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Development and validation of a simple UV spectrophotometric method for the determination of antihyperlipidemic drugs both in bulk and marketed dosage formulations

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Abstract
A rapid, specific and economic UV spectrophotometric method has been developed using Methanol as a solvent to determine the simvastatin and ezetimibe content in bulk and pharmaceutical dosage formulations at a pre-determined λmax of Simvastatin and Ezetimibe 249 nm and 228 nm respectively, it was proved linear in the range of 2.0–10.0 µg/mL, and exhibited good correlation coefficient (R^2=0.999 for Simvastatin and R^2=0.998 for Ezetimibe) and excellent mean recovery (99.65–99.98 % for Simvastatin and 98.23-99.11 % for ezetimibe). This method was successfully applied to the determination of simvastatin and ezetimibe content in marketed brands and the results were in good agreement with the label claims. The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of simvastatin and ezetimibe in bulks as well as in the commercial formulations.

Keywords: simvastatin, ezetimibe, UV spectrophotometric method

1. Introduction
Simvastatin chemically known as butanoic acid, 2, 2-dimethyl-1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-[2(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester (Figure 1), is an anti-lipidemic drug which is derived synthesized from fermentation products of Aspergillus terreus [1]. Simvastatin mainly used for the treatment and management of dyslipidemia and the prevention of cardiovascular disease.[2]It is instructed to use only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels [3]. General adverse reactions may include abdominal pain, diarrhoea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps [4]. Cholestatic hepatitis, hepatic cirrhosis, rhabdomyolysis and myositis have been reported in patients receiving the drug chronically [5]. Ezetimibe (Figure 2) is a drug that decreases cholesterol. It decreases absorption of cholesterol in the intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol lowering medications are not tolerated, or simultaneously with statins (ex-simvastatin/ezetimibe marketed as vytorin) when statins alone don't suppress cholesterol. Although ezetimibe controls cholesterol, the outcomes of two clinical trials (2008 and 2009) proved that it was not having any improvement, like major coronary events, and shown some outcomes, like thickening of artery wall, worse. Eventually, a panel of experts concluded in 2008 that it should "can be the last resort" [6].
Simvastatin was estimated by several methods including liquid chromatography with UV detection (LC–UV) [7, 9], gas chromatography-mass spectrometry (GC-MS) [10]. Ezetimibe was estimated alone or without combination of several drugs by high performance liquid chromatography and spectrophotometrically [11, 12].
Literature investigations reveal some HPLC methods have been reported for the estimation of these two drugs in combined dosage forms. Preliminary separation enforces pursuing of present research work.

2. Materials and methods
2.1 Apparatus
a) Shimadzu UV–visible spectrophotometer (UVmini-1601, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.
b) Precision balance model Citizen Cy 220 having sensitivity 0.1 mg was used for weighing the substances.
c) pH meter model Electronic India was used for measuring pH of solvents.

2.2 All reagents and chemicals used were of HPLC and analytical grade
All HPLC solvents and solution were filtered through membrane filter (ultipore'n86', Nylon 66, 0.45µm pore size) and degassed before use. The pure drugs Simvastatin (100.07 %) and Ezetimibe (99.75 %) were gifted by Zim Lab, Kalmeshwar, Nagpur and Blue Cross Lab Nashik were used as reference standard, respectively.
The tablets formulation was purchased from local market, its details are given in Table 1.

Table 1: Details of marketed tablet formulation

Brand Name	Drugs	Label claim (mg/tablet)	Manufacturer
SIMVOTIN EZ	Simvastatin (SEM)	10 mg	Hetero Labs Ltd., Kalyanpur (village). chakkan Road; Baddi (tehsil); Solan (Dist) Himachal Pradesh-173 205
	Ezetimibe (EZE)	10 g	

2.3 Determination of wavelength of maximum absorption

A standard stock solutions of SEM (100 µg/mL) and EZE (100 µg/mL) was prepared using methanol and 1 mL of both solution in 1:1 ratio was then diluted to 10 mL with the same diluents to obtain 10 µg/mL mixed standard stock solution. An UV spectroscopic scanning (200-350 nm) was carried out to determine the λmax for the detection of both using methanol as blank.

2.4 Linearity and range

Accurately measured aliquot portion of SIM, EZE and laboratory mixture (1:1) were diluted with Methanol to get the concentration in the range 2-10 µg/mL for both drug solutions. The obtained data were used for the linearity calibration plot. Limit of detection (LOD) and limit of quantification (LOQ) for the assay were also calculated [13]

2.5 Stability study

Samples prepared for repeatability study were preserved for 24 h at room temperature and analyzed on the following days to test for short-term stability.

2.6 Assay of content of SIM and EZE in selected marketed brand

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 10 mg of SIM and 10 mg of EZE was taken in volumetric flask (50 mL) and dissolved in about 10 mL of methanol and sonicated for 15 minutes, it was further diluted up to mark with methanol. The resulting solution was filtered through Whatmann filter paper no.41 and the filtrate was further diluted with methanol to obtain concentration of about 10 µg/mL of SIM and 10 µg/mL EZE. The absorbance of solution was measured at two selected wavelengths against blank.

Simultaneous equations were formed using these absorptivity coefficient values as follows. Quantitative estimation of SIM and EZE were carried out by solving following simultaneous equations.

$$Cx = A_1 \text{ X } 0.02065 - A_2 \text{ X } 0.02971 \quad \text{. . .} \quad [Eq.1]$$

$$Cy = A_2 \text{ X } 0.02265 - A_1 \text{ X } 0.01434 \quad \text{. . .} \quad [Eq.2]$$

Where,

A_1 and A_2 are absorbances of mixture at 228 nm and 249 nm respectively.

Cx and Cy are concentration of X and Y components estimated respectively.

2.7 Accuracy/recovery study

Recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. In 80 %, 100 % and 120 % recovery study for SIM, amount of standard drug solutions added was 8, 10 and 12 µg/mL of SIM respectively. In 80 %, 100 % and 120 % recovery study for EZE, amount of standard drug solution added 8, 10 and 12 µg/mL of EZE respectively. The mixed samples solution was analyzed to get the spectrum; absorbance measured at 249 nm and 228 nm. At each level minimum three determinations were performed.

2.8 Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

SIMVOTIN EZ tablets were finely powdered and the sample stock solution of 100 µg/mL was prepared following the same dilution pattern as above. Three different aliquots of stock were then diluted to 10 mL to obtain the concentrations of 10 µg/mL. This procedure was repeated in the following days.

3. Results and discussion

3.1 Method development and optimization

SEM and EZE are almost insoluble in aqueous medium and freely soluble in organic solvents like methanol and acetonitrile. During the development phase, the use of a few milliliters of methanol with water as the diluents resulted in preferable outcome in UV analysis. The use of pure methanol as solvent was optimized for better results. Wavelength 249 nm (λ_{max} of SIM) and 228 nm (λ_{max} of EZE) were selected for derivation of equation.

3.2 Method validation

3.2.1 Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the SIM and EZE in the range of 2-10 µg/mL for both drug solutions was linear with a correlation coefficient (R^2) near to requisite value.

3.2.2 Ruggedness studies

The studies of ruggedness were carried out under different conditions i.e. different elapsed times (interday, intraday) and different analysts (Table 2) confirmed adequate sample stability and method reliability with the entire % RSDs were < 2%.

3.2.3 Stability

Stability study's results were within the acceptance range (Table 3) and indicated the samples stability over 24 h (short-term).

3.2.4 Accuracy/recovery

Results within the range 99.65–99.98 % for Simvastatin and 98.23-99.11 % for ezetimibe, ensure an accurate method (Table 4) as well as indicate non-interference with the excipients of formulation.

3.2.5 Specificity in the presence of excipients

The specificity of the analytical method was proved by subjecting sample under various vigorous conditions. The results are shown in Table 5.

3.2.6 Content of SEM and ERE in marketed brands

SEM and ERE content of marketed products determined by the proposed method (Table 6) was in good agreement with the label claims and was in the range of 100.01 and 99.10 % for SEM and EZE with the RSD values of 1.702–1.176 % respectively.

4. Conclusion

The results and the statistical parameters reveal that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of antihyperlipidemic drugs, simvastatin and ezetimibe either in bulk or in the marketed dosage formulations without interference with commonly used excipients and related substances. This method can be further explore for dtermination of these drugs in usual pharmacokinetic and pharmacodynamic studies in biological fluids.

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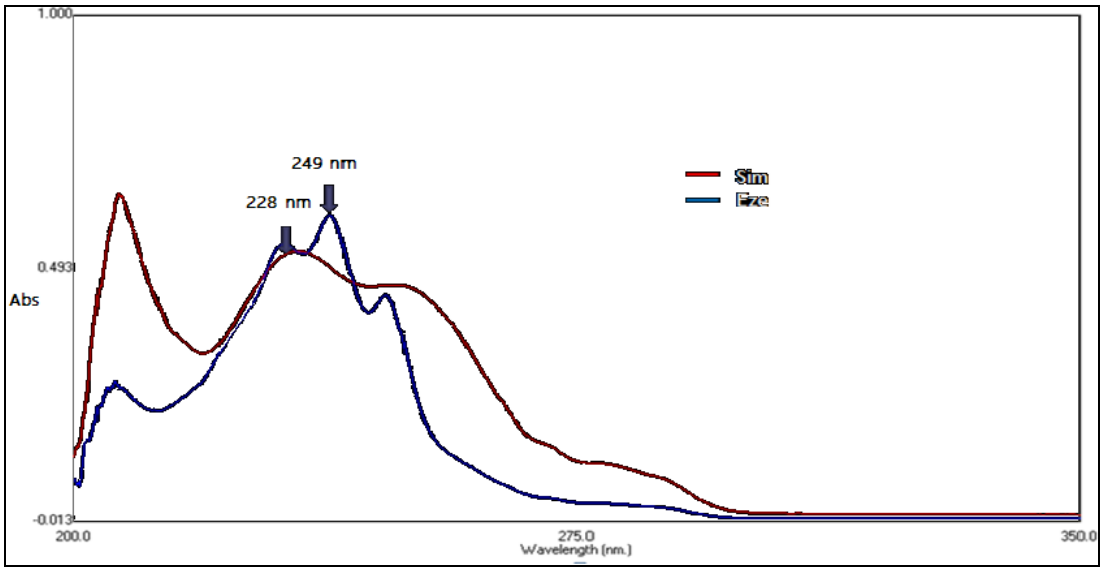

Fig 1: Overlain spectrum of SIM and EZE (10 µg/mL)

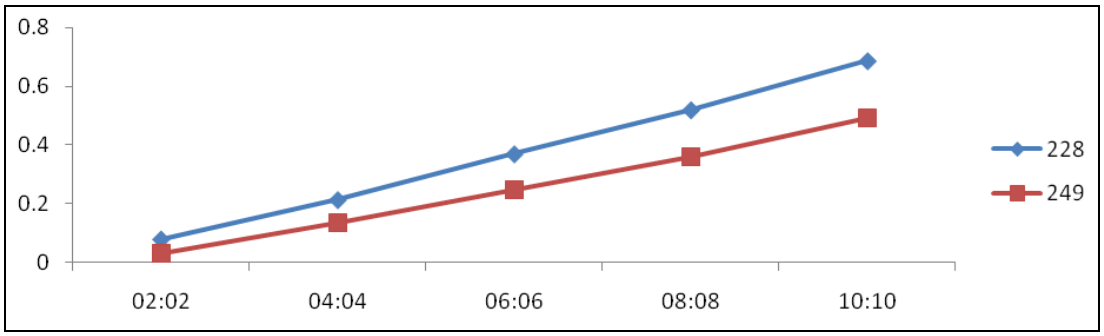

Fig 2: Plot of Beer- Lambert's law

Table 2: Ruggedness studies of SIM and EZE (n=3)

Drugs	Parameter	Intermediate Precision		
		Interday	Intraday	Different Analysts
SIM	Mean	99.98	97.36	98.37
	±SD	0.953	0.911	1.000
	% RSD	0.964	0.920	0.989
EZE	Mean	98.90	97.81	98.12
	±SD	0.812	0.826	0.833
	% RSD	0.832	0.844	0.837

Table 3: Short term stability (24 h) determined by the proposed method (n=3)

SEM			
Conc. Declared (µg/ mL)	**Conc. found (mean±SD, µg/mL)**	**Average potency (%)**	**RSD (%)**
5	5.00±0.0289	100.20	0.5773
10	10.08±0.1024	102.30	1.0166
15	14.94±0.0562	99.07	0.3762
EZE			
5	5.04±0.0551	99.60	1.094
10	9.96±0.0578	98.70	0.580
15	15.01±0.1333	99.67	0.888

Table 4: Recovery/accuracy for five different concentrations of SEM and ERE by the proposed method

Dosage form	Label claim	Amount added (%)	Recovery (%)
SEM	10 mg	80	99.98
		100	99.72
		120	99.65
EZE	10 mg	80	99.11
		100	98.23
		120	98.33

Table 5: Data of specificity study

Drugs	% Mean				
	Room Temperature	Acid (0.1N HCl)	Alkali (0.1N NaOH)	Oxide 3% H_2O_2	Heat 60ºC
SEM	99.14	98.27	97.86	96.63	99.38
EZE	98.94	96.14	96.79	95.92	98.08

Table 6: Content of SEM and EZE in marketed products determined by the proposed method

Drug	Label claim (mg)	Amount Tablet Powder taken (g)	Amount of drug estimated (g/tablet)	Potency (%)	RSD (%)
SEM	10	0.1709 to 0.1712	0.1000	100.01	1.702
EZE	10		0.9985	99.10	1.176

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