Research Article

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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETERIC METHOD FOR ESTIMATION OF EZETIMIBE IN TABLET FORMULATION

C. Sridhar, A**. Sivarami Reddy, L.Sivasankar Reddy, Y.PradeepKumar*, M.C.Mohan Reddy, M.Ramya, S.Madhava Swamy, T.Ramasekhar**

Creative Educational Society's College of Pharmacy, N.H 7, Chinnatekur, Kurnool-518 218 Andhra Pradesh, INDIA.

***Corresponding Author E-mail: y.pradeepkumar36@gmail.com**

ABSTRACT:

A versatile, accurate, precise and economic method for determination of ezetimibe products was developed. The absorbance value at 745nm of over line spectrum was used for the estimation of ezetimibe, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 10 µg /ml for ezetimibe. The results of analysis have been validated statistically for linearity, accuracy and precision of the proposed method.

Key words: Ezetimibe (EZE), Methanol, Ultraviolet Spectrophotometry, Absorption ratio Method.

Introduction:

Ezetimibe is the first lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol. Ezetimibe was originally discovered by a team of four Schering-Plough research chemists: Drs. Stuart B. Rosenblum, Duane A. Burnett, John W. Clader and Brian A. McKittrick. It was approved by the FDA in October 2002 for hypercholesterolemia. (elevated blood cholesterol) alone or associated with statins. Since then, the use of ezetimibe has increased, especially in the United States and is chemically 1-(4- fluorophenyl)-(3R)-

[3-(4 fluorophenyl) - 3S)-hydroxyphenyl]-4S-(4-hydroxyphenyl)-2-azetidinone.It is not official in any pharmacopoeia. HPLC [1] and LC-MS [2] methods have been reported for the estimation of EZE in pharmaceutical formulations and in plasma. Also, HPLC [3, 4], methods were reported for the estimation of EZE in tablet dosage form. The review of literature revealed that no method is reported for the estimation of EZE in fixed dose products by UV spectrophotometry. The present research describes a simple, rapid, accurate and reproducible method for the estimation of EZE in tablet formulation by Absorption ratio method.

Experimental Work:

Pharmaceutical grade of Ezetimibe were kindly supplied as gift sample by Aurobindo Pharmaceuticals pvt.ltd. Methanol (AR grade),Deionised water, Folin-Ciocalteu reagent (a mixture of Phosphomolybdate and Phosphotungstate),Sodium carbonate were all of analytical grade and used without any further processing. The marketed formulations were purchased from local market (Lupin Laboratories) were obtained from retail pharmacies.

Equipments:

Double beam UV-Visible Spectrophotometer-ELICO SL 164 with two matched cuvette cells of one cm light path were used for the measurement of absorbance. Electronic Dhona Balance 200D was used for weighing the samples. Class 'A' volumetric glassware were used.

Procedure:

UV Spectrometric methods for determination of Ezetimibe:

Selection of Solvent [6]

The solvent was selected by determining the solubility of ezetimibe in various solvents namely Distilled water, Hydrochloric Acid, Sodium Hydroxide Solution, Methanol. Finally, Methanol was chosen as the solvent for Ezetimibe depending on absorption at its analytical wavelength.

Preparation of standard stock solution:

An accurately weighed quantity of about 40.9 mg Ezetimibe was taken in a 100 ml volumetric flask and was dissolved in methanol. The volume was made upto mark with methanol to get the concentration of 409mcg/ml.

Study of spectra:

The aliquot portion of standard stock solution of Ezetimibe was diluted appropriately with methanol obtaining concentration 10 µg/ml. Solution was taken in 1 cm cell and scanned in the range 180 nm to 400 nm and spectrum was recorder as showed in Fig1.

Fig-1: Determination of λ-max of ezetimibe (in visible region)

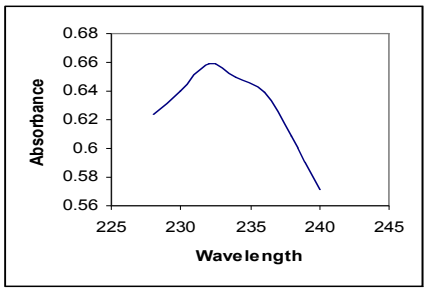


Fig 2: Determination of λ-max of ezetimibe

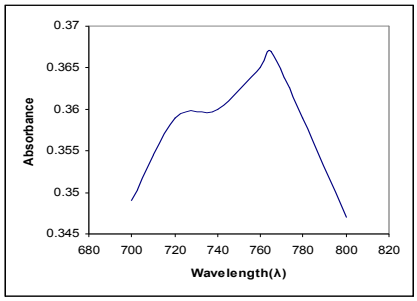


Table: 1 Determination of λ max for ezetimibe

S.NO.	Concentration (µg)	Wavelength (λ)	Absorbance
1	10	228	0.624
2	10	230	0.640
3	10	232(λ max)	0.659
4	10	234	0.650
5	10	236	0.639
6	10	238	0.609
7	10	240	0.571

Study of spectra and selection of wavelength:

The aliquot portion of standard stock solution of Ezetimibe was diluted appropriately with methanol obtaining a concentration of 40µg/ml, Then one ml of this solution was diluted with 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent[5] (Phenolic reagent) was added. This solution was kept aside for 5 minutes and 4 ml of 20% of sodium carbonate was added to it and then the solution was made upto 25 ml with water and incubated for 30minutes, this Solution was taken in 1 cm cell and scanned in the range 380 nm to 800 nm using similarly prepared blank and spectrum was recorded as showed in Fig:2.

Table: 2 scanning of (λ) max of ezetimibe (in visible region)

S.NO	Concentration(µg)	Wavelength(λ)	Absorbance
1	40	700	0.349
2	40	720	0.359
3	40	740	0.360
4	40	760	0.365
5	40	765(λ max)	0.367
6	40	780	0.359
7	40	800	0.349

UV spectrum of Ezetimibe:

The λ_{max} of Ezetimibe was found to be at 765nm (in visible region). Hence the estimation of ezetimibe was done at its λ_{max} i.e. 765nm.

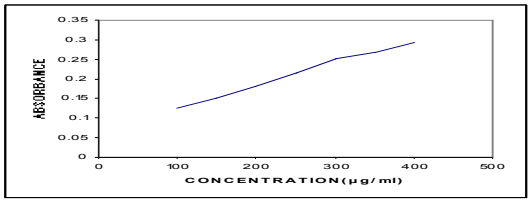
Study of Beer-Lambert's law [7]:

Accurately measured aliquot of standard stock solution ranging from 0.25 ml to 1ml (Concentration range of 100-400 µg/m) were taken in series of 25 ml volumetric flasks, diluted with 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent (phenolic reagent) was added, this solution was kept aside for 5 minutes and 4 ml of 20% of sodium carbonate was added to it and then the solution was made upto 25 ml with water and incubated for 30minutes. The absorbance of each solution was measured at 765 nm against blank.

Table: 3- observations of optical densities of concentration of µg/ml and

Fig 3: Plot of Beer-Lambert's for Ezetimibe

S.NO	Concentration (µg/ml)	Absorbance
1	100	0.125
2	150	0.152
3	200	0.181
4	250	0.215
5	300	0.252
6	350	0.268
7	400	0.292



Determination of E (1%, 1cm) values of drug at selected wavelength [8]:

An accurately weighed quantity of about 10mg Ezetimibe was transferred to a 100 ml volumetric flask dissolved in methanol and diluted upto the mark. The 1 ml of this solution of Ezetimibe was diluted appropriately with methanol to obtain concentration of 10 µg/ml. absorbance of final dilution was measured at 765 nm and E (1%, 1cm) values was calculated by using formula

.

$$E\ (1\%, 1cm) = \frac{\text{Absorbance}}{\text{Concentration in g/100ml}}$$

The results are recorded in the following table:

Table no. 4: Observation and results of E(1%,1cm)

S.No.	Concentration(g/100ml)	Absorbance	E(1%,1cm)
1	0.0010	0.070	70
2	0.0010	0.072	72
3	0.0010	0.072	72
4	0.0010	0.072	72
5	0.0010	0.073	73

Application of proposed method to the marketed formulation:

Preparation of the sample solution:

About 20 tablets each tablet contains 10 mg of ezetimibe were weighed and thoroughly powdered. The amount of powder equivalent to about 10 mg was added to 10 ml of methanol and is stirred on magnetic stirrer for 5 minutes and again add 10 ml of methanol to it and allow for mixing on the magnetic stirrer, allow the mixture to centrifugation at 1000 rpm for 5 minutes collect the supernatant and fill it into the 100 ml volumetric flask through whatman filter paper 40, wash the residue thrice with methanol and add the filtrate to the volumetric flask and make up the volume to 100 ml with methanol. Absorbance of standard solution and sample solution (350µg) was measured at 765 nm.

Table No.5: Observation of optical density for ezetimibe formulation (10 mg)

Brand Name: Ezedoc-10 mg Average weight: 0.1262 gm.

S.no:	Wt of std.(mg)	Wt.of Sample.(mg)	Absorbance of std.	Absorbance of Sample
1	100	126.2	0.659	0.268
2	100	126.2	0.656	0.270
3	100	126.2	0.658	0.280
4	100	126.2	0.658	0.268
5	100	126.2	0.659	0.269

Estimation of ezetimibe using A (1%, 1cm)

$$\text{Concentration (g/100ml)} = \frac{\text{A (unknown) x D}}{\text{A (1\%, 1cm)}}$$

Where, A**(unknown)** = Absorbance of unknown

D = Dilution factor

A(1%,1cm) = 72

Further % label claimed was determined using the formula:

$$\textbf{\%label claim} = \frac{\text{Amount estimated in Avg.wt.of tablet}}{\text{Label claim}} \times 100$$

Table No.6:- Results of estimation of ezetimibe for marketed formulation

Brand name:- Ezedoc-10 mg **Average weight:-** 0.1262 gm

S.no:	Weight taken (mg)	Absorbance at 765 nm	% Label claim
1	126.2	0.268	93%
2	126.2	0.270	93.75%
3	126.2	0.280	97.22%
4	126.2	0.268	93%
5	126.2	0.269	93.4%

Recovery studies:

Standard solution:

standard solution was prepared in the similar way as in the estimation for marketed formulation. Accurately weighed quantity of pre-analyzed tablet power equivalent to 10 mg of Ezetimibe was taken in a 100 ml volumetric flask and 350 µg of pure Ezetimibe was added to it. Then it was dissolved in methanol and the volume was adjusted up to the mark. The solution was filtered using grade-1 filter paper. A 1.0 ml portion of this solution was further diluted to 100 ml to get the final solution .The absorbance of the samples was measured at 765 nm against the blank.

Table No 7: Results of recovery studies

Brand name: - Ezedoc-10 mg **Average weight: -** 0.1262 gm

S.no	Concentration of power taken(µg)	Weight of pure drug added (µg)	% recovery
1	350	280	99.2
2	350	350	99.5
3	350	420	102

Validation of proposed method [9]:

Ruggedness: The studies on ruggedness were carried out under two different conditions:

Days and Analyst

Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. Interval within a day. The % label claim was calculated using same formula as for marketed formulation analysis. Results and statistical data are shown in table.

Table No.8: Result and statistical data of intraday study:

Obs.	Weight of powder (mg)	Absorbance at 765 nm	%label claim
Obs.1	126.2	0.281	97.5%
Obs.2		0.283	98.2%
Obs.3		0.281	97.5%

Results are the mean of three replicates.

Interday (Different days):

Same procedure was performed as under marketed formulation analysis and absorbance of same sample were recorded on different days. The % label claim was calculated using same formula as for marketed formulation analysis. Results and statistical data are shown in table.

Table No.9: Result and statistical data of interday study:

Obs.	Weight of powder (mg)	Absorbance at 765 nm	%label claim
Day.1	126.2	0.281	97.5%
Day.2		0.274	95.1%

Different analyst:

The sample solutions were prepared by two different analysts and same procedure was followed as described earlier. The %label was calculated as done in marketed formulation estimation. Results and statistical data are shown in Table.

Table No.10*:* Results of different analyst study

Analyst	Weight of powder (mg)	Absorbance at 765 nm	%label claim
1.	126.2	0.265	92.01%
2.		0.259	89.9%
3.		0.263	91.3%

Linearity and range:-

Accurately weighed quantities of tablet power equivalent to 10 mg of ezetimibe were taken in a volumetric flask and were dissolved in methanol with vigorous shaking. The solutions were then filtered and aliquots of filtrate were made to get a final concentration of about 100, 150, 200, 250, 300, 350, 400 µg/ml of ezetimibe, The one ml of each solution was diluted with 10 ml of water in different 25 ml volumetric flasks and 1.5 ml of Folin-Ciocalteu reagent (phenolic reagent) was added, these solution was kept aside for 5 minutes and 4 ml of 20% of sodium carbonate was added to them and then the solution in each volumetric flask was made upto 25 ml with water and incubated for 30minutes, these solution were taken in 1 cm cell and absorbance was read at 765nm.The graph was plotted as concentration vs absorbance and the slope and correlation coefficient were calculated.

Table No.11*:* Study of linearity and range:

S.no:	Concentration in µg/ml	Absorbance at 765 nm
1	100	0.075
2	140	0.113
3	180	0.15
4	220	0.196
5	260	0.217

Fig 4: The plot of linearity and range is study for ezetimibe

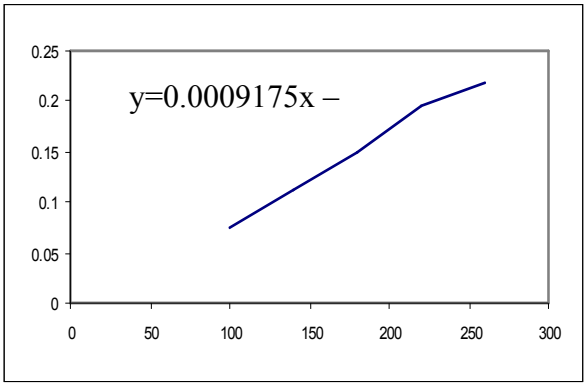


Table No.12: Results of linearity and range study:

Parameters	At 765nm
Linear dynamic range (µg/ml)	100-260µg/ml
Slope	0.0009175
Correlation coefficient	0.99521

Results and Discussion:

Determination of λ-max (by visible spectroscopy):

The λ-max of ezetimibe has been determined and was found to be 765nm

Study of Beer's Lambert's law:

Different concentrations of ezetimibe solutions were prepared ranging from 100-400µg/ml linearity curve was constructed and regression coefficient was found to be 0.99521

Determination of $E^{1\%}_{1cm}$:

s.no	Parameters	Result
1	λ-max	765
2	Beer's Lambert's plot	100-400µg/ml
3	$E^{1\%}_{1cm}$	72

Application of the present method for the estimation of the Formulation

Ezetimibe formulation (Lupin-10 mg) has been estimated by using the present method and the label claimed was found to be 98.2%

Validation of the developed method was done according to the USP 2006, Asian edition. The method is validated according to ICH guidelines and all the parameters are within the specified limit.

Method validation:

Validation Parameter:

1	I) Raggudness a) intraday b)interday	a)0.4 b)1.762
2	Different analyst	1.178
3	Linearity and range	100-400µg/ml

CONCLUSION:

Uv-Visible spectrophotometric method for ezetimbe has been developed using methanol and λ-max was found to be 765 nm. Beer's Lambert's law has been satisfied at a linearity range of 100-400 µg/ml.$E^{1\%}_{1cm}$ was 72. Ezetimibe formulation (lupin-10mg) has been estimated using the present method and %label claim is 98.2%. The method is validated according to ICH guidelines and all the parameters are within the range.

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