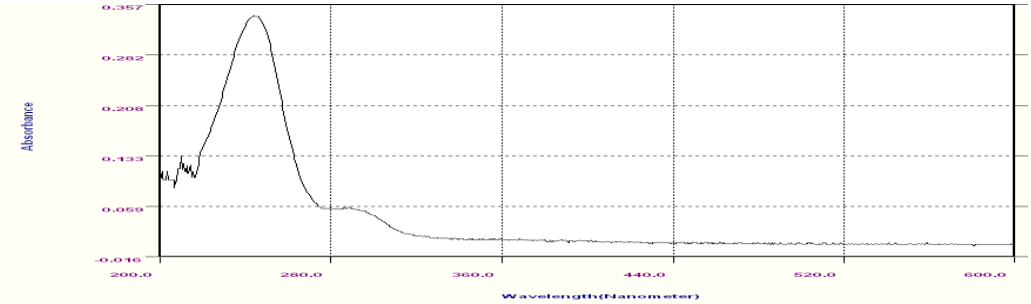

Fig. 3: Spectra of Ezetimibe

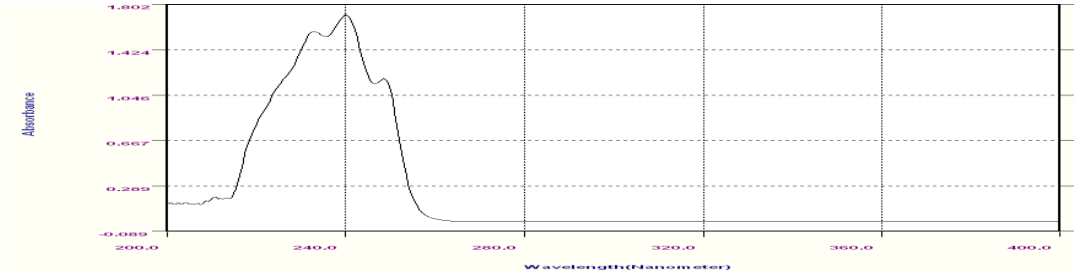

Fig. 4: Spectra of Simvastatin

Table 1: Linearity of Ezetimibe (pure drug) at 244nm in 0.1N NaOH

S. NO.	CONCENTRATION (mcg/ml)	ABSORBANCE
1	0.5	0.031
2	1	0.065
3	2	0.132
4	5	0.323
5	10	0.624
6	15	0.926
7	20	1.231
8	30	1.853

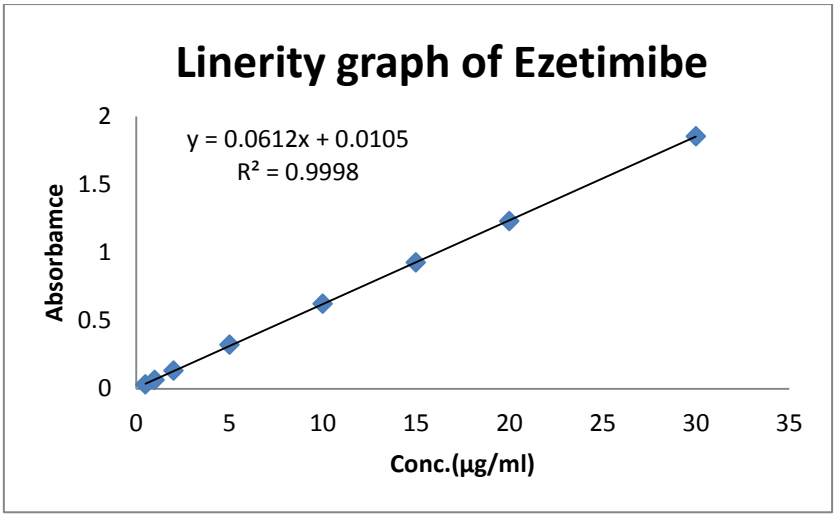

Fig. 5: LINEARITY GRAPH OF EZETIMIBE

Table 2: Linearity of Simvastatin (pure drug) at 248nm in 0.1N NaOH

S.L. NO.	CONCENTRATION (mcg/ml)	ABSORBANCE
1	1	0.04
2	2	0.09
3	5	0.201
4	10	0.412
5	15	0.601
6	20	0.804
7	25	1.041
8	30	1.198
9	40	1.610

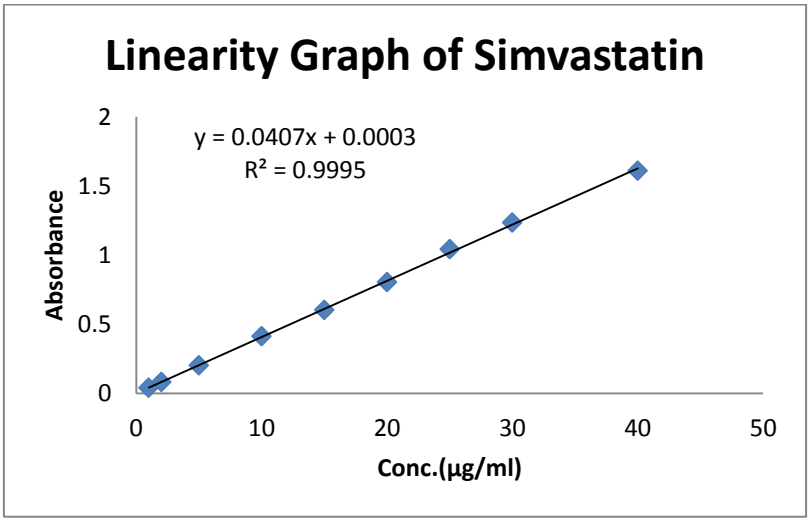

Fig. 6: LINEARITY OF SIMVASTATIN

Table 3: Recovery from the Marketed formulation

Formulation	Labelled amount		Observed amount* (± S.D) mg		%Recovery by proposed method		%R.S.D	
	EZE	SIM	EZE	SIM	EZE	SIM	EZE	SIM
ZOSTAMAX	10	10	9.96±0.005	9.89±0.005	99.6	98.9	0.10142	0.05793

Table 4: Precision readings

Conc(µg/ml)		Absorbance at 244nm	Statistical analysis EZE	Absorbances at 248nm	Statistical analysis SIM
EZE	SIM				
10	10	0.624	Mean=0.625	0.412	Mean=0.413
10	10	0.624		0.413	
10	10	0.625	S.D=0.00155	0.417	S.D=0.00168
10	10	0.626		0.413	
10	10	0.623		0.415	
10	10	0.628	%R.S.D=0.2399	0.414	%R.S.D=0.4068
10	10	0.625		0.413	
10	10	0.626		0.412	

Table 5: Accuracy readings

Sample	Concentration(µg/ml)				%Recovery		Statically analysis	
	Pure drug		Formulation		EZE at 244nm	SIM at 248nm	EZE at 244nm	SIM at 248nm
	EZE	SIM	EZE	SIM				
S1:80%	8	8	10	10	98.89%	99.597%	Mean=98.83	Mean=99.168
S2:80%	8	8	10	10	98.02%	99.753%	S.D=0.786	S.D=0.8798
S3:80%	8	8	10	10	99.59%	98.154%	%R.S.D=0.795	%R.S.D=0.887
S4:100%	10	10	10	10	98.62%	100.015%	Mean=98.576	Mean=99.418
S5:100%	10	10	10	10	99.77%	99.27%	S.D=0.876	S.D=0.5380
S6:100%	10	10	10	10	100.34%	98.97%	%R.S.D=0.888	%R.S.D=0.5411
S7:120%	12	12	10	10	98.958%	99.72%	Mean=99.299	Mean=99.9533
S8:120%	12	12	10	10	99.979%	100.03%	S.D=0.5886	S.D=0.2059
S9:120%	12	12	10	10	98.961%	100.11%	%R.S.D=0.592	%R.S.D=0.20612

(S1, S2 …S9 denote different samples)

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