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Development of RP-HPLC method for simultaneous estimation of ezetimibe and simvastatin in tablet formulation

Esmail O. Elakesh
Faculty of Pharmacy, University of Zawia, Az Zawiyah, Libya.
E-mail id: esmail_elakesh@yahoo.co.uk

ABSTRACT
A new, accurate, precise and robust HPLC method was developed and validated for the determination of Ezetimibe and Simvastatin in tablet dosage form. The chromatographic separation was achieved on an X-Terra C_{18} (150mm x 4.6mm, 3.5 µm) stationary phase maintained at ambient temperature with a mobile phase combination of 0.1% TEA and Acetonitrile (30:70) at a flow rate of 1.4 mL/min, and the detection was carried out by using UV detector at 238 nm. The total run time was 8 min. The retention time of Ezetimibe and Simvastatin were found to be 1.721 min. and 4.618 min. respectively. The performance of the method was validated according to the present ICH guidelines.
Keywords: Ezetimibe, Simvastatin, HPLC, Validation.

INTRODUCTION
Ezetimibe is an anticholesteremic agent. Chemically, it is known as (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one.Ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. This leads to a decrease in the delivery of intestinal cholesterol to the liver.[1]
Simvastatin is also anticholesteremic agent. Chemically, it is known as (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8 a hexahydro naphthalene-1-yl 2,2-dimethylbutanoate.

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 (hydroxyl methyl glutary 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.[2]
The literature survey revealed that there are several HPLC, UPLC, spectroscopic methods and HPTLC methods [5-18] available for the determination of Ezetimibe

and Simvastatin in their individual and combined dosage forms. The existed methods uses mobile phase of phosphate buffer having various pH range, acetate buffer, OPA buffer using different columns like Supelcosil, Synergi fusion, LiChrospher, Luna, etc. The present study was aimed to develop a new simple, precise, accurate and robust method for simultaneous estimation of Ezetimibe and Simvastatin in combined pharmaceutical dosage form.

EXPERIMENTAL

Instrumentation

The Waters HPLC system equipped with auto sampler and UV or DAD was used for method development, and method validation. The output signal was monitored and processed by using Empower software.

Materials

Ezetimibe and Simvastatin bulk drugs were made available from Pharmatrain Ltd. Orthophosphoric acid, methanol, acetonitrile were obtained from Merck. Commercially available Ezetimibe and Simvastatin tablets were used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment. The pharmaceutical dosage form assayed in the study is Vytorin 10/10 containing 10 mg of Ezetimibe and 10 mg of Simvastatin.

Chromatographic conditions

The mobile phase used was mixture of buffer of 0.1% TEA (pH 2.5) and acetonitrile in the ratio of 30:70 employing isocratic elution at a flow rate of 1.4 mL/min and the injection volume was 20 µL. The analytical column used was X-Terra C_{18} (150 mm x 4.6mm, 3.5 µm) at ambient temperature. The detection was carried out at a wavelength of 238nm for a run time of 8 min.

The Standard and sample stock solution were prepared in methanol and the working solution were prepared in the mobile phase. They were injected into the HPLC system by using above mentioned chromatographic conditions.

Preparation of standard stock solution

Accurately weigh and transfer 10 mg of Ezetimibe and 10 mg of Simvastatin working standard into two separate 10 mL clean dry volumetric flask add Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 mL of Ezetimibe and 0.3 mL Simvastatin of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Assay of pharmaceutical dosage form: (Sample preparation)

Take 20 tablets of Vytorin 10/10 and calculate the average weight. Crush the tablets and accurately weigh 77.5 mg of tablet powder and transfer it into 10 mL volumetric flask and add methanol to extract Ezetimibe and Simvastatin by ultra sonication for 10 min. The resultant mixture was filtered through 0.45 µ filter. From this take 0.3 mL and transfer it to 10 mL volumetric flask and make up the volume using mobile phase.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Several HPLC methods were developed for the estimation of Ezetimibe and Simvastatin using methanol, water, acetonitrile and phosphate, acetate, OPA buffer. Hence we have selected TEA buffer and X-Terra C_{18} column to decrease the retention time and to obtain symmetric peaks having good resolution. Different trails were performed using different proportions of TEA buffer having different pH with methanol, acetonitrile. The mobile phase, 0.1% TEA (adjusted to pH 2.5):acetonitrile (30:70 v/v) was found to be satisfactory and gave two symmetric and well-resolved peaks for Ezetimibe and Simvastatin.

The retention time of Ezetimibe and Simvastatin were found to be 1.721 and 4.618 respectively. The USP plate count and tailing factor were 2015, 2746 and 1.40, 1.32 for Ezetimibe and Simvastatin respectively. The USP resolution between Ezetimibe and Simvastatin was 10.81. The standard chromatogram was shown in Fig-1.

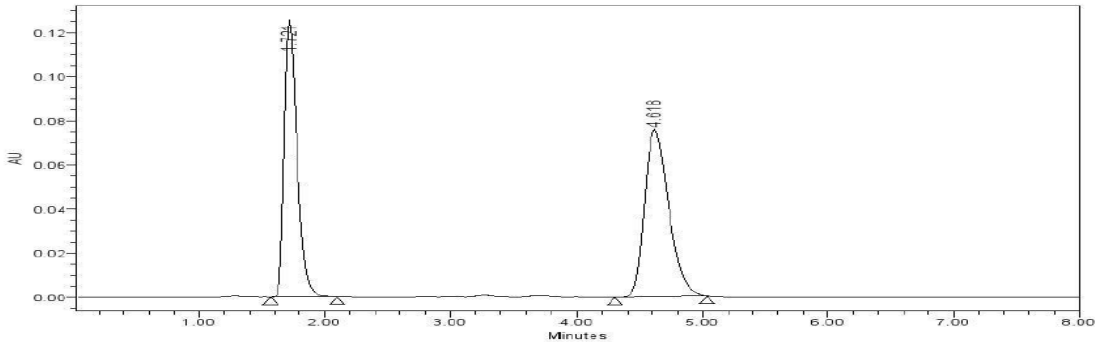

Fig-1: Standard chromatogram of Ezetimibe and Simvastatin

Validation of proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[5,6].

Linearity

Linearity test solutions of Ezetimibe and Simvastatin were prepared from the stock solution at five different concentration levels. The calibration curves were constructed by plotting peak areas versus its corresponding concentrations. The slope, Y-intercept and correlation coefficient of the calibration curve were calculated. The correlation coefficient was found to be 0.999 for both the drugs. The results were shown in Table 1 and the calibration curve for Ezetimibe and Simvastatin were shown in Fig-2 and Fig-3.

Table 1: Regression and statistical parameters

Parameter	Ezetimibe	Simvastatin
Concentration Range (µg/ mL)	10-50	10-50
Correlation coefficient	0.999	0.999
Intercept	62522	71559
Slope	9281	10535

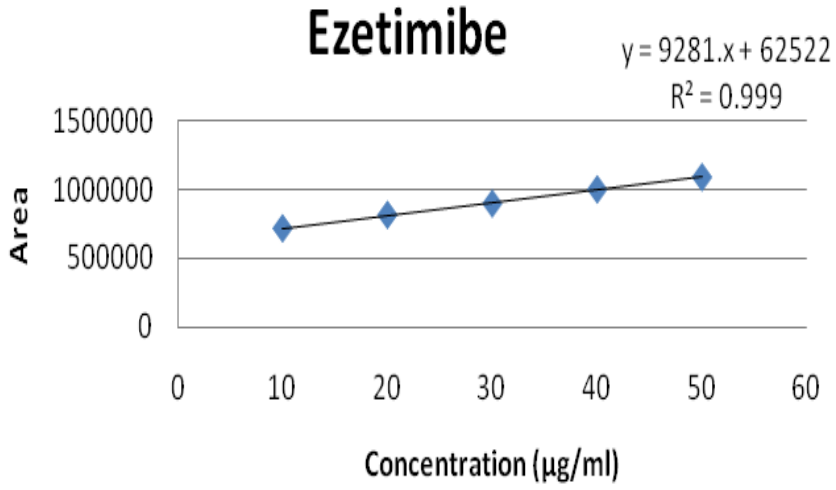

Fig-2: Calibration curve of Ezetimibe

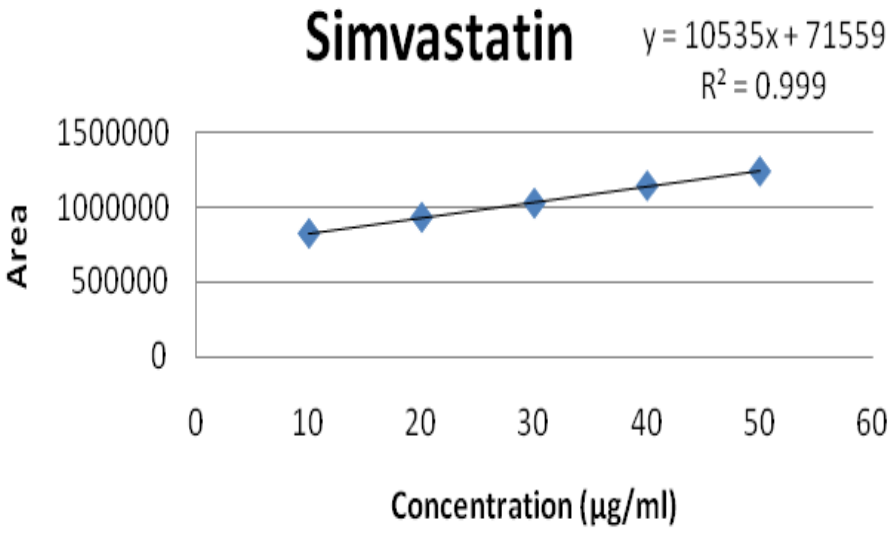

Fig-3: Calibration curve of Simvastatin

Precision

Precision was evaluated by injecting five replicate injections of Ezetimibe and Simvastatin of standard concentration under the same chromatographic conditions and calculated the % RSD. The %RSD indicates that the developed method is repeatable. The %RSD for assay of Ezetimibe and Simvastatin were found to be 0.09 and 0.10 respectively. The results were shown in Table-2.

Table 2: Precision Results for Ezetimibe and Simvastatin

S.No.	Ezetimibe	Simvastatin
1	882524	1018279
2	881215	1016821
3	882709	1019092
4	882812	1018954
5	883364	1019187
Average	882525	1018467
Standard deviation	796.1	986.3
% RSD	0.09	0.10

Intermediate precision/ruggedness

The intermediate precision of the method was checked by determining precision on same instrument, using same chromatographic conditions in different day. The % RSD of Ezetimibe and Simvastatin was found to be below 2 even when it is performed in different day. The method is said to be precise with respect to the criteria of the intermediate precision. The results were shown in Table-3.

Table 3: Intermediate Precision Results for Ezetimibe and Simvastatin

S.No.	Ezetimibe	Simvastatin
1	882222	1017321
2	883098	1018064
3	883441	1017142
4	882989	1017653
5	885032	1019474
Average	883356	1017931
Standard deviation	1037.2	931.4
% RSD	0.12	0.09

Accuracy

In order to judge the quality and applicability of method the recovery analysis was performed at three levels 50%, 100% and 150% by standard addition method. The % recovery for Ezetimibe and Simvastatin were calculated by injecting the samples and it was found to be within the limits for both the drugs. The results were shown in Table-4.

Table 4: Accuracy Results for Ezetimibe and Simvastatin

Analyte	% Level	Nominal Value (mg)	Found (mg)	% Recovery	Mean % Recovery
Ezetimibe	50%	5	4.95	99.03	100.15
	100%	10	10.18	101.8	
	150%	15	14.94	99.6	
Simvastatin	50%	5	4.96	99.2	99.62
	100%	10	10.12	101.1	
	150%	15	14.77	98.44	

Robustness

The robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase composition (10% absolute change in organic phase) and flow rate (± 0.1 mL min^{-1}). In all robust conditions, the resolution between Ezetimibe and Simvastatin has been found to be greater than 2.0. The USP plate count and USP Tailing were within the limits. So, the method was found to be robust with respect to variability in all robust conditions.

LOD and LOQ

The LOD and LOQ of Ezetimibe and Simvastatin were determined by using the signal to noise approach as defined in ICH guidelines. The concentration with signal to noise ratio of at least 3 was taken as LOD and concentration with signal to noise ratio of at least 10 was taken as LOQ. The results were shown in Table-5.

Table 5: LOD and LOQ Results for Ezetimibe and Simvastatin

Analyte	LOD (µg/ mL)	LOQ (µg/ mL)	LOD S/N Ratio	LOQ S/N Ratio
Ezetimibe	0.014	0.048	3.08	10.34
Simvastatin	0.024	0.072	3.14	10.16

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine Ezetimibe and Simvastatin in their tablet dosage form. The result obtained for Ezetimibe and Simvastatin was comparable with the corresponding labeled amounts and they were shown in Table-6.

Table 6: Assay Results for Ezetimibe and Simvastatin

Analyte	Label Claim (mg)	Amount found (mg)	% Assay
Ezetimibe	10	10	100.09
Simvastatin	10	9.88	98.77

CONCLUSION

In the present work a new, accurate, precise, and robust HPLC method was developed and validated for estimation of Ezetimibe and Simvastatin in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (8 min). The method was validated and found to be simple, accurate, and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Ezetimibe and Simvastatin in their combined dosage form.

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