D. Sathis Kumar
*Department of Pharmaceutical
Analysis, Aditya Institute of
Pharmaceutical Sciences and
Research, Surampalem, Andhra
Pradesh, India-533437.*

Lps. Nainesha Allada
*Department of Pharmaceutical
Analysis, Aditya Institute of
Pharmaceutical Sciences and
Research, Surampalem, Andhra
Pradesh, India-533437.*

Y. Narayudu
*Department of Pharmaceutical
Analysis, Aditya Institute of
Pharmaceutical Sciences and
Research, Surampalem, Andhra
Pradesh, India-533437.*

Spectrophotometric Method Development and Validation for Montelukast Sodium and Simvastatin in Bulk and Tablet Dosage Form Using Absorption Ratio Method

D. Sathis Kumar, Lps. Nainesha Allada, Y. Narayudu

ABSTRACT

A simple, economic, and accurate absorption ratio method was developed for the simultaneous estimation of Montelukast Sodium (MTK) and Simvastatin (SMV) in bulk and tablet dosage form. 0.1M NaOH was used as a diluent to dissolve MTK and SMV. The absorptions were observed at 244 nm (Isosbestic point) and 295 nm (λmax of MTK) which were selected based on overlapping spectra of SMV and MTK. The linearity range was found to be 2-10 µg/ml at 244 nm (r^2= 0.998±0.001) and 295 nm (r^2= 0.998±0.0008). The method was found to be simple, precise, accurate and rapid for the simultaneous determination of SMV and MTK in bulk and tablet dosage form using absorption ratio method. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Keywords: Montelukast Sodium, Simvastatin, Absorption ratio, Isosbestic point.

1. Introduction

Montelukast Sodium (MTK) is chemically 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl}-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl] acetic acid. Montelukast belongs to a class of leukotriene receptor antagonist (LTRA), used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene, and results in less inflammation [1]. Its molecular formula and molecular weight are $C_{35}H_{36}ClNO_3S$ and 586.18 g/mol, respectively [2]. The structural formula of MTK is in Figure 1. Literature survey reveals few analytical methods for the determination of Montelukast Sodium alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. Spectrophotometry [3-6], HPLC [7-8] and HPTLC [9-10].

Simvastatin (SMV) is chemically [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl] ethyl]-3, 7-dimethyl-1, 2, 3, 7, 8, 8a-hexahydronaphthalen-1-yl] 2, 2-dimethylbutanoate. Simvastatin is a Supplied as a crystalline Solid with the molecular weight of 418.56g/mol and the molecular formula $C_{25}H_{38}O_5$ [11]. Simvastatin is a prodrug in which the 6-membered lactone ring of simvastatin is hydrolyzed *in vivo* to generate the beta, delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme. Interference with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol. The structural formula of SMV is in Figure 1. Literature survey reveals few analytical methods for the determination of Simvastatin alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. Spectrophotometry [12-15] and HPLC [16- 17].

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that simultaneous analytical methods are not available for the drug combination *SMV and MTK*. Hence it is proposed to develop new methods for the assay of *SMV and MTK* in pharmaceutical dosage forms adapting UV visible spectrophotometry. The objective of the study was to develop a simple and accurate method for the determination of *SMV and MTK* simultaneously using absorption ratio method by UV-spectrophotometry in pharmaceutical dosage form.

Correspondence:
Lps. Nainesha Allada
Department of Pharmaceutical
Analysis, Aditya Institute of
Pharmaceutical Sciences and
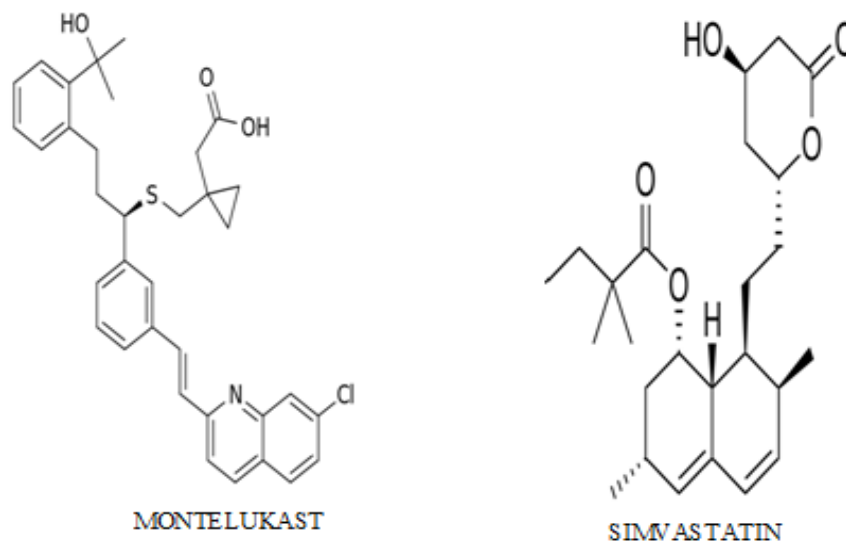Research, Surampalem, Andhra
Pradesh, India-533437.

Fig 1: Structures of drugs Montelukast and simvastatin (images are derived from http://en.wikipedia.org).

2. Materials and Methods
2.1. Materials
SMV and MTK obtained from pharmaceutical market were of analytical grade. A commercial sample *SMV and MTK* tablets were procured from local market and used within their shelf-life period. Sodium Hydroxide (S. D. Fine Chemical Limited, Mumbai) was of pharmaceutical or analytical grades.

2.2. Instrumentation
Quantitative estimation was performed on Labindia UV 3000+ double beam UV visible spectrophotometers (Maharashtra, India) with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UV-Win (Maharashtra, India) software was used along with quartz cuvette for the λmax and absorption prediction.

2.3. Trial and error method
To develop a suitable and robust absorption ratio method for the determination of *SMV and MTK,* different diluents like methanol, 0.1M HCl, etc., were tried based on the solubility and functional group present in the compound. Finally 0.1M NaOH was selected as a diluent due to its reproducible results. Absorbance was measured at selected λmax (244 nm and 295 nm) based on the overlap spectrum of both drugs. The data were collected and analyzed with software (Labindia UV-Win, Maharashtra, India) in a computer system.

2.4. Preparation of Standard Stock Solutions of SMV and MTK
Stock solution of MTK (1000 µg/mL) was prepared by dissolving 100 mg of drug in 100 ml of volumetric flask containing 50 mL of 0.1M NaOH. The solution was sonicated for about 15 minutes and then made up to 100 ml with 0.1 M NaOH. From the stock solution, 1 mL was pipette out and transferred into the 10 mL volumetric flask to get 100 µg/mL concentrations. From the second dilution, 0.5 mL was pipette out and transferred into the 10 mL volumetric flask to get 5 µg/mL concentrations. The same procedure was followed for SMV standard. The final solutions of both standard drugs solutions were scanned individually and spectra obtained were overlapped. From the overlap spectrum, two wavelengths were selected. Among the two, 295 nm is a λmax of SMV and 244 nm is an Isosbestic point (The wavelength at which both the drugs show same absorbance). Then the absorbance was measured at 244 nm and 295 nm and calculated the absorptivity from the formula $\mathcal{E} = A/cl$ where A is absorbance; c is concentration; l is path length.

2.5. Preparation of standard mixture
From 100 µg/mL of MTK and SMV standard second dilution, 0.5 ml was pipette out individually and mixed in 10 ml volumetric flask then it was made up to the mark with 0.1M NaOH. Absorbance was measured at selected λmax (244 nm and 295 nm).

2.6 Preparation of tablet Stock Solutions of MTK and SMV
20 MTK tablets were weighed and powdered. The amount of powder equivalent to 100 mg of MTK was weighed and transferred into the 100 ml of volumetric flask containing 50 ml of 0.1 M NaOH. The solution was sonicated for about 20 minutes and then made up to volume with 0.1 M NaOH. The solution was filtered using 0.25 µ filter paper and vacuum-associated filtration unit. From the filtrate, 1ml was pipette out and transferred into the 10 ml volumetric flask then made up to the mark with 0.1 M NaOH to get 100 µg/ml concentrations. 20 SMV tablets were weighed and powdered. The amount of powder equivalent to 100 mg of SMV was weighed. Then same procedures were followed to get the 100 µg/ml concentration.

2.7. Preparation of tablet mixture
From 100 µg/ml concentration of tablet second dilutions, 0.5 ml was pipette out individually and transferred combine in to a 10 ml volumetric flask then it was made up to the mark with 0.1M NaOH. The amount of drug present in pharmaceutical formulation was calculated [18] using the following formula:

Cy = (A1/ax1)-Cx
Cx = ((Qm-Qy)/Qx-Qy)) (A1/ax1)

where, Cx is a concentration of drug SMV in mixture; Cy is a concentration of MTK in mixture; Qx (absorption ratio of SMV) = ax2/ax1; Qy (absorption ratio of MTK) = ay2/ay1; Qm (absorption ratio of mixture) = A2/A1; A1 is absorption at 244 nm in mixture; A2 is absorption at 295 nm in mixture; ax1 and ax2 are absorptivities of SMV at 244 nm and 295 nm respectively. ay1 and ay2 are absorptivities of MTK at 244 nm and 295 nm, respectively.

2.8. Validation
The described method has been validated for the assay of *SMV and MTK* using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with absorbance. Six different concentrations of *SMV and MTK* drug mixtures (2 to 10 µg/ml of each drug in the mixture) were employed i.e., 2, 4, 6, 8, 10 µg/ml. All solutions were scanned and absorbance measured at 244 nm and 295 nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of *SMV and MTK* (6 µg/ml) on the same day. The precision of each method was ascertained separately from the absorbance obtained by actual determination of five replicates of a fixed amount of drug (6µg/mL). The % RSD (percentage relative standard deviation) was calculated for precision. The accuracy of the method was shown by analyzing the model mixtures containing 80, 100 and 120% of both *SMV and MTK* along with 6 µg/mL of placebo. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different normality of NaOH like 0.05 and 0.15M.

3. Results
An absorption ratio method procedure was proposed as a suitable method for the analysis of drugs *SMV and MTK* in dosage forms. A typical overlap spectrogram of standard *SMV*

and MTK and their mixture is shown in Figure 2. The λmax was found to be 244 nm and 295 nm. The regression equation for the method at 244 nm for SMV was found to be y = 0.696x+0.004(r=0.998) (slope, intercept and correlation coefficient were found to be -0.696±0.539, 0.004 ±0.016, and 0.998±0.0008) and linear over Beer's range 2-10 µg/ml. The regression equation for the method at 295 nm for MTK was found to be y =0.015x+0.0035 (r=0.998) (slope, intercept and correlation coefficient were found to be 0.0154±0.0004, 0.004±0.0023 and 0.998±0.0012) and linear over Beer's range 2-10 µg/ml. The linearity graph of *SMV and MTK* mixtures is shown in Figure 3. A typical overlap spectrogram of different concentration of mixture of *SMV and MTK* is shown in Figure 4.

The percentage of content of *SMV and MTK* in tablet dosage form was 101.44 ±1.13% and 99.29 ±1.46% respectively. The precision and ruggedness were determined using the % RSD of the absorbance for five replicate preparations of the drug. The % RSD of precision of *SMV and MTK* were found to be 1.65 and 1.52 respectively. The calculated RSD values were less than 2. Precision data are presented in Table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 80%, 100% and 120% of standard solution of drug MTK and drug SMV and along with 6 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 90.35±0.15, 101.20±1.25 and 91.89±0.72%w/w for 80%, 100% and 120% respectively for SMV. The mean percentage recoveries were found to be 93.28±1.46, 99.4±1.76 and 91.009±0.72%w/w for 80%, 100% and 120% respectively for SMV. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. Accuracy data are presented in Table 2. LOD for SMV and MTK was found to be 0.078 µg and 0.505 µg respectively. LOQ for SMV and MTK was found to be 0.237 µg and 1.530 µg respectively. Robustness was performed by following the same method with 0.05M and 0.15M NaOH. Percentage content of SMV and MTK in 0.05M NaOH diluents were 80.88±0.59 and 112.94±2.15 respectively; for 0.15M NaOH were 91.35±1.504 and 92.51±0.911 respectively. The results of robustness shown that in 0.05M and 0.15M NaOH, percentage content was reduced between 80 to 93% but percentage relative standard deviations values were less than 2.
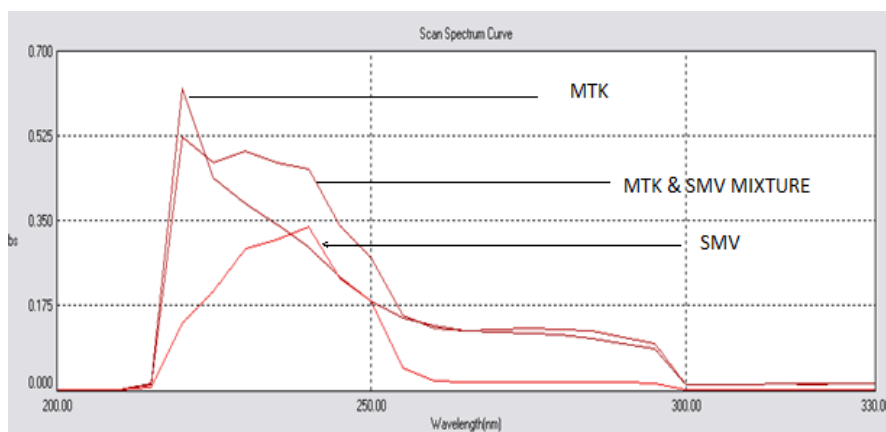

Fig 2: Overlap spectrogram of standard drugs Montelukast (MTK) and simvastatin (SMV) and their mixture
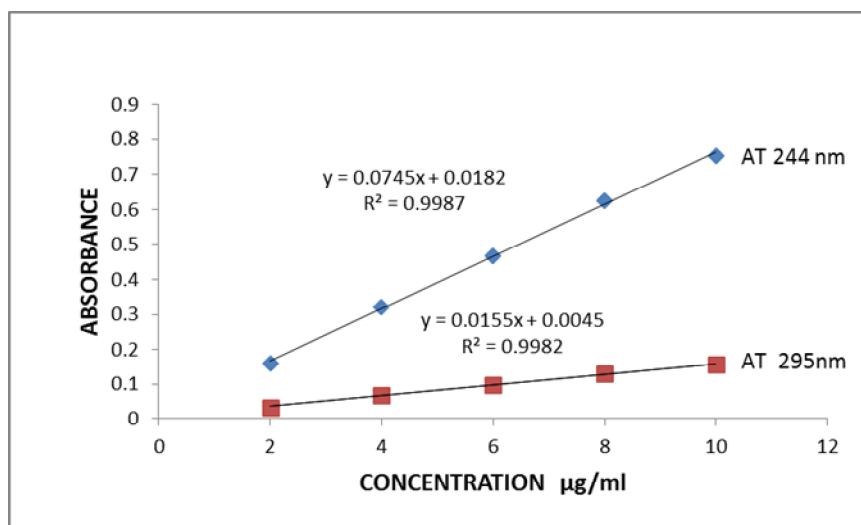
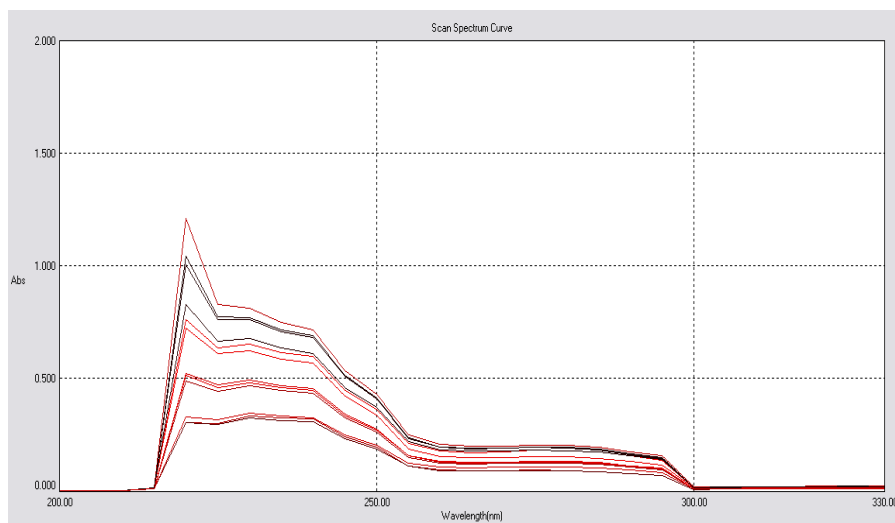**Fig 3:** Linearity graph of Montelukast (MTK) and simvastatin (SMV)

Fig 4: Overlap linearity Spectrogram of mixture of Montelukast (MTK) and simvastatin (SMV)

Table 1: Data for Precision SMV and MTK.

	Simvastatin	Montelukast	Simvastatin	Montelukast
	Percentage content		**Absorbance**	
	94.10743	92.61132	0.478	0.1
	90.18076	89.89737	0.461	0.096
	92.95343	92.98407	0.476	0.099
	91.18158	90.45905	0.465	0.097
	92.25899	90.55351	0.468	0.098
SD	1.525103	1.395301	0.007232	0.001581
MEAN	92.13644	91.30106	0.4696	0.098
%RSD	1.655266	1.528242	1.540007	1.613407
SMV- Simvastatin; MTK- Montelukast; SD- standard deviation; %RSD-Percentage relative standard deviation				

Table 2: Data for accuracy of SMV and MTK

	Accuracy 80%		Accuracy 100%		Accuracy 120%	
	SMV	MTK	SMV	MTK	SMV	MTK
	absorbance					
SD	0.002646	0.000577	0.008021	0.003055	0.004163	0.003606
MEAN	0.333	0.136667	0.545667	0.226667	0.663333	0.275
%RSD	0.79452	0.422451	1.46991	1.347816	0.627638	1.31111
	% recovery					
SD	0.150199	1.462955	1.258277	1.765791	1.870633	0.726514
MEAN	90.35685	93.28285	101.203	99.40974	91.8949	91.00951
%RSD	0.166228	1.5683	1.24332	1.776276	2.035622	0.798283
	% content					
SD	0.100132	0.975303	1.258277	1.765791	2.494177	0.968685
MEAN	60.2379	62.18857	101.203	99.40974	122.5265	121.346
%RSD	0.166228	1.5683	1.24332	1.776276	2.035622	0.798283
	% relative error					
SD	0.150214	1.463102	1.258277	1.765791	1.871101	0.726695
MEAN	-9.63411	-6.70782	1.203004	-0.59026	-8.08212	-8.96773
SD- Standard deviation; %RSD- percentage relative standard deviation						

4. Discussion
The developed method can be used for routine analysis because the linearity found in SMV and MTK is nearby to 1 that is 0.998 and 0.998 which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recoveries for each component are nearby 100%. So, method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods [3-6, 12-15] consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like Sodium Hydroxide, and distilled water, which are very simple, economical and also easily available. And also our proposed method requires less time for the determination of SMV and MTK simultaneously compared to other methods and even these other methods required reagents which is costly and time taking for the reaction.

5. Conclusion
The presented method was found to be precise, sensitive and accurate. This method has simple sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of SMV and MTK in pharmaceuticals.

6. Acknowledgement

7. References
1. Montelukast Sodium Monograph. http://www.drugbank.ca/drugs/DB00471. 4 Nov, 2013.
2. Montelukast Sodium Monograph. http://pubchem.ncbi.nlm.nih.gov/summary/summar.cgi?cid=5281040, 4 Nov, 2013.
3. Jyoti JS, Pratik PG, Parula BP. Development and validation of simultaneous equation method for estimation of ebastine and montelukast sodium in combined tablet dosage form, Pelagia Research Library 2012; 3(6):690-698.
4. Pallavi K, Srinivasa BP. Validated UV Spectroscopic Method for Estimation of Montelukast Sodium from Bulk and Tablet Formulations. International Journal of Advances in Pharmacy Biology and Chemistry 2012; 1(4):434–37.
5. Shanmukha KJV, Ramachandran D, Sushma K, Vijaya SS. Visible Spectrophotometric Methods For Estimation Of Montelukast Sodium In Bulk Dosage Forms And Formulations. Oriental Journal of Chemistry 2010; 26(1):293-296.
6. Madhuri AN, Shweta VP, Swetal PV, Simultaneous Spectrophotometric Estimation of Levocetrizine Dihydrochloride, Montelukast Sodium and Ambroxol Hydrochloride in Bulk and Capsule Dosage Form. Journal of Advances in Pharmacy and Healthcare Research 2012; 2(4):3-8.
7. Lakshmi MY, Rao SN, Meghana R. Method Development and Validation for Simultaneous Estimation of Fexofenadine Hcl and Montelukast Sodium by Rp-Hplc in Pure and Combined Tablet Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences 2013; 2(6):5948-5965.
8. Sandeep KBVV, Pratima M, Rajesh N, Rao ND, Satyanarayana P. Analytical Method Development and Validation of Levocetirizine Hydrochloride and Montelukast Sodium In Combined Tablet Dosage Form By Rp-Hplc, 2011; 2(7):380–396.
9. Rathore AS, Sathiyanarayanan L, Mahadik KR,

Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form, Pharmaceutica Analytica Acta 1:106.

10. Sonali R, Pallavi P, Vittal C. Development and Validation of HPTLC method for the estimation of Sitagliptin Phosphate and Simvastatin in bulk and MarketedFormulation. International Journal of Drug Development & Research 2012; 4(3):292-297.

11. Simvastatin Monograph http://www.drugbank.ca/drugs/DB00641. 4 November, 2013.

12. Varsha BM, Surekha B, Nita K. Development of UV Spectrophotometric method for the simultaneous estimation of Simvastatin and Ezetimibe in tablet dosage form by simultaneous Equation and Absorbance ratio method. International Journal of PharmTech Research 2011; 3(3):1459-1466.

13. Asha JP, Tejaswi K, Sujitha PS. Spectrophotometric Estimation of Simvastatin in Bulk and Tablet Dosage Form. International Journal of Innovative Pharmaceutical Research 2013; 4(1):284-287.

14. Ankur K, Sheetal S, Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Tablet Dosage Form. Int J Pharm 2012; 2(3):609-612.

15. Sujani P, Reddy PY, Devanna N, Phanindra S, Anasuya P. New Validated Spectroscopic Method For The Simultaneous Estimation Of Simvastatin and Sitagliptin. Research & Reviews: Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(1):32-36.

16. Jat RK, Sharma S, Chhipa R, Singh R, Alam I. Development and Validation of Reversed Phase Hplc Method for Estimation of Simvastatin In Pharmaceutical Dosage Form. Journal of Drug Delivery & Therapeutics 2012; 2(3):121-124.

17. Krishnaveni G, Sathyannarayana PVV. Method Development and Validation for Simultaneous Determination of Ezetimibe and Simvastatin In Combined Pharmaceutical Dosage form by Rp-Hplc Method. International Journal of Pharmaceutical and Life Sciences 2013; 2(2):60-69.

18. International Conference on Harmonization (ICH), 1995.