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STABILITY INDICATING VALIDATED METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF EZETIMIBE & SIMVASTATIN BY RP-HPLC

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ABSTRACT

Simple, specific, economical and precise high performance liquid chromatographic method for the simultaneous determination of Ezetimibe and Simvastatin in API (active pharmaceutical ingredient) and formulation has been developed and validated. Chromatography was carried out at 30˚C on a prepacked Zorbax SB C18 (5 mm, 250 x 4.6 mm) column with a mobile phase consisting of Acetonitrile: Methanol: Orthophosphoric acid (0.1%, pH 4), (70:25:5 v/v) . The UV detection was carried at 236 nm. The results obtained showed good agreement with the declared contents. Ezetimibe and Simvastatin separated in less than 10 minutes with good resolution and minimal tailing and without interference of excipients. The retention times of Ezetimibe and Simvastatin were 2.75 min and 6.9 min, respectively. The method was linear in the range of 2.5–15 µg/ml for Ezetimibe concentration with a correlation co-efficient 0.9996 and in the range 2.5–25 µg/ml for Simvastatin concentrations having correlation co-efficient 0.9998 and the recovery was 99-102%. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The proposed method can be used for quantitative determination of Ezetimibe and Simvastatin combination from API and formulations.

KEYWORDS: Ezetimibe, Simvastatin, Method Development, Validation, RP-HPLC.

INTRODUCTION

Simvastatin is -(+)-{1*S*,3*R*,7*S*,8*S*,8*aR*)-1, 2, 3, 7, 8, 8*a*-hexahydro-3,7-dimethyl-8-[2-(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-I-naphthyl-2,2-dimethyl butanoate. It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into β hydroxy acid of simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver.[1]

Ezetimibe is [(3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone]. It is a selective cholesterol absorption inhibitor used in the treatment of primary hypercholesterolemia. It inhibits the absorption of biliary and dietary cholesterol from small intestine without affecting absorption of fat soluble vitamins, triglycerides and bile acids. After oral administration, Ezetimibe is metabolized into its glucuronide in the liver and small intestine, which is also active in prevention of absorption of cholesterol. EZE does not have significant pharmacokinetic interactions with other lipid lowering drugs as it does not influence the activity of cyotochrome P450. , Ezetimibe is administered at the dose of 10 mg with and without Simvastatin.[2] Both the drugs are marketed as a combination therapy in USA as a trade name Vytorin™ for treatment of hyperlipidemia.

Simvastatin alone can be estimated by various methods reported in the literature such as high performance liquid chromatography (HPLC) with UV detection[3], Liquid chromatography coupled with tandem mass spectroscopy[4], UV Spectrophotometry[5] HPLC method for determination of Ezetimibe from pharmaceutical dosage form has been reported in the literature.[6] Reports are available which describe a stability indicating method for determination of Simvastatin as well as for Ezetimibe with its degradation products and impurities.[7-8] Simultaneous determination of Simvastatin and Ezetimibe from pharmaceutical dosage form by dual mode gradient liquid chromatography was also reported.[9] The method involved use of C8 column and elution was accomplished by the application of a dual-mode solvent and flow-rate gradient system. Detection was carried out using a diode-array detector set at 240 nm. A Stability indicating HPTLC method for simultaneous estimation of Simvastatin and Ezetimibe was also reported.[10]

The present work is aimed at development of a sensitive, specific and validated reverse phase high performance liquid chromatographic method for simultaneous determination of

Simvastatin and Ezetimibe from the dosage form and its degradation products formed under stress degradation of both Simvastatin and Ezetimibe.

MATERIALS AND METHODS

Simvastatin and Ezetimibe reference standard (label claim 99.8% pure) was provided by Ranbaxy Pharmaceuticals Ltd. Tablets of, Simvastatin and Ezetimibe Tonact-EZ with 10 mg each label claim manufactured by Lupin Pharmaceutical Pvt. Ltd, Ahmedabad, India were procured from a local pharmacy. HPLC grade acetonitrile, Orthophosphoric acid water, methanol and sodium phosphate acid were obtained from Merck India Limited, Mumbai, India. Analytical grade hydrochloric acid, sodium hydroxide pellets and hydrogen peroxide solution 30% (v/v) were obtained from Ranbaxy Fine Chemicals, New Delhi, India and 0.45 µm nylon membrane filter was obtained from Pall Life Sciences, Mumbai.

Chromatography: The chromatographic system used to perform development and validation of this assay method consisted of an perkin elemer series 200 LC pump, and turbochrom series 200 UV\VIS detector (Perkin elmer). Chromatographic analysis was performed on Zorbax SB C18 (250mm x 4.6mm), 5 µm particle size) column.

Preparation of Diluent: 70:25:5 Acetonitrile: methanol orthophosphoric acid (0.1%) % v/v was used as the diluent. methanol was used since the drug under question are soluble in methanol, while acetonitrile was used to increase the solubility of ezetimibe and orthophosphoric acid was used to adjust the pH approximately near 4.

Preparation of Ezetimibe stock solution: Accurately about 40 mg of Ezetimibe working standard was weighed and transferred to a 100ml volumetric flask and about 50 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with diluent. The solution contained 400 µg/ml of Ezetimibe.

Preparation of Simvastatin stock solution: Simvastatin working standard equivalent to 40 mg was accurately weighed and transferred to a 100 ml volumetric flask and about 100 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with diluent. The solution contained 400 µg/ml of Simvastatin.

Preparation of Simvastatin standard solution: 5 ml of Ezetimibe stock solution, 5 ml of Simvastatin stock solution, was accurately transferred to a 100 ml volumetric flask and the volume was made up with diluent and mixed well. The solution was then filtered through

0.2µm glass nylon filter. This final solution contains 20µg/ml, 20µg/ml, of Ezetimibe and Simvastatin respectively.

Selection of Wavelength: The standard solution of the ezetimibe and Simvastatin was subjected to Ultraviolet scanning and the λ max of ezetimibe and Simvastatin, were found to be at 232, 244 nm, respectively as shown in figure 1-2.

The maximum absorbance for the Ezetimibe and Simvastatin together was found to be at around 236 nm as shown in the spectra of the drugs. Hence the wavelength selected for the chromatography was optimized at 236 nm.

Chromatographic conditions: Different chromatographic conditions were tried to optimize the method, which included the following.

70:25:5 acetonitrile: methanol orthophosphoric acid (0.1%) % v/v was used as the mobile phase. A suitable High Performance Liquid Chromatography (HPLC) system equipped with the following was used.

Column	Zorbax SB C18 (250mm x 4.6mm) 5µm
Flow rate	1 ml/min
Detector	UV at 236
Injection volume	20µl
Run time	10 min

Procedure: The standard was injected into the HPLC and the chromatogram was recorded. The column efficiency determined for Ezetimibe and Simvastatin peaks was not less than 10000 theoretical plates. The tailing factor for Ezetimibe and Simvastatin peaks was not more than 2. The relative standard deviation for five replicate injections was not more than 2%. The sample solution was injected into the chromatograph and the peak area counts of the peaks were measured. The retentions times of Ezetimibe and Simvastatin were 2.75 and 6.9 minutes respectively as shown in figure 3.

VALIDATION

Specificity: Placebo of the tablets, equivalent to the sample weight was taken and solution prepared similarly to the sample solution. The solution was analyzed as per the proposed method. Sample solution was also analyzed as per the proposed method. No interference

from placebo was observed at the retention time of the drugs peaks. Peak purity plots also indicated that the peaks of the Ezetimibe and Simvastatin are pure and don't have any co eluting peaks. Therefore it is concluded that the method is specific. Chromatogram of placebo and sample along with peak purity plots are given in Figure 4-5.

Linearity and range

Preparation of stock solution

25 mg of Ezetimibe and 25 mg of Simvastatin were taken in a 100 ml flask. About 50 ml diluent was added and sonicated to dissolve. The volume was made up with diluent. 1 ml of this solution was pipette out in 10 ml volumetric flask the volume was made up with diluent. The solution was contains 25µg/ml of Ezetimibe and Simvastatin respectively.

Preparation of working solution

Different aliquots, .5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of the stock solution were taken in a series of 10 ml volumetric flasks and diluted up to the mark with the diluent to get required concentration range of 70% to 130%. The solutions were then filtered through 0.2µm glass nylon filters and injected into the HPLC system.

Accuracy

Three different sample solutions at each 80%, 100%, 120% levels were prepared.

Stock solution

20 mg of Ezetimibe and 20 mg of Simvastatin were taken in a 100 ml flask. About 50 ml diluent was added and sonicated to dissolve. The volume was made up with diluent. 1 ml of this solution was pipette out in 10 ml volumetric flask the volume was made up with diluent. The solution was contains 20µg/ml of Ezetimibe and Simvastatin respectively.

Test solution

4, 5 and 6 ml of the stock solution was taken in triplicate in nine 10 ml volumetric flasks containing about 0.4 g of placebo in it. Then sufficient diluent was added to the flasks and sonicated for 20 to 25 min. to dissolve. The volume was made up with diluent and mixed. The solutions were filtered through 0.2µm glass nylon filters and injected into the HPLC system. The chromatograms were recorded and the percentage recovery was calculated. Results shown in Table 3 - 4 indicate that the method has an acceptable level of accuracy.

The % content of Ezetimibe in the tablet sample was 100 % as per the label claim. Hence the acceptance mean recovery ought to be 98 – 102 %[5]. The % recovery was found to be 100.11 ± 1.8 % which was within the acceptance limit.

Precision

System Precision

4 ml of the stock solution was taken in six 10 ml volumetric flasks .Then sufficient diluent was added to the flasks and sonicated for 20 to 25 min. to dissolve. The volume was made up with diluent and mixed. The solutions were filtered through 0.2µm glass nylon filters and injected into the HPLC system. The chromatograms were recorded. Results shown in Table 5.

Method Precision

Six different sample solutions were prepared by taking accurately 6ml of standard stock solution in six 10 ml volumetric flasks. The volume was then made up with more diluent and mixed. The sample solutions were injected into the HPLC system and chromatograms were recorded. Results are shown in Table 6. The% RSD value indicates that the method has an acceptable level of precision. (Acceptance Criteria: RSD ≤ 2 % for Ezetimibe, ≤ 2% Simvastatin,).

Stress Degradation Study

A stress degradation study was carried out on the Ezetimibe and Simvastatin.

Hydrolytic and Oxidative degradation

A sample solution of the drugs was prepared and treated with 1N hydrochloric acid, 1N sodium hydroxide and 30% hydrogen peroxide solutions and immediately was followed by analysis as.

Thermal degradation

A sample solution of the drugs was prepared and subjected to thermal degradation by keeping at 105˚C for 1 hr, followed by analysis as per the proposed method.

Photolytic degradation

Photolytic degradation study was carried out by exposing the sample to light in a photolytic chamber at 2600 lux for 24 hr, followed by analysis as per proposed method. Using the peak purity test, the purity of the drugs peaks were checked at every stage of above-mentioned

studies. Table 7 shows the degradation of drugs. Chromatograms of sample are shown in Figure 8-12. Table 7 shows the purity of the peaks at various degradation parameters.

Application to the Marketed Products

The developed and validated HPLC method was applied for determination of Simvastatin and Ezetimibe from dosage forms. The tablet contains 10 mg Simvastatin and 10 mg Ezetimibe. in two marketed products viz. STARSTAT-EZ (Lupin Pharmaceutical Pvt. Ltd), SIMVAS-EZ (Micro Lab Pvt. Ltd).

Assay of tablet Starstat-EZ – B.No. SE-076875

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg Ezetimibe and Simvastatin was taken in 50 ml volumetric flask. To the above flask, 20 ml of methanol was added and the flask was sonicated for 5 minutes. The solution was filtered in another 50 ml volumetric flask using Whatman filter paper (No.1) and volume was made up to the mark with the same solvent. Appropriate dilutions were made to obtain to obtain a solution containing 10 µg/ml of Simvastatin.The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak area was recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve. The assay percentage obtained is shown in Table 7.

Assay of tablet Simvas-EZ – B.No. SE-077563

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg Ezetimibe and Simvastatin was taken in 50 ml volumetric flask. To the above flask, 20 ml of methanol was added and the flask was sonicated for 5 minutes. The solution was filtered in another 50 ml volumetric flask using Whatman filter paper (No.1) and volume was made up to the mark with the same solvent. Appropriate dilutions were made to obtain to obtain a solution containing 10 µg/ml of Simvastatin.The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak area was recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve. The assay percentage obtained is shown in Table 8.

RESULT AND DISCUSSION

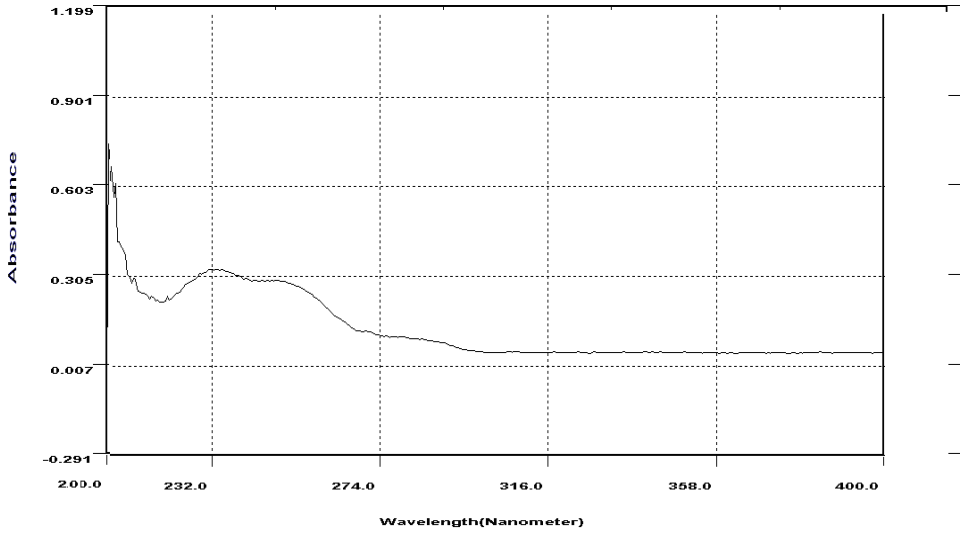

Figure 1: UV Spectra of Ezetimibe

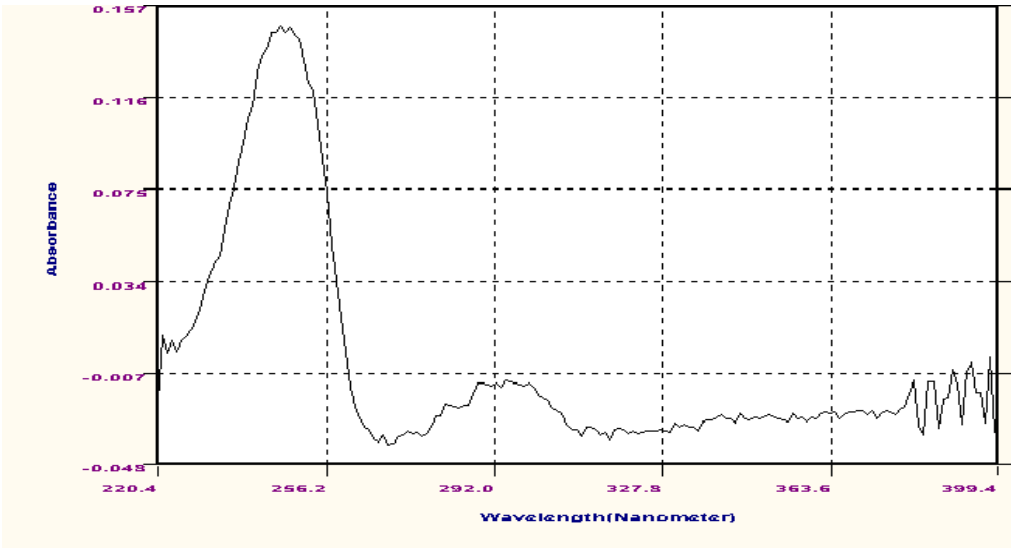

Figure 2: UV Spectra of Simvastatin

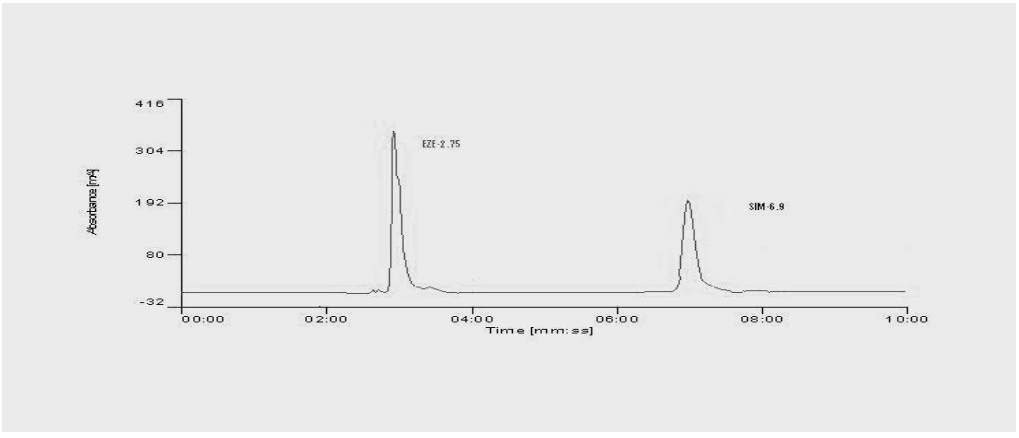

Figure 3: Chromatogram of the standard solution of Ezetimibe and Simvastatin

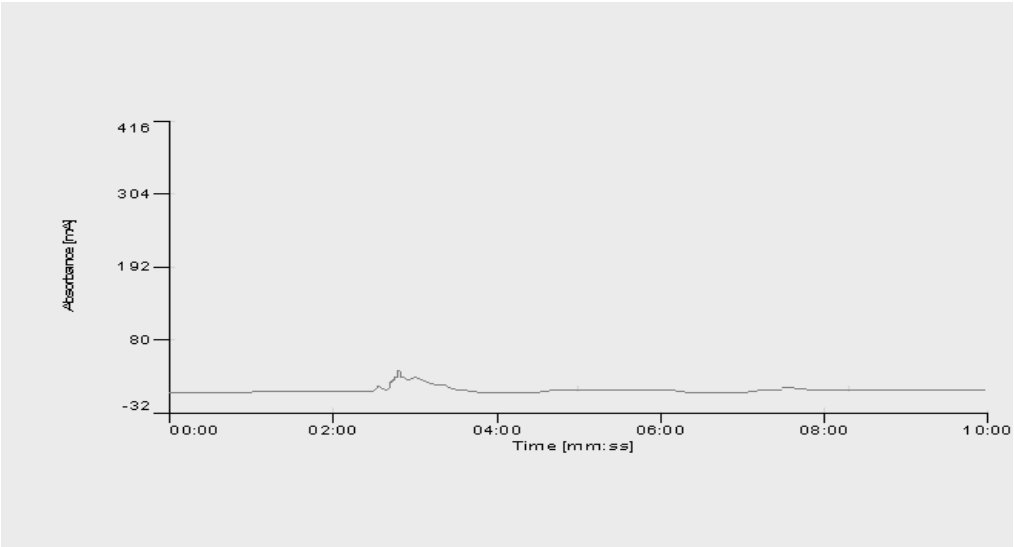

Figure 4: Chromatogram of placebo (specificity)

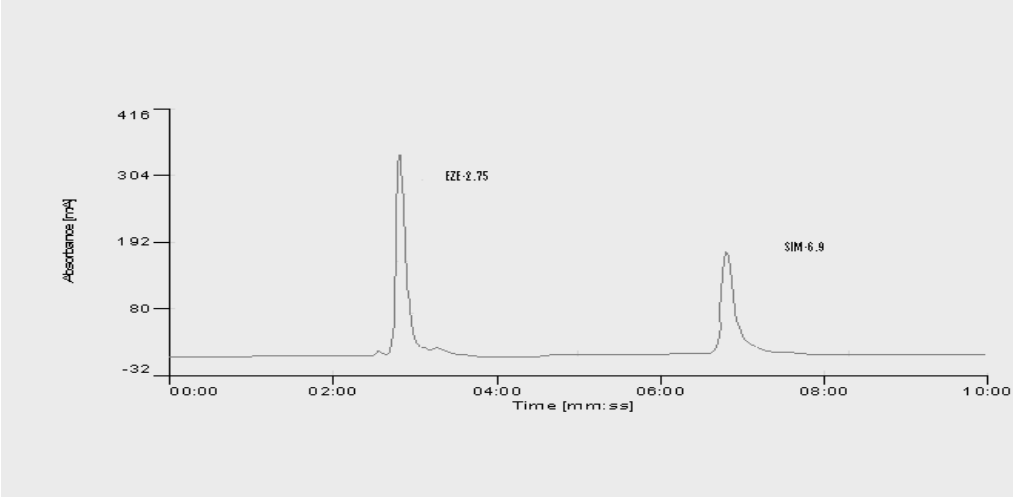

Figure 5: Chromatogram of sample (specificity)

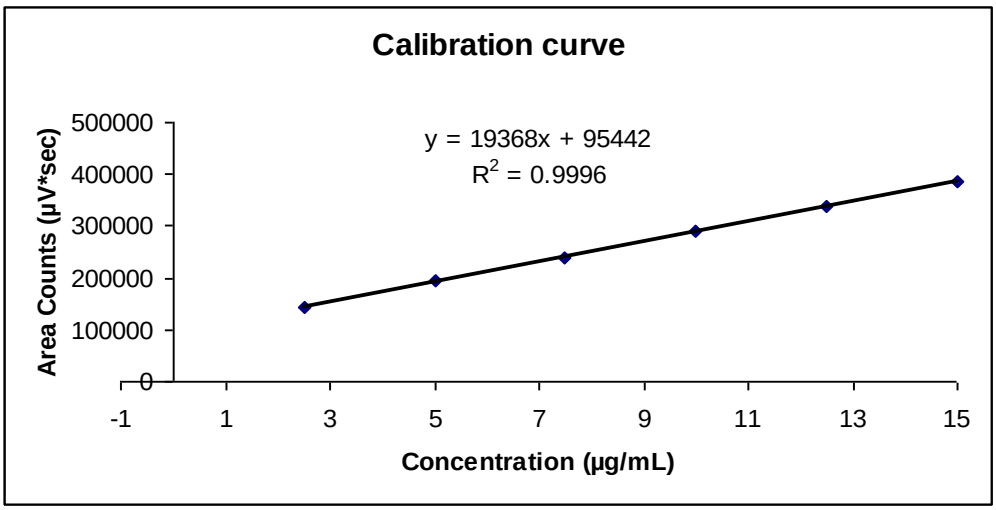

Figure 6: Calibration curve of Ezetimibe

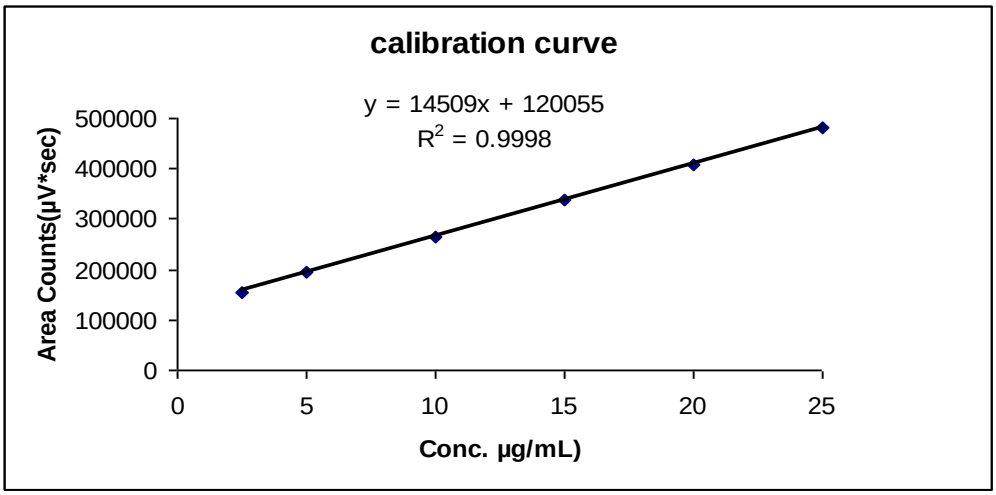

Figure 7: Calibration curve of Simvastatin

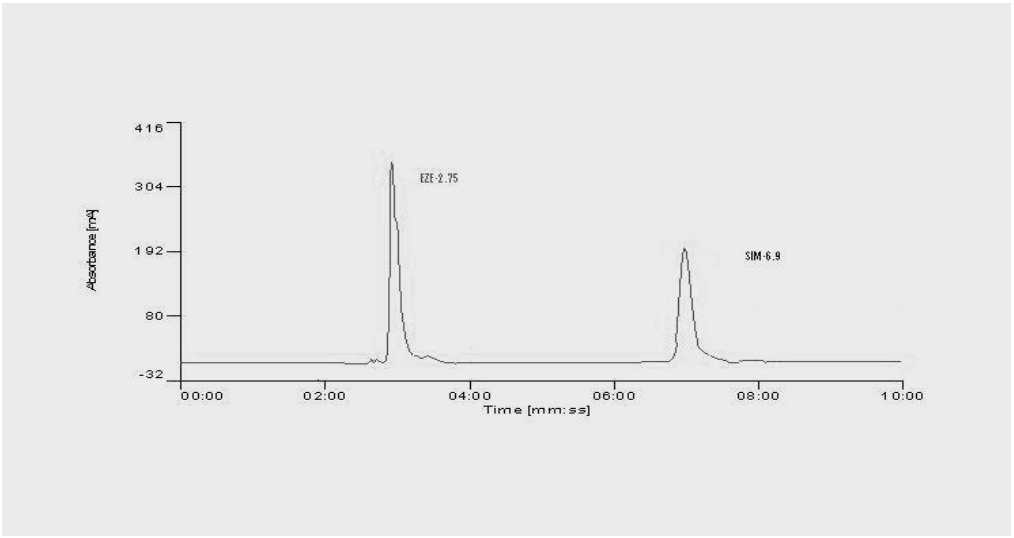

Figure 8: Chromatogram of Ezetimibe and Simvastatin under acid degradation

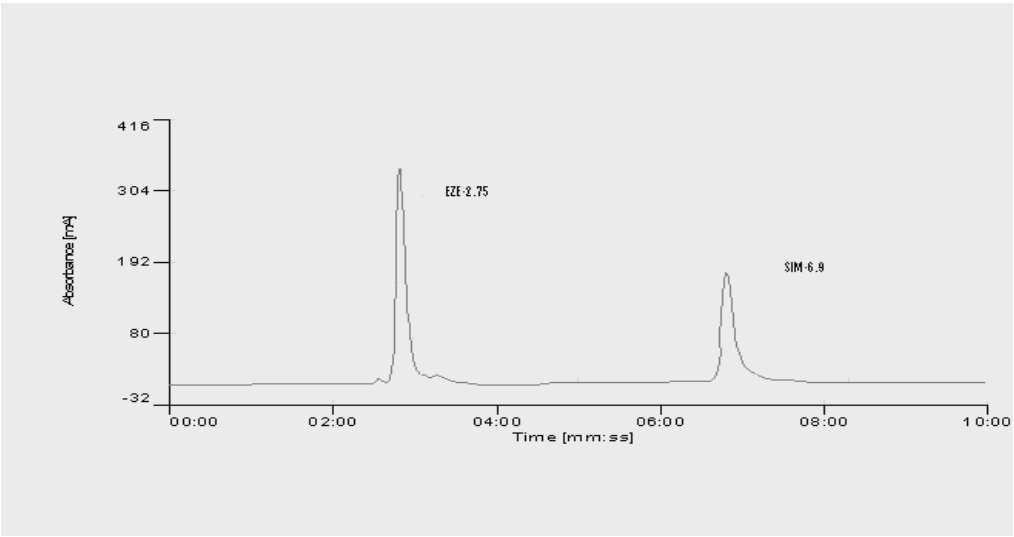

Figure 9: Chromatogram of Ezetimibe and Simvastatin under alkali degradation

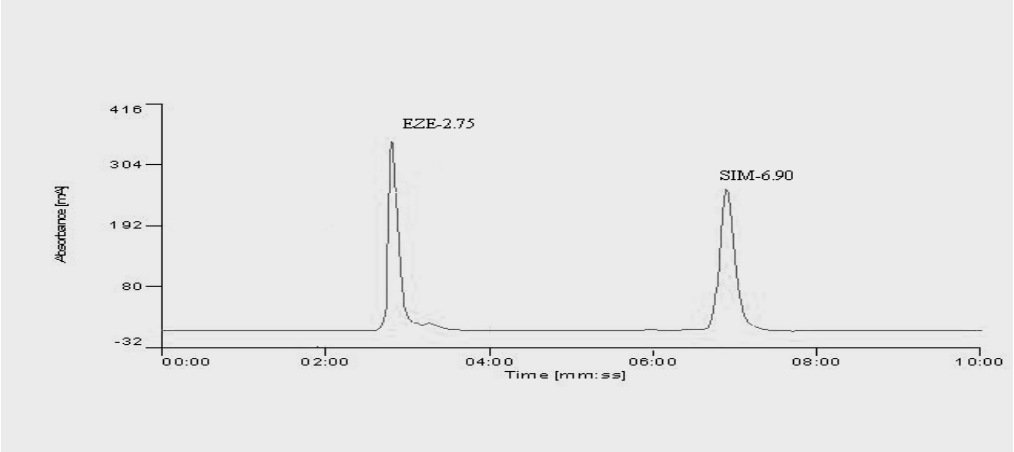

Figure 10: Chromatogram of sample Ezetimibe and Simvastatin oxidation degradation (peroxide

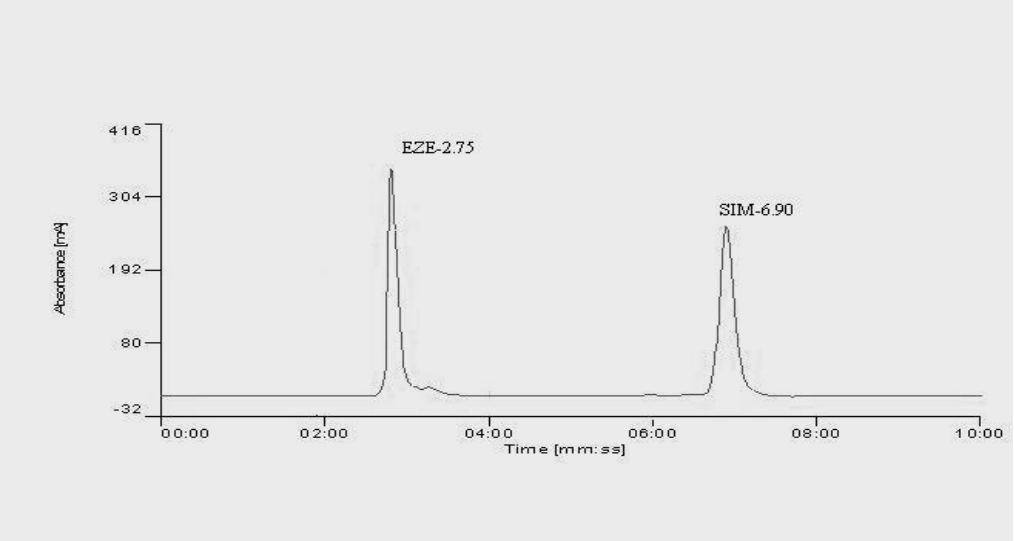

Figure 11: Chromatogram of Ezetimibe and Simvastatin under photolytic degradation

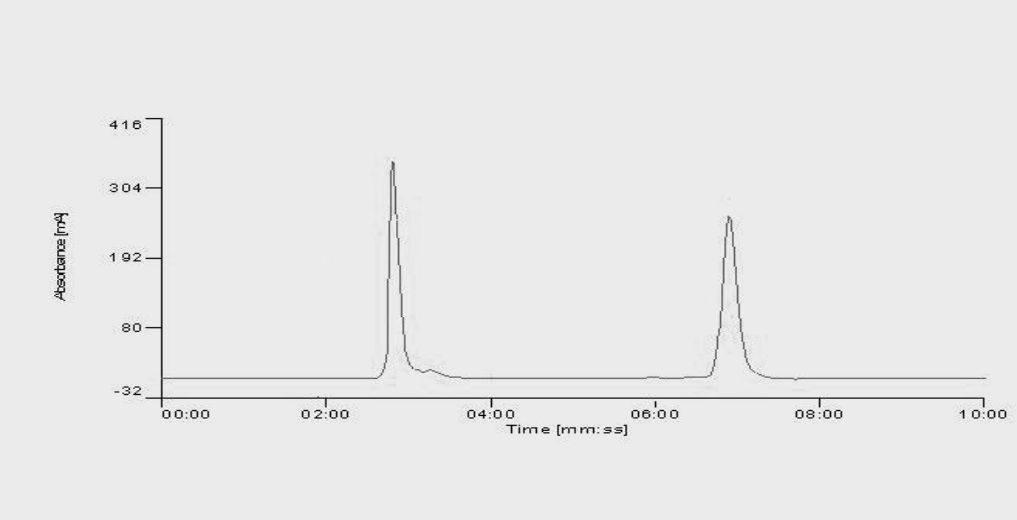

Figure 12: Chromatogram of Ezetimibe and Simvastatin under thermal degradation.

Table 1: Data for calibration curve for Ezetimibe

Sample ID	Conc. (µg/mL)	Area Counts (µV*sec)		
		Inj#1	Inj#2	Mean
L-1	2.5	141681	143075	142378
L-2	5	196213	192279	194246
L-3	7.5	238578	239556	239067
L-4	10	291022	292612	291817
L-5	12.5	337624	335678	336651
L-6	15	389221	381439	385330
			Slope	**19368**
			Intercept	**95442**
			r	**0.9996**

Table 2: Data for calibration curve for Simvastatin

Sample	Conc. µg/mL)	Area Counts(µV*sec)		
		Inj#1	Inj#2	Mean
L-1	2.5	151548	157472	154510
L-2	5	194844	192392	193618
L-3	10	267616	264398	266007
L-4	15	337935	337945	337940
L-5	20	408357	408115	408236
L-6	25	482458	483446	482952
			Slope	**14509**
			Intercept	**12055**
			r	**0.9998**

Table 3: Accuracy for Ezetimibe

Sample	Mean area counts	Amt. Recovered (mg)	Actual amt. Added (mg)	% Recovery
80%-Rec-1	235191	20.12	20	100.60
80%-Rec-2	236007	20.50	20	102.50
80%-Rec-3	236327	20.80	20	104.00
100%-Rec-1	297474	20.12	20	100.60
100%-Rec-2	297115	20.08	20	100.40
100%-Rec-3	296664	19.80	20	99.00
120%-Rec-1	338250	19.75	20	98.75
120%-Rec-2	339536	19.85	20	99.25
120%-Rec-3	341307	20.80	20	101.00
Mean ± SD				**100.67 ± 1.7**

Table 4: Accuracy for Simvastatin

Sample	Mean area counts	Amt. Recovered (mg)	Actual amt. Added (mg)	% recovery
80%-Rec-1	235191	20.04	20	100.2
80%-Rec-2	236007	20.15	20	100.75
80%-Rec-3	236327	20.18	20	100.9
100%-Rec-1	297474	20.22	20	101.1
100%-Rec-2	297115	20.20	20	101.05
100%-Rec-3	296664	19.11	20	95.55
120%-Rec-1	338250	20.25	20	101.25
120%-Rec-2	337536	20.15	20	100.75
120%-Rec-3	336536	19.90	20	99.5
Mean ± SD				**100.11 ± 1.8**

Table 5: System Precision for Ezetimibe and Simvastatin

Inj#	Area counts(µV*sec)	
	Ezetimibe	**Simvastatin**
1	297738	265545
2	296622	264953
3	301843	266191
4	300604	266011
5	293315	266808
6	296323	266678
Mean	297740	266031
SD	3090	699

Table 6: Method precision for Ezetimibe and Simvastatin

Sample	Ezetimibe Mean Area Counts (µv*sec)	Simvastatin Mean Area Counts (µv*sec)	Ezetimibe		Simvastatin	
			Assay	%Assay	Assay	%Assay
MP-1	334941	305315	25.46	101.84	25.05	100.20
MP-2	333302	304677	25.09	100.36	25.01	100.04
MP-3	332387	305674	25.09	100.36	25.03	100.12
MP-4	336446	305976	25.53	102.12	25.04	100.16
MP-5	338168	308282	25.41	101.64	25.06	100.24
MP-6	338187	306188	25.4	101.60	25.05	100.20
Mean		**25.32**		**25.04**		**Mean**
SD		**0.337**		**0.016**		**SD**
RSD		**1.27**		**0.53**		**RSD**

Table 7: Degradation study for Ezetimibe and Simvastatin

Sample	Ezetimibe Area counts (µv*sec)	Simvastatin Area counts (µv*sec)	Ezetimibe		Simvastatin	
			25mg	Percent Degradation	25mg	Percent Degradation
Sample (1 N HCl, 5ml)	257865	242001	23.63	5.46	22.60	9.6
Sample (1 N NaOH, 5ml)	325122	262735	24.75	1.00	23.09	7.64
Sample (H_2O_2 30%, 5ml)	336802	260685	24.88	0.48	23.07	7.72
Sample Thermal Deg.(105˚C/1hr)	305449	253395	23.63	5.448	22.79	8.84
Sample photolytic Deg. (2600 Lux/24hr)	297514	452202	24.19	3.484	21.76	12.96

Table 8: Assay of tablet Starstat-EZ

Sample	Ezetimibe, 10 mg per tablet			Simvastatin, 10 mg per tablet		
	Area Counts (µV*sec)	Assay (mg)	% Assay	Area Counts (µV*sec)	Assay (mg)	% Assay
1	289455	10.15	101.50	270455	10.05	100.50
2	297674	10.02	100.20	267674	9.90	99.00
3	298234	10.12	101.10	271234	10.12	101.2
Mean	**295121**	**10.09**	**100.93**	**269787**	**10.02**	**100.23**
SD	**4914**	**0.06**	**0.66**	**1871.47**	**0.11**	**1.11**
RSD	**1.66**	**0.67**	**0.65**	**0.693683**	**1.12**	**1.10**

Table 9: Assay of tablet Simvas-EZ

Sample	Ezetimibe, 10 mg per tablet			Simvastatin, 10 mg per tablet		
	Area Counts (µV*sec)	Assay (mg)	% Assay	Area Counts (µV*sec)	Assay (mg)	% Assay
1	284565	10.08	101.880	268455	9.98	99.80
2	292144	10.15	101.50	267674	9.92	99.20
3	291673	10.11	101.10	271234	10.08	100.8
Mean	**289460**	**10.11**	**101.49**	**269121**	**9.99**	**99.93**
SD	**4246**	**0.035**	**0.390**	**1871**	**0.079**	**0.79**
RSD	**1.46**	**0.34**	**0.38**	**0.69**	**0.80**	**0.80**

Table 10: Summary of Validation Parameters

PARAMETERS	OBSERVATION	
	Ezetimibe	Simvastatin
SPECIFICITY	No Interference was found w.r.t. excipients	
LINEARITY (R)[5]	0.9996	0.9998
RANGE	70 – 130 % of test concentration	
PRECISION (RSD)*		
a) Repeatability (n=6)	1.03	0.28
(system precision)		
c) Method Precision (n=6)	1.276	0.53
ACCURACY (% Recovery)**	100.67 ± 1.70	100.11±1.8
STABILITY IN ANALYTICAL SOLUTION	Stable	

STRESS DEGRADATION	The Peaks were **Pure** without any interference	
ROBUSTNESS (Overall RSD)* **	Less than 2%	
a) Change in Wavelength	0.64	0.28
• 231 nm	1.09	0.29
• 241 nm		
b) Change in Flow rate	1.9	0.12
• 0.9 ml/min	0.74	0.15
• 1.1 ml/min		
c) Change in Organic Conc.	1.32	0.08
• - 2%	1.17	0.06
• + 2%		
d) Change in Column Temp.	1.05	0.17
• 25°C	0.31	0.10
• 35°C		
e) Change in pH of Buffer	0.29	0.02
• 3.8 • 4.2	1.92	0.12
RUGGEDNESS(Overall RSD)* **	1.07	1.32

Acceptance Criteria**:** RSD ≤ 2 %.

** Acceptance mean recovery**:** for 90 – 110 %.

*** Acceptance Criteria**:** RSD ≤ 2 %

Both Simvastatin and EZE have limited aqueous solubilities hence methanol was used for the extraction of drugs from the formulations and for preparation of stock solutions. Ezetimibe and Simvastatin have λ_{max} at 232 nm and 238 nm respectively, so HPLC analysis was done at 236 nm for Ezetimibe and 238 nm for SMV. Mobile phase optimization was consisting of Acetonitrile: Methanol: Orthophosphoric acid (0.1%, pH 4), (70:25:5 v/v) . at the flow rate of 1. ml/min . The flow rate was decreased from 1.5 ml/min to 1.0 ml/min to resolve the degradation product from main drug peaks. The peak shape and separation was found to be good when a mobile phase mobile phase consisting of Acetonitrile: Methanol: Orthophosphoric acid (0.1%, pH 4), (70:25:5 v/v) was used at the flow rate of 1.0 ml/min. Although a HPLC gradient method using UV detector has been reported for the determination of Simvastatin and Ezetimibe from pharmaceutical dosage form, the above developed method has few advantages on the reported method [9] . The method developed above is simple, uses isocratic flow system and UV detector for detection of drug. The chromatograms are given in the Figure3.

The calibration curve constructed was evaluated by its correlation coefficient. The peak area was linear in the range of 2.5 to 15 µg/ml for Ezetimibe and 2.5 to 25 µg/ml for Simvastatin. The correlation coefficients for both the calibration plots of drugs were more than 0.999.

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. The amount of Ezetimibe and Simvastatin was recovered, in the presence of placebo interference, was calculated. The mean recovery of Ezetimibe and Simvastatin was 100.55 %, 100.49% respectively which is satisfactory and result were shown in table 2.

Precision of the method were done in the % RSD for repeatability was found 0.63, 0.25 and Intermediate Precision was found 1.27, 0.53 for Ezetimibe and Simvastatin respectively. The method precision was found 1.70 and 1.23 for both drugs respectively.. The mean recovery of Ezetimibe and Simvastatin was found 100.55, 100.49 %, respectively which is satisfactory table 5. The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value.

System suitability data were also found to be satisfactory during variation of the analytical conditions table 10. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions. During study of the stability of stored solutions of standards and test preparations for assay determination the solutions were found to be stable for up to 13 h. Assay values obtained after 13 h were statistically identical with the initial value without measurable loss result were shown in table 10.

This furnished evidence the method was suitable for its intended purpose. The specificity of the method was determined by checking for interference with the drug from placebo components. The specificity of the method was also evaluated by checking the peak purity of the analyte peak during the forced degradation study.

The peak purity of the Ezetimibe and Simvastatin peak under different stress conditions was 1.00, which is satisfactory and indicates there was no interference with the analyte peak from degradation products result were shown 7. The degradation of Ezetimibe and Simvastatin was found up to 5.46 and 9.6 % occurred under acidic conditions, Under alkaline conditions the drug was degraded by approximately 1.00 and 7.64. The drug was approximately 0.48% and 7.72 % degraded under oxidizing conditions. The drugs was degraded 5.44% and 8.84%

under thermal condition. The 3.48% and 12.96% degradation occurred under photolytic conditions.

CONCLUSION

The intensive approach described in this manuscript was used to develop and validate a liquid chromatographic analytical method that can be used for both assay and determination of content uniformity of Ezetimibe and Simvastatin in a pharmaceutical dosage form. Degradation products produced as a result of stress did not interfere with detection of Ezetimibe, Simvastatin and the assay method can thus be regarded as stability indicating. This HPLC method for assay and determination of content uniformity of Ezetimibe and Simvastatin in a tablet formulation was successfully developed and validated for its intended purpose. The method was shown to specific, linear, precise, accurate, and robust. Because the method separates Ezetimibe and Simvastatin and all the degradation products formed under variety of stress conditions it can be regarded as stability indicating. Because there is no pharmacopeial method for assay and determination of content uniformity of Ezetimibe and Simvastatin in pharmaceutical dosage forms, this method is recommended to the industry for quality control of drug content in pharmaceutical preparations.

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