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First derivative UV-spectrophotometric method for simultaneous determination of simvastatin and ezetimibe in tablet dosage form

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ABSTRACT

A rapid, precise, accurate and specific first derivative UV spectrophotometric method was developed for the simultaneous estimation of simvastatin and ezetimibe in tablet dosage form. The first derivative spectrum was recorded between 200 and 350 nm and a zero-crossing technique for first-derivative measurement at 235 nm and 266 nm of simvastatin and ezetimibe, respectively were selected. Methanol was used as solvent. The developed method was validated for linearity, accuracy and precision as per ICH guidelines. The method illustrated excellent linearity (correlation coefficient (r^2 > 0.999) in the concentration range of 2-20 µg/mL for simvastatin and ezetimibe. Precision (%R.S.D. < 1.50) and analytical recovery was found in the range of 91-101%, show the suitability of the method for determination in quality control analysis. The described UV spectrophotometric method can be successfully employed for the quantitative analysis of simvastatin and ezetimibe as in bulk drug and in pharmaceutical formulations.

Key words: Simvastatin, Ezetimibe, First-derivative spectrophotometry, Validation.

INTRODUCTION

Simvastatin (SIM) is chemically 2,2-dimethylbutanoic acid (1S, 3R, 7S, 8S, 8aR)- 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R, 4R)-tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl] ethyl]-1-napthalenyl ester (Fig.1). It is used for the treatment of hypercholesterolemia. It competitively inhibit HMG co-enzyme-A reductase, a rate limiting step in cholesterol synthesis. Reduce cholesterol synthesis results in compensatory increase in uptake of plasma cholesterol mediated by increase in number of LDL receptors. Therefore, LDL level in plasma reduces [1, 2].

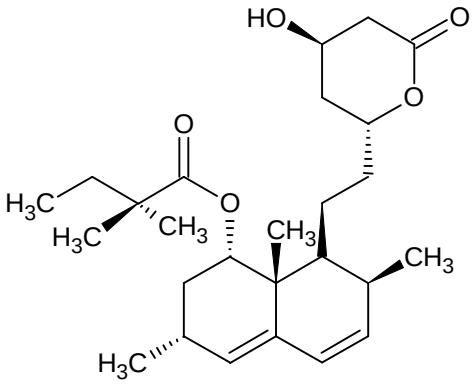

Fig. 1: Chemical structure of simvastatin

Ezetimibe (EZE), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is chemically designated as (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4 -hdroxyphenyl)-2-azetidinone (Fig. 2). It prevents transport of cholesterol through the intestinal wall by selectively blocking the absorption of cholesterol from dietary and billiary sources. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and a subsequent reduction in serum LDL-C [3, 4].

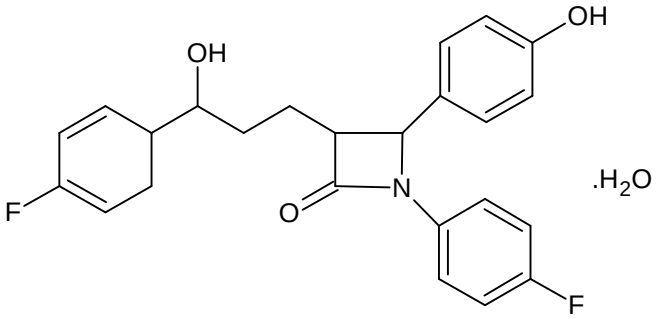

Fig. 2: Chemical structure of ezetimibe

In literature survey some analytical methods were reported for the quantitative determination of SIM, alone or in combination with other drugs by spectrophotometry [5-14], voltammetry [15], micellar enhanced kinetic chromatography (MEKC) [16], UPLC-MS [17], HPLC [18-21], HPTLC [22-26], LC-MS/MS [27]. Various methods have been reported for determination of EZE, individually or in combination with other drugs. These methods include spectrophotometry [28, 29], spectrofluorimetry [30], MEKC [31], gas chromatography-mass spectrometry (GC-MS) [32], UPLC [33], HPLC [34–35] and LC-MS/MS methods [36].

This paper reports the development and validation of a simple, rapid and sensitive first order derivative spectrophotometric method for the simultaneous determination of SIM and EZE in pharmaceutical formulation. The proposed method was validated as per ICH [37].

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutically pure drug samples of SIM and EZE, were obtained as gift samples from Micro Labs, Bangalore, India. Commercial tablet formulations containing SIM (10 mg) and EZE (10 mg) were purchased from the local market. All chemicals and reagents used were of Analytical Grade, obtained from E. Merck, Mumbai, India.

Instrumentation

UV spectrophotometric analyses was carried out on Shimadzu 1601 Double beam UV-Vis spectrophotometer, with pair of 1.0 cm matched quartz cells.

Preparation of standard solutions and calibration curve

The standard stock solutions (1 mg/mL) of SIM and EZE were prepared separately in methanol, which were further diluted with the same solvent to a concentration of (0.1 mg/mL) of each drug as working standard solutions.

For calibration, series of SIM and EZE solutions containing 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 µg /mL were prepared by diluting the standard solutions of SIM and EZE with methanol in volumetric flasks (10 mL) for the UV derivative-spectrophotometric method.

Study of spectra and selection of wavelength

Aliquot of each working standard solution were transferred into 10 mL volumetric flasks and then diluted with the methanol to obtain a concentration of 10 µg/mL of each drug. The final solutions were scanned in spectrum mode of the instrument from 350-200 nm. The first derivative spectrum of standard solutions was recorded. Upon examining the first derivative spectra of the two drugs (Fig. 3), it can be noticed that SIM can be determined at 235 nm (zero crossing point of EZE) and EZE can be determined at 266 nm where SIM shows a zero crossing point. The concentrations of drugs were determined from the standard calibration curve of EZE and SIM, respectively by interpolation method.

$A_1 = 0.0038C_{SIM} + 0.0004$ $r^2 = 0.9994$ ($\lambda = 235$ nm) -------(1)

$A_2 = 0.0026C_{EZE} + 0.0003$ $r^2 = 0.9991$ ($\lambda = 266$ nm) -------(2)

where,
C_{SIM} and C_{EZE} = concentration in µg/mL.
A_1 and A_2 = peak amplitude of the first derivative curves at 235 nm and 266 nm for SIM and EZE respectively.
r^2 = correlation coefficient.

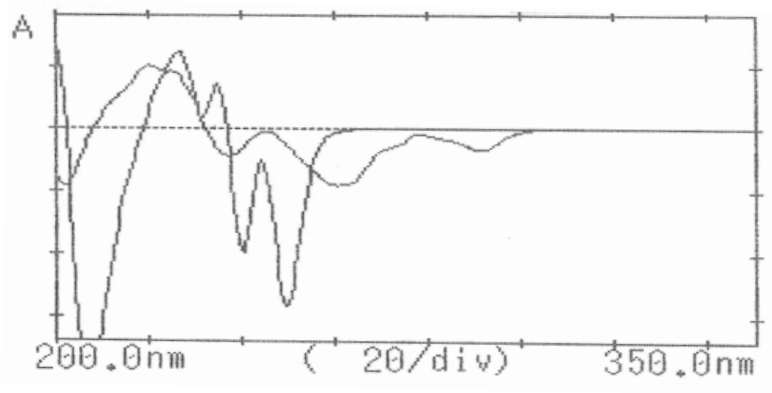

Fig. 3: Overlain first order derivative spectra of simvastatin (SIM) and ezetimibe (EZE) in methanol

Assays of the pharmaceutical preparations by the proposed method
Twenty tablets were individually weighed to get the average weight of the tablet. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 10 mg of SIM and EZE was transferred to 50 mL volumetric flasks, sonicated for 20 minutes with 20 mL methanol, then the volume was brought to 50 mL with the same solvent and filtered to prepare stock solution each drug having a concentration 0.2 mg/mL.

Aliquots portion of filtrate was diluted with the same solvent to produce solution of 10 µg/mL of SIM and 10 µg/mL of EZE. The first-derivative spectrum of sample solution was recorded and peak amplitude (*D*1) of first derivative spectra was measured at 235 nm and 266 nm for SIM and EZE, respectively. The amount of the two drugs was calculated from the computed regression eqn. (1) and (2). The results are represented in Table 1.

Table1: Results of analysis of tablet formulation

Drugs	% ± SD (n=6)
SIM	99.45±0.425
EZE	99.02±0.621

SD: Standard deviation.

Precision: The sample solutions of SIM and EZE were analyzed six times within the same day to obtain the repeatability. Each assay was carried out on a different sample of SIM and EZE. The percentage relative standard deviation (RSD %) of the data obtained was calculated.

Accuracy: The accuracy of the proposed methods was demonstrated by recovery experiments, using a standard addition technique to pre-analyzed tablet sample solution at three different concentration levels taking into consideration percentage purity of added bulk drug sample.

Linearity: Linearity of first derivative spectra of SIM and EZE was established by preparing standard solutions in concentration ranging from 2 to 20 µg/ml of SIM and EZE. The first-derivative spectra were recorded using the diluents as blanks and *D*1 values were determined at 235 nm and 266. Graphs were constructed by plotting *D*1 against standard concentrations.

Ruggedness: Ruggedness of the proposed method was determined by analysis of sample solution prepared by proposed methods between different time intervals, days and analysts. The % R.S.D. was determined.

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of SIM and EZE. The data of regression analysis of the SIM and EZE were found to be linear with correlation coefficient (r^2) = 0.9994 and 0.9991, respectively. The results of analysis of pharmaceutical dosage form by the proposed

method (Table 1), expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The intra-day and inter-day precision study (Table 2) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were <2%. Other validation parameters were found to be satisfactory and are shown in Table 2. The recovery studies of SIM and EZE were found in the range of 99.3 to 100.2% and 99.0 to 100.4%, respectively. Percentage of assay recovery shows that the method is free from interference of the excipients used in the formulation (Table 3).

Table 2: Recovery study results

Level of % recovery	Amount spiked (µg/mL)	%Recovery (n=3) ±S.D.	
		SIM	EZE
80	8	99.31±0.412	99.04±0.623
100	10	100.22±0.651	99.06±0.752
120	12	99.45±0.497	100.46±0.458

S.D.: Standard deviation.

Table 3: Validation parameters

Parameters	First order derivative method	
	SIM	EZE
Correlation coefficient	0.9989	0.9991
Linearity range	2-20 µg/mL	2-20 µg/mL
LOD(µg/ml)	0.7	1.0
LOQ(µg/ml)	0.5	0.9
Precision (% RSD)	0.425	0.621
Intra day (n=3) %± SD	100.34±0.623	99.61±0.791
Inter day (n=3) %± SD	99.45±0.991	100.11±0.842
Different analyst (n=3) %± SD	100.51±0.548	99.23±0.495

CONCLUSION

Based on the results and the statistical parameters obtained, it was concluded that the proposed method of analysis is simple, rapid, accurate, precise and economical. The method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and their by decreased the degree of error, time in estimation of drugs and the overall cost of the analysis. The developed method can be employed for routine quality control analysis of SIM and EZE in bulk and pharmaceutical formulations.

REFERENCES

[1] MJ O'Neil. The Merck index, In: An Encyclopedia of Chemicals, Drugs and Biologicals. Edn. 14th, Merck Research Lab, Whitehouse Station, NJ, USA, **2006**; 1471.
[2] SC Sweetman. Martin Dale, The Complete Drug Reference. Edn. 35th London: Pharmaceutical Press, **2002**; 1313.
[3] MJ O'Neil. The Merck index, In: An Encyclopedia of Chemicals, Drugs and Biologicals. Edn. 14th, Merck Research Lab, Whitehouse Station, NJ, USA, **2006**; 668.
[4] L Melani; R Mills; D Hassman. *Eur. Heart J.*, **2003**; *24*, 717-728.
[5] V Chavan; N Naghbidkar; M Shukla; V Singh. *PHARMANEST - An Int. J. Adv. Pharm. Sci.*, **2014**; 5, *1*, 1740-1750.
[6] L Wang; M Asgharnejad. *J. Pharm. Biomed. Anal*., **2000**; 21, *6*, 1243–1248.
[7] MM Sharaf El-Din; KA Attia; MW Nassar; MM Kaddah. *Spectrochimica Acta A*., **2010**; 76, *3*, 423–428.
[8] N Erk. *Der Pharmazie*, **2002**; 57, *12*, 817–819.
[9] S Sharma; MC Sharma; R Sharma; AD Sharma. *J. Pharma. Res.*, **2010**; 3, *5*, 1063-1067.
[10] VB Mane; S Babar; N Kulkarni. *Int. J. ChemTech Res.*, **2011**; 3, *3*, 1459-1466.
[11] V Singla; R Bhaskar. *Rasayan Journal*. **2010**; 3, *3*, 507-513.
[12] D Phaneemdra, V Venkatesh, N Ramarao. *Int. J. Adv. Pharm. Anal*., **2012**; 2, *1*, 19-23.
[13] IM Palabiyik; F Onur; C Yardimci; N Ozaltin. *Química Nova*., **2008**; 3, *5*, 1121-1124.
[14] G Phaneemdra, V Nagamalleswari, AE Venkatesh, PV Prabahar, NR Suresh. *Asian Pacific J. Trop. Biomed.*, **2012**; 1-6.
[15] O Coruh; SA Ozkan. *Der Pharmazie*. **2006**; 61, *4*, 285–290.
[16] MK Srinivasu; AN Raju; GO Reddy. *J. Pharm. Biomed. Anal.*, **2002**; 29, *4*, 715–721.
[17] L Novakova; H Vlckova; D Satınsky. *J. Chromatography B.*, **2009**; 877, *22*, 2093–2103.
[18] NS Sultana; M Arayne; SS Naz; N Shafi; SJ Naveed. *Chinese Chem. Soc*., **2010**; 57, *6*, 1286-1288.
[19] DA Kumar; DP Sujan; V Vijayasree; JV Rao. *J. Chem.*, **2009**; 6, *2*, 541-544.
[20] N Ozaltin; E Ucakturk; N Ozaltin; E Ucakturk. *Chromatographia*, **2007**; 66, 587-591.

[21] E Leitersdorf. *Int. J. Clin. Pract.*, **2002**; 56, *2*, 116-119.
[22] PR Dixit; RC Barhate; SM Nagarsenker. *J. Chromatographia.*, **2008**; 67, *1*, 101-107.
[23] B Shrestha; B Stephenrathinaraj; S Patel; N Verma; R Mazumder. *E-Journal of Chemistry.*, **2010**; 7, *4*, 1206-1211.
[24] SS Dhaneshwar; P Deshpande; M Patil; G Vadnerkar. *Acta Chromatographia*., **2008**; 20, *1*, 71-79.
[25] S Rathinaraj. *Int. J. Pharm. Bio. Arch.*, **2010**; 1, *4*, 222-227.
[26] S Rathod; P Patil; V Chopade. *Int. J. Drug Dev. Res.*, **2012**; 4, *3*, 292-297.
[27] B Barrett; J Huclova; V Borek-Dohalsky; B Nemec; I Jelinek. *J. Pharm. Biomed. Anal.*, **2012**; 41, *2*, 517-526.
[28] M Sharma; DV Mhaske; M Mahadik; SS Kadam; SR Dhaneshwar. *Indian J. Pharm. Sci*., **2008**; 70, *2*, 258–260.
[29] M Imran; RS Singh; S Chandran. *Der Pharmazie*., **2006**; 61, *9*, 766–769.
[30] NA Alarfaj; FA Aly. *J. Fluorescence.*, **2012**; 22, *1*, 9–15.
[31] SL Dalmora; PR Oliveira; T Barth; V Todeschini. *Analytical Sciences*, **2008**; 24, *4*, 499–503.
[32] E Ucakturk; N Ozaltin; B Kaya. *J. Separation Sci.*, **2009**; 32, *11*, 1868–1874.
[33] A Goel; S Baboota; JK Sahni. *J. Chromatogr. Sci.*, **2012**; 51, *3*, 222–228.
[34] R Sistla; VS Tata; YV Kashyap; D Chandrasekar; PV Diwan. *J. Pharm. Biomed. Anal.*, **2005**; 39, *3*, 517–522.
[35] A Ajmera; S Deshpande; P Patel; K Patel; S Solanki; K Rathod. *Int. J. Pharm. Pharm Sci*., **2012**; 4, *1*, 206–209.
[36] S Li; G Liu; J Jia; X Li; C Yu. *J. Pharm. Biomed. Anal.*, **2006**; 40, *4*, 987–992.
[37] ICH Guidelines, Validation of Analytical Procedures: Methodology Q2 (R1), International Conference on Harmonization, IFPMA, Geneva; **1996**.