ISSN NO. 2320–7418 **Available online at www.jmpas.com** **DOI: 10.22270/jmpas.V10I3.1187**
Research article

COST EFFECTIVE STABILITY INDICATING RAPID, EFFICIENT ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RELATED SUBSTANCES METHOD FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND EZETIMIBE IN TABLET FORMULATIONS

Neelu Jain, Srinivasarao Kosanam*, Surendra Singh Rao, Asheesh Singh

Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India

ABSTRACT
An efficient, cost effective, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the determination of Ezetimibe and Simvastatin in pharmaceutical dosage forms. Chromatography was carried out by using X-terra C18, 150 x 4.6mm, 3.5µ or equivalent internal diameter with a mixture of 0.5 mL glacial acetic acid in 2000 mL water as mobile phase A and Acetonitrile as mobile phase B. Analytical method validation parameters such as specificity, linearity, precision, accuracy, solution stability and robustness, limit of detection (LOD) and limit of quantification (LOQ) was done. The correlation coefficient was found to be linear for each analyte in the desired concentration range. The average recovery was found with range of 96.7 and 107.7 for Ezetimibe and Simvastatin and their respective impurities respectively. The proposed method is highly sensitive, precise and accurate, which was evident from the LOD value range of minimum 0.135 ppm for Ezetimibe and 0.356 ppm for Lovastatin & Epilovastatin for Simvastatin impurities. Hence the present method can be applied successfully for the quantification of finished dosage form in the combined formulations of Ezetimibe and Simvastatin.

KEYWORDS: Ezetimibe, Simvastatin, HPLC, Development, Validation.

Received - 05/06/2021, **Reviewed -** 12/06/2021, **Revised/ Accepted -** 24/06/2021

CORRESPONDENCE:
SrinivasaraoKosanam* ✉ kosanamsrinivasarao@gmail.com
Address – Department of Chemistry, Sri SatyaSai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India

INTRODUCTION
Ezetimibe is a drug that lowers cholesterol. It decreases cholesterol absorption in the intestine. Ezetimibe used alone, when other cholesterol-lowering medications are not tolerated, or together with statins (e.g., ezetimibe/simvastatin) when statins alone do not control elevated cholesterol level. Even though ezetimibe decreases cholesterol levels, the results of two major, high-quality clinical trials showed that it did not improve clinically significant outcomes, such as major coronary events and actually made some outcomes, such as artery wall thickness, worse. Indeed, a panel of experts concluded in 2008 that it should "only be used as a last resort"[(1-5)].One more study which was conducted by Britain's NICE statement which however was published in 2007 and may not have been updated to reflect the results of the above mentioned trials[(6-10)].

Simvastatin is a hyperlipidemia drug used to control increased cholesterol, or hypercholesterolemia. It is a member of the statin class of Anti-hyperlipidemic dugs. Simvastatin is a synthetically derive from fermentation product of Aspergillus terreus. Simvastatin is widely used for the treatment of dyslipidemia and the prevention of cardiovascular disease. It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels[(11-13)].The present study describes the development and validation of a new rapid, simple, sensitive and reproducible chromatographic related substances method for the analysis of ezetimibe and simvastatin in tablet dosage form that offer certain advantages in its simplicity, sensitivity, effectiveness and applicable in routine analysis. It also describes the development of validation work as per ICH guidelines recommended by the Food and Drug Administration (FDA) of the United States[(14-15)].

METHODOLOGY
Instruments, Reagent and Materials Used:
Instruments, Materials and reagent were used for the validation studies mentioned in table 1 to 2.

Table 1: Instruments, Materials and Working Standard

Instrument Name	Make & Model
HPLC	Waters 2489 dual wavelength
HPLC	Waters 2998 PDA detector
Balance	Metlar Toledo
pH meter	Metrohm

Table 2: Reagents & solvents used in validation study

Reagent & Solvents	Grade
Orthophosphoric acid	AR grade
Glacial acetic acid	AR grade
Sodium hydroxide	AR grade
Acetonitrile	HPLC grade
Water	Milli Q or equivalent

Mobile Phase A:
Add 0.5 mL glacial acetic acid in 2000 mL water. Mix well and degas it.
Mobile Phase B:
Acetonitrile

Preparation of Buffer:
Add 3 mL glacial acetic acid in 900 mL water. Adjust the pH 4.0 ± 0.05 with Sodium hydroxide make up volume up to 1000mL with water. Use mixture of Buffer and Acetonitrile in the ration 20: 80 as diluent.

Chromatographic Condition

Column	:	X-terra C18, 150 x 4.6mm, 3.5µ or equivalent
Flow Rate	:	1.4 mL / min
Detection	:	215 nm
Column Temp	:	50°C
Sample Temp	:	15°C
Injection volume	:	20 µL
Run time	:	90 minutes

Gradient Programme

Time (minute)	Mobile phase A	Mobile phase B
0	75	25
70	25	75
75	25	75
80	75	25
90	75	25

Preparation of system suitability solution:
Weigh accurately about 25 mg of Ezetimibe working standard, 200 mg of Simvastatin Working Standard and 1.0 mg each of Acetyl Simvastatin impurity and Anhydrous Simvastatin impurity into 25 mL volumetric flask. Add 20 mL diluent sonicated to dissolve. Add 1 mL of Ezetimibe impurity B and Ezetimibe impurity C stock solution into this and make up to the volume with diluent and mix.

Preparation of standard solution A:
Weigh accurately about 50 mg of Ezetimibe working standard into 50 mL volumetric flask. Add 35 mL diluent sonicated to dissolve. Make up to the volume with the diluent.

Preparation of standard solution B:
Weigh accurately about 80 mg of Simvastatin Working Standard into 50 mL volumetric flask. Add 35 mL diluent sonicated to dissolve. Make up to the volume with the diluent.

Preparation of standard solution:
Further dilute 1 mL of standard solution A and 2 mL of standard solution B to 200 mL with diluent and mix.

Preparation of sample solution:
Crush 10 tablets to a fine powder using motor and pestle. Transfer the powder equivalent to 50 mg of Ezetimibe and 50 mg of Simvastatin into a 50 mL volumetric flask. Add 35 mL of diluent, sonicated in cool water for 15 minutes with intermittent shaking. Dilute to volume with diluent at room temperature. Filter the sample through 0.45µ nylon filter.

Preparation of placebo solution:
Transfer the placebo powder equivalent to 50 mg of Ezetimibe and 50 mg of Simvastatin into a 50 mL volumetric flask. Add 35 mL of diluent, sonicated in cool water for 15 minutes with intermittent shaking. Dilute nylon filter.

Evaluation of System suitability:
Inject System suitability solution into the chromatograph and record the chromatogram. The resolution between Ezetimibe Impurity B and Ezetimibe Impurity C should not be less than 1.2 and the resolution between Acetyl Simvastatin impurity and Anhydrous Simvastatin impurity should not be less than 1.2. The Number of theoretical plates for Ezetimibe should not be less than 20000 and tailing factor should not be more than 2.0. Inject Standard Solution six times into the chromatograph and record the chromatograms. Measure the area counts of Ezetimibe and Simvastatin peaks. The % RSD should not be more than 5.0 for six replicates injections.

Table 3: Response Factor of Ezetimibe and simvastatin Impurities

S.No.	Sample	RF
1	Ezetimibe impurity A	0.95
2	Ezetimibe impurity B	1.18
3	Ezetimibe impurity C	0.79
4	Ezetimibe	1.00
5	Ezetimibe impurity D	0.76
6	Simvastatin hydroxy acid	1.02
7	Epilovastatin and Lovastatin	1.02
8	Methylene simvastatin	1.18
9	Simvastatin	1.00
10	Acetyl simvastatin	1.24
11	Anhydro simvastatin	1.16
12	Simvastatin dimer	2.34

RESULT AND DISCUSSION

Method Development Study:
The aim of our research work study was to develop a simple, robust, accurate and sensitive HPLC method for the simultaneous determination of ezetimibe and simvastatin in their fixed dose combination. Initially various mobile phases and stationery phases were tested to obtain the best separation and resolution between ezetimibe and simvastatin.

In development trail 01 peak of ezetimibe and simvastatin was separated clearly but no impurities peak eluting in that chromatographic condition so we stop the development trail run after 30 minutes of run time and make new trail with some modified chromatographic condition as well as sample preparation and different mobile phase.

In development trail 02 peak of ezetimibe and simvastatin was separated clearly and peak of ezetimibe related impurities were eluted but no impurities peak of simvastatin diluted in that chromatographic condition so we stop the development trail 02 run after 60 minutes of run time and make new trail with some modified chromatographic condition as well as sample preparation and different mobile phase.

In development trail 03 we tried mobile phase A as 0.5 mL glacial acetic acid in 2000 mL water and mobile Phase Bas Acetonitrile. Column X-terra C18, 150 x 4.6mm, 3.5µ or equivalent, Flow Rate 1.4 mL / min Detection 215 nm and Run Time 90 minutes we find peak of ezetimibe and simvastatin was separated clearly (About 22.0 minutes to 25.0

minutes for Ezetimibe and About 33.0 minutes to 37.0 minutes for Simvastatin) and peak of ezetimibe and simvastatin related impurities were eluted clearly. So we finalized development trail 03 for further study and perform method validation based on result obtained from development trail 03.

Method Validation Study

Specificity:

Prepared a representative Standard solution and Sample solution of Ezetimibe and Simvastatin Tablets and injected System suitability solution, Diluted Standard Solution, Sample Solutions, individual impurity solutions and Spiked Sample Solutions in HPLC using the Chromatographic system described in the procedure by using a photodiode array detector. Result of specificity given in table 4 and figure 1 & 2.

Table 4: Peak purity of spiked samples

Impurities	Spike Sample	
	Purity angle	Purity Threshold
Ezetimibe Impurity A	3.827	21.665
Ezetimibe Impurity B	0.769	6.229
Ezetimibe Impurity C	1.004	5.774
Ezetimibe	0.093	1.095
Simvastatin hydroxy acid	0.389	3.428
Lovastatin &Epilovastatin	0.490	4.162
Ezetimibe Impurity D	5.093	23.483
Methylene Derivative	1.254	8.753
Simvastatin	0.249	1.417
Simvastatin acetate ester	1.765	13.156
Anhydrosimvastatin	2.349	15.661
Simvastatin Dimer	3.374	24.984

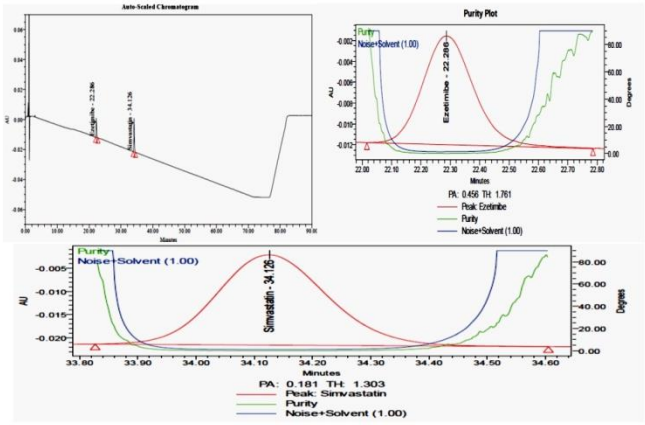

Figure 1: Chromatogram of standard solution

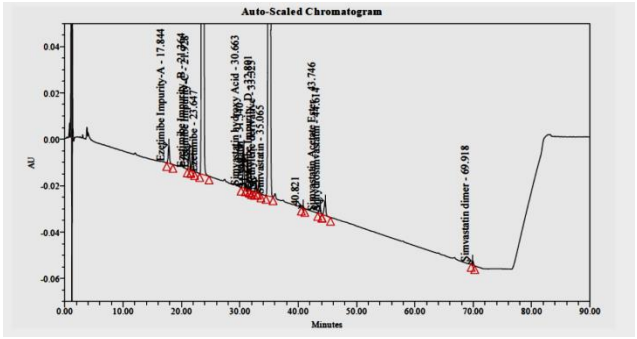

Figure 2: Reference Spike sample solution Chromatogram

Forced Degradation Studies:

Result of Force degradation studies in different degradation conditions is given below in table 5.

Table 5: Table for impurities in Forced Degradation Studies

Experiment	% Single max. unknown	%Total Impurities
Control	0.059	0.959
Acid Degradation	0.068	16.088
Base Degradation	1.140	30.462
	0.640	16.936
Peroxide Degradation	3.680	17.264
Thermal Degradation	0.711	4.511
Humidity Degradation	0.061	1.041
Photolytic Degradation	0.074	1.002

LOD & LOQ:

Based on response of Prediction linearity, LOD and LOQ were determined. Six replicate injections were made for LOD & LOQ. Results of LOQ and LOD given in table 6.

Table 6: Table of LOQ and LOD Study

Name of Impurity	LOD	LOQ	Name of Impurity	LOD	LOQ
Ezetimibe	0.135	0.449	Simvastatin	0.257	0.858
Ezetimibe Imp. A	0.187	0.622	Hydroxy acid	0.354	1.178
Ezetimibe Imp. B	0.215	0.718	Lova&Epilova	0.356	1.187
Ezetimibe Imp. C	0.124	0.412	Methylene Derivative	0.224	0.745
Ezetimibe Imp. D	0.156	0.520	Simvastatin acetate ester	0.309	1.031
			Anhydro simvastatin	0.304	1.014

Linearity:

A series of Standard preparations (minimum of five preparations) in duplicate of Ezetimibe, Simvastatin and impurity working standards were prepared over a range of the LOQ to 150% of specification limits. Linearity of ezetimibe and simvastatin given in figure 3 to 4.

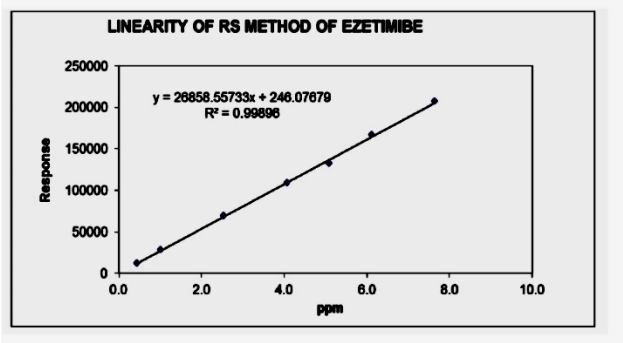

Figure 3: Linearity graph of Ezetimibe

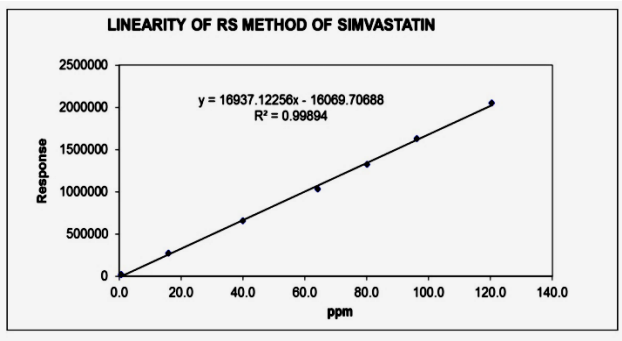

Figure 4: Linearity graph of Simvastatin

Discussion:

Linearity of Ezetimibe, Simvastatin and impurity working standards were prepared over a range of the LOQ to 150% of specification limits. Correlation coefficient of Ezetimibe,

Simvastatin and their related impurities were found within acceptance criteria. Therefore we concluded that method is linear for above given concentration.

Accuracy:

Sample of Ezetimibe and Simvastatin Tablets, were spiked with Known impurities at four different levels: LOQ, 50%, 100%, and 150% of specification limit. Results obtained from accuracy were given in table 7.

Table 7: Table for Recovery

Impurity name	% Mean Recovery	Impurity name	% Mean Recovery
Ezetimibe		Simvastatin	
Impurity A	97.5	Hydroxy acid	96.7
Impurity B	99.8	Lova&Epilova	104.5
Impurity C	102.0	Methylene Derivative	101.3
Impurity D	100.9	Acetate ester	101.9
		Anhydrosimvastatin	107.7
		Dimer	96.5

Discussion:

Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels. The Mean Recovery for all impurities is within limits. Therefore, the HPLC method for the determination of related substances in Ezetimibe and Simvastatin Tablets is accurate.

Precision:

Six replicate injections of the standard preparation were made into the HPLC using the method as described under procedure section. Result of system precision given in blow table 8.

Table 8: Table for System Precision

Injection	Area Ezetimibe	Area Simvastatin
1	136590	247245
2	130542	247314
3	129528	243981
4	133855	245806
5	137269	243830
6	133292	247342
Mean	133513	245920
SD	3112.823	1665.038
%RSD	2.33	0.68

Discussion:

RSD should not be more than 5.0%. The %RSD of system precision is within limits. Therefore, the HPLC method for the determination of related substances in Ezetimibe and Simvastatin Tablets is precise.

Method Precision & Ruggedness:

Six test solutions of Ezetimibe and Simvastatin Tablets and one sample spiked with known impurities at specification limit along with mix impurity standard at specification limit were prepared and injected into the HPLC along with standard preparation (in ruggedness different analyst and analysed using different column on a different day and injected into a different HPLC along with Standard preparation). Results of precision study were given in table 9.

Discussion:

Overall RSD should not be more than 10.0% for impurities observed above 50% of specification limit. Overall RSD should not be more than 15.0% for impurities observed between LOQ to 50% of specification limit. RSD is less than 10.0%.Therefore, the HPLC method for the determination of related substances in Ezetimibe and Simvastatin Tablets is rugged.

Table 9: Table for method Precision & Ruggedness Study

Sr. No.	% Single max unknown	% Single max unknown	% Total Impurity	% Total Impurity
1	0.054	0.050	0.910	0.854
2	0.058	0.052	0.853	0.869
3	0.054	0.053	0.904	0.849
4	0.060	0.054	0.878	0.850
5	0.051	0.052	0.906	0.847
6	0.060	0.054	0.897	0.855
Mean	0.054		0.873	
SD	0.003		0.025	
% RSD	5.56		2.86	

Robustness:

System suitability solution, Standard solution, control sample and placebo prepared and sample spiked with known impurities were injected under different chromatographic conditions. Result obtained from different parameter of robustness is given in table 10.

Table 10: Robustness, RRT

Parameter	Variation	RRT of Related Compounds				
		Ezetimibe			Simvastatin	
		Imp-A	Imp-B	Imp-C	Hydroxy Acid	Lova &Epilova
Sample-1	-	0.49	0.59	0.60	0.87	0.89
Sample-2	-	0.51	0.61	0.63	0.87	0.89
Column Temp	+5°C	0.47	0.58	0.59	0.88	0.89
	- 5°C	0.50	0.60	0.61	0.87	0.89
Flow rate	-0.1ml/min	0.49	0.59	0.61	0.87	0.89
	+0.1ml/min	0.48	0.58	0.60	0.87	0.89
Wavelength	-5 nm	0.51	0.61	0.63	0.87	0.89
	+5 nm	0.51	0.61	0.63	0.87	0.89

Discussion:

Based on the result obtained from different variable condition we concluded that method is robust i.e system suitability criteria found within limit in different variable condition.

Filter Equivalency:

Sample of Ezetimibe and Simvastatin Tablet spiked with Known impurities were prepared. Centrifuged in triplicate and filter in triplicate through one or more different membrane filters such as Nylon 0.45µ, Teflon 0.45µ filters discarding first few mL of the filtrate. Results of filter equivalency are given in table 11.

Table 11: Table for Filter equivalency

Sample	Ezetimibe				Simvastatin	
	Imp-A	Imp-B	Imp-C	Imp-D	Lova &Epilova	Hydroxy Acid
Centrifuge -1	0.472	0.534	0.464	0.212	1.163	1.544
Centrifuge -2	0.471	0.536	0.459	0.210	1.169	1.576
Centrifuge -3	0.468	0.511	0.463	0.214	1.156	1.564
Teflon -1	0.468	0.532	0.465	0.209	1.151	1.547
Teflon -2	0.471	0.519	0.470	0.204	1.160	1.542
Teflon -3	0.471	0.513	0.468	0.207	1.170	1.552
Mean	0.470	0.524	0.465	0.209	1.162	1.554
SD	0.0017	0.0112	0.0039	0.0036	0.0074	0.0133
%RSD	0.36	2.14	0.84	1.72	0.64	0.86
Nylon -1	0.467	0.536	0.466	0.210	1.173	1.533
Nylon -2	0.463	0.529	0.470	0.208	1.158	1.563
Nylon -3	0.470	0.533	0.467	0.210	1.157	1.576
Mean	0.469	0.530	0.465	0.211	1.163	1.559
SD	0.0033	0.0096	0.0038	0.0021	0.0070	0.0174
%RSD	0.70	1.81	0.82	1.00	0.60	1.12

Discussion:

Based on the result obtained from Centrifuged and different membrane filters such as Nylon 0.45µ, Teflon 0.45µ filters. We concluded that method is compatible for Nylon Teflon filter system suitability criteria found within limit in different filter.

SUMMARY AND CONCLUSION

All the method validation parameter performed for development trail 03 and found all parameter within acceptance criteria. During method validation system suitability of each parameter was found within limit. In specificity no placebo and other interference was found and in force degradation studies we obtained desired degradation on Acid, base and peroxide conditions. In Linearity both ezetimibe and simvastatin with their relevant impurities correlation coefficient within acceptance criteria simultaneously we performed Accuracy, precision, solution stability and filter equivalency and result obtained in each of parameter was well within limit. Method validation performed As per ICH guidelines Q2 (R1).

ACKNOWLEDGEMENTS

Authors would like to thanks the Sri Satya Sai University of Technology & medical science, department of science, chemistry division, Sehore Madhya Pradesh, India for giving us an opportunity to carry out validation & provide necessary facilities in Laboratories.

REFERENCES

1. Taizia DS, Marcelo AO, Renata BO, Cristina DVS, 2012. Development and Validation of a Simple and Fast HPLC Method for Determination of Lovastatin, Pravastatin and Simvastatin. Journal of Chromatographic Science. 50, 831–838.
2. Hassan A. Alhazmi, Ahmed M. Alnami, Mohammed A. A. Arishi, Raad K. Alameer, Mohammed Al Bratty, Zia urRehman, Sadique A. Javed, Ismail A. Arbab, 2018. A Fast and Validated Reversed-Phase HPLC Method for Simultaneous Determination of Simvastatin, Atorvastatin, Telmisartan and Irbesartan in Bulk Drugs and Tablet Formulations.Sci Pharm. 80, 11-21.
3. Goel A,Baboota S, Sahni JK,Srinivas S, Gupta RS, Gupta A, Semwal VP, Ali J, 2013. Development and Validation of Stability-Indicating Assay Method by UPLC for a Fixed Dose Combination of Atorvastatin and Ezetimibe. JChrom Sci. 51, 222–228.
4. Malenovic D, Ivanovic M, Medenica B, Jancic S, Markovic, 2014. HPLC determination of ezetimibe and simvastatin in pharmaceutical formulations. J of Sep Sci. 27, 1087-1095.
5. Paulo RO, Thiago B, Vitor T, Sergio LD, 2017. Simultaneous liquid chromatographic determination of ezetimibe and simvastatin in pharmaceutical products. J AOAC Inter. 90, 1566-1572.
6. Zhao JJ, Xie IH, Yang AY, Roadcap BA, Rogers JD, 2000. Quantitation of simvastatin and its β-hydroxy acid in human plasma by liquid-liquid cartridge extraction and liquid chromatography/tandem mass spectrometry, J of Mass Spectro. 35, 1133-1143.
7. Oliveira PR, Barth T, Todeschini V, Dalmora S, 2007. Simultaneous liquid chromatographic determination of ezetimibe and simvastatin in pharmaceutical products. J AOAC Inter. 90, 1566–1572.
8. Melani L, Mills R, Hassman D, 2013. Efficacy and safety of ezetimibe co-administered with pravastatin in patients with primary hypercholesterolemia: A prospective, randomized, double-blind trial, Eur Heart J. 24, 717-728.
9. Kadav AA, Vora DN, 2008. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets, J Pharm and Biom Ana. 48, 120-126.
10. Novakova L, Satinsky D, 2008. HPLC methods for the determination of simvastatin and atorvastatin, Trends in Ana Chem. 27, 352-367.
11. Seshachalam U, Kothapally CB, 2008. HPLC analysis for simultaneous determination of atorvastatin and ezetimibe in pharmaceutical formulations. J Liq Chroma and Rel Tech. 31, 714–721.
12. Chavhan V, Reddy K, Ahhirao K, 2014. Development of UV spectrophotometric methods and validation for estimation of simvastatin in bulk and tablet dosage form by absorbance maxima and area under the curve method. J App Pharm Sci. 6, 55–64.
13. Erturk S, Aktas ES, Ersoy L, 2003. A HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm and Biomed Anal. 33, 1017-1023.
14. Center for Drug Evaluation and Research, US Food and Drug Administration. Reviewer Guidance, Validation of Chromatographic Methods. FDA; Rockville, MD, USA: 2017.
15. International Conference on Harmonization, ICH harmonized tripartite guideline validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, 2005.

How to cite this article

Neelu Jain, Srinivasarao Kosanam, Surendra Singh Rao, Asheesh Singh, 2021. Cost effective stability indicating rapid, efficient analytical method development and validation of related substances method for simultaneous determination of simvastatin and ezetimibe in tablet formulations. Jour. of Med. P'ceutical &Alli. Sci. V 10 - I 3, 1187 P-3024-3028. DOI: 10.22270/jmpas.V10I3.1187.