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Derivative spectrophotometric method for simultaneous determination of ezetimibe and simvastatin in combined tablets

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ABSTRACT

The introduced study portrays the development and validation of an effortless, speedy and selective first order derivative zero-crossing spectrophotometric method to estimate ezetimibe and simvastatin simultaneously in combined drug products over and above in the presence of ezetimibe alkaline degradation products. Ezetimibe and its alkaline degradates spectra showed zero-crossing point at 249 nm, so the amplitude at 249 nm could be used for calculating the concentration of simvastatin. While simvastatin and ezetimibe alkaline degradates spectra showed a zero-reading value at 261 nm, so ezetimibe could be determined by measuring the amplitude at 261 nm. Regression plots revealed good linear relationships in the concentration range 1-16 µg/mL with accuracy checked by conducting recovery studies; average recovery was 99.61 ± 1.183 and 100.47 ± 1.310 for ezetimibe and simvastatin, respectively. The suggested first derivative spectrophotometric method was successfully applied for the determination of the cited drugs in "Inegy 10/10, 10/20 and 10/40 tablets". Satisfactory results were obtained for the recovery of both drugs and were in good agreement with the labeled amounts. Method validation was estimated according to USP guidelines.

1. Introduction

Ezetimibe (EZE); Sch-58235 [(3R,4S)-1-(*p*-fluorophenyl)-3-[(3S)-(*p*-fluorophenyl)-3-hydroxypropyl]-4-(*p*-hydoxyphenyl)-2-azetidinone] (Figure 1a) is an inhibitor of intestinal cholesterol absorption by enterocytes rather than inhibition of intestinal acyl coenzyme A: cholesterol actyl transferase causing selective inhibition of intestinal absorption of dietary and biliary cholesterol leading to a decrease in the delivery of intestinal cholesterol to the liver. Simvastatin (SIM); [2,2-dimethylbutanoic acid (1*S*,3*R*,7*S*,8*S*,8a*R*)-1,2,3,7,8,8a-hexa hydro-3,7-dimethyl-8-[2-[2*R*,4*R*)-tetrahydro-4-hydoxy-6-oxo-2H-pyran-2-yl]ethyl]-1-napthalenylester] (Figure 1b) is 3-hydoxy-3-methylglutaryl coenzyme A reductase inhibitor which is the rate limiting step in the biosynthesis of cholesterol [1,2].

Ezetimibe is more effective when used in a combination therapy with statins rather than when used as a monotherapy where the dual therapy with these two classes of drugs prevents the enhanced cholesterol synthesis by ezetimibe and the increase in cholesterol absorption induced by statins so the combination therapy is used to reduce total cholesterol, LDL-cholesterol and apolipoprotien B in the management of hyperlipidaemia and to reduce sitosterol and camposterol in patients with homozygous familial sito-sterolaemia [1,2].

Owing to the curative value of EZE and SIM, various numbers of analytical procedures have been established for the quantitative determination in drug substances, drug products and/or biological fluids, over and above in binary mixtures. These procedures include spectrophotometric comprising simultaneous determination of EZE and atorvastatin calcium in pharmaceutical dosage form [3], also, simvastatin and fluvastatin in human plasma and pharmaceutical formulation [4], TLC [5-7], LC [3,8-26], Capillary Zone Electrophoresis (CZE) [27,28] and GC [29] methods.

Derivative spectrophotometry is considered one of the most convenient analytical techniques, because of its inherent simplicity, low cost and wide availability in most quality control laboratories especially in developed countries. Also, some reported techniques were found to be time consuming, expensive and relatively complicated. For these reasons, the present study describes direct, simple, rapid and economical derivative spectrophotometric method for the simultaneous assay of EZE and SIM in drug substance and in drug products without inference of EZE degradation products and tablet excipients.

2. Experimental

2.1. Chemicals and reagents

All the solvents and chemicals used were of analytical grade. EZE was kindly supplied by Marcyrl Pharmaceutical Industries (Cairo, Egypt); its purity was found to be 99.23 ± 1.074 %, according to Raw Material Specification (IN HOUSE) [30] sent by Glenmark Pharmaceutical, Mumbai, India; by applying HPLC technique using C_{18} column; Water: Acetonitrile (60:40, v:v) as mobile phase and UV detector at 225 nm applying isocratic elution mode. SIM was nicely supplied by SIGMA Pharmaceutical Industries (Cairo, Egypt). Its purity was

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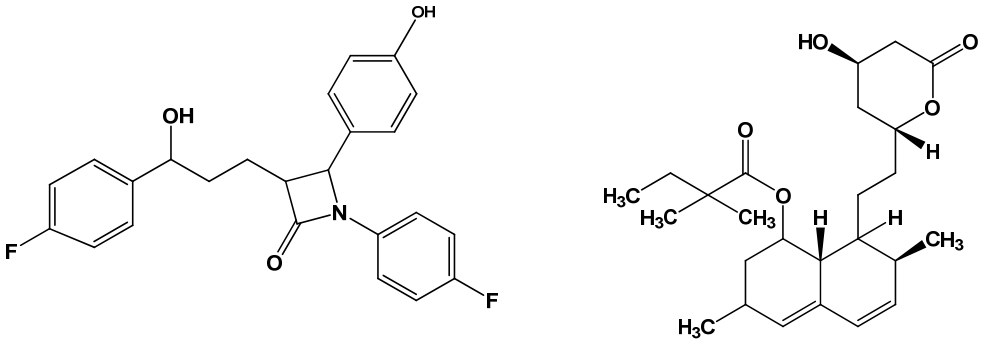

(a) (b)

Figure 1. Structure of ezetimibe ($C_{24}H_{21}F_2NO_3$) (a) and simvastatin ($C_{25}H_{38}O_5$) (b).

found to be 99.40 ± 1.148 % according USP pharmacopoeia [31] by performing HPLC technique using ODS column and mixture of acetonitrile: 0.1 % (v:v) phosphoric acid (50:50 – v:v) (mobile phase A) and 0.1 % (v:v) phosphoric acid in acetonitrile (mobile phase B) as mobile phase and UV detector at 238 nm applying gradient elution mode. Inegy tablets; produced by Global Napi Pharmaceuticals (GNP, Cairo, Egypt) under license from Merck & Co. Inc., Schering-Plough Co., USA. Each tablet was labeled to contain 10/10 mg, 10/20 mg and 10/40 mg of EZE and SIM, respectively. Sodium hydroxide pellets: El-Nasr Pharmaceutical Chemicals, Co. Egypt.

2.2. Apparatus

Unicam UV 300, thermospectronic, vision 32 software equipped with IBM PC computer used for all the absorbance measurements and treatment of data, HP laser jet 1000 series printer and 1 cm path length quartz cuvettes. Thin-layer chromatographic glass plates, precoated with silica gel GF- 50 UV_{254}, 20×20 cm, 0.5 mm thickness fluorescent at 254 nm (Macherey- Na Gel MN, Germany). UV Lamp. short wavelength, 254 nm. Sonamak, Ultrasonic Cleaner, Falc Instrument, Italy.

2.3. Standard solutions

EZE stock solution (1 mg/mL) and working solution (20 µg/mL) ethanol 95%. SIM stock solution (1 mg/mL) and working solution (20 µg/mL) in ethanol 95 %. EZE alkaline degradation products stock solutions (0.1 mg/mL) and working solutions (20 µg/mL and 0.4 µg/mL) in ethanol 95 %.

2.4. Preparation of EZE alkaline degradation products

100 mg of ezetimibe was dissolved in 100 mL of methanolic 1 M sodium hydroxide and refluxed for one hour. The solution was neutralized using methanolic 1 M hydrochloric acid, filtered and the filtrate was evaporated to about 10 mL. The filtrate was spotted on silica gel TLC glass plates (0.5 mm thickness) in form of bands, developed in mobile phase iso-propanol: ammonia 33% (9:1; v:v) showing partial degradation. Each developed band was scratched from silica gel TLC plates and extracted with methanol several times, filtered, followed by evaporation obtaining two degradation products.

2.5. Preparation of combined standard solutions of EZE and SIM

Synthetic mixtures were set up by preparing three different solutions of ezetimibe and simvastatin in ethanol 95 %. *First solution* containing EZE (10 µg/mL) and SIM (10 µg/mL), then different aliquots (1-4 mL) equivalent to (20-80 µg/mL) of each EZE and SIM were transferred. *Second solution* containing EZE (10 µg/mL) and SIM (20 µg/mL), then different aliquots (1-3 mL) equivalent to (20-60 µg/mL) of EZE and (40-120 µg/mL) of SIM were transferred. *Third solution* containing EZE (10 µg/mL) and SIM (40 µg/mL), then different aliquots (0.5-1.5 mL) equivalent to (10-30 µg/mL) of EZE and (40-120 µg/mL) of SIM were transferred, into a series of 10 mL volumetric flasks. The volume was completed with ethanol 95 %, taking in consideration that the final concentration ratio of EZE to SIM in each solution was the same as that of the dosage form (1:1, 1:2, 1:4).

2.6. Sample Preparation

Ten tablets of each 10/10 mg, 10/20 mg and 10/40 mg were accurately weighed and finely powdered. An amount of powder equivalent to 10 mg of EZE and 10 or 20 or 40 of SIM was weighed and transferred to three 25 mL volumetric flasks. 20 mL of ethanol 95 % was added and sonicated for 30 min, the volume was completed with ethanol 95 %, mix well and filter through a Whatman no. 1 filter paper, discarding the first few milliliters. The procedure was completed as mentioned under preparation of combined standard solutions of EZE and SIM.

2.7. Analytical performance

Aliquots (0.5-8 mL) of EZE working solution (20 µg/mL) equivalent to 10-160 µg EZE and aliquots (0.5-8 mL) of SIM working solution (20 µg/mL) equivalent to 10-160 µg SIM were separately transferred into two series of 10 mL volumetric flasks and the volume was completed with ethanol 95 %. The first derivative spectrum of each solution was recorded against ethanol 95 % as blank at a range of 200-400 nm; band width: 1.5 nm; scan speed: intelliscan; data interval: normal; smoothing: high. The amplitude of different concentrations at 261 nm for EZE and 249 nm for SIM were measured, then two calibration curves were constructed and the regression equations were calculated.

3. Results and discussion

3.1. Methods development

Derivative spectrophotometry is a useful technique for identifying and quantitating combination of drugs with overlapping spectra and for eliminating interference from formulation matrix by using the zero-crossing point (ZCP) technique [32-37]. The zero-order absorption spectra of EZE, its alkaline degradation products and SIM in ethanol 95% showed an extensive overlap of spectral bands, Figure 2.

Accordingly, a derivative spectrophotometric method was developed for the evaluation of EZE and SIM in their binary mixture and in presence of EZE alkaline degradation products.

Table 1. Analytical and validation parameters for determination of ezetimibe & simvastatin using first derivative spectrophotometric method.

Parameters	First Derivative Spectrophotometric Method	
	Ezetimibe	**Simvastatin**
Wavelength (nm)	261	249
Linearity range	1-16 µg/mL	1- 6 µg/mL
Specificity (mean ± R.S.D. %)[a]	100.14 ± 1.141	100.14 ± 1.219
Slope (b)	0.1146	0.2845
Intercept (a)	0.0177	0.0155
Regression coefficient (r^2)	0.9999	0.9997
LOD	0.132 µg/mL	0.123 µg/mL
LOQ	0.439 µg/mL	0.410 µg/mL
	Precision (± R.S.D. %)	**Precision (± R.S.D. %)**
Intraday[b]	± 0.832	± 1.114
Interday[b]	± 1.109	± 1.315
Synthetic mixture (Mean ± R.S.D. %)	99.48 ± 1.150	100.24 ± 1.196
	100.38 ± 1.269	99.74 ± 1.142
	99.15 ± 1.221	99.93 ± 1.286
	Accuracy (Mean ± R.S.D. %)	**Accuracy (Mean ± R.S.D. %)**
Drug substances	99.61 ± 1.183	100.47 ± 1.310
Drug products	102.43 ± 1.097	102.64 ± 1.299
	102.66 ± 1.244	104.23 ± 1.193
	103.08 ± 1.272	103.38 ± 1.174
Standard added	100.09 ± 1.291	100.34 ± 1.343
	99.42 ± 1.573	99.57 ± 1.530
	100.54 ± 1.344	100.02 ± 1.259

[a] Average of 6 experiments.
[b] Average of 9 experiments.

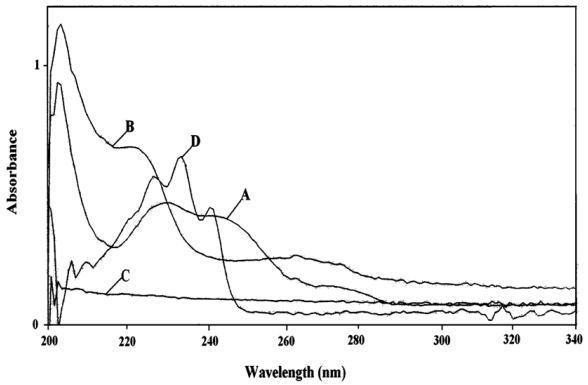

Figure 2. The zero-order absorption spectra of: (A) - Ezetimibe Intact (10 µg/mL); (B) and (C) - Ezetimibe Alkaline Degradation Products (10 µg/mL); (D) - Simvastatin Intact (10 µg/mL).

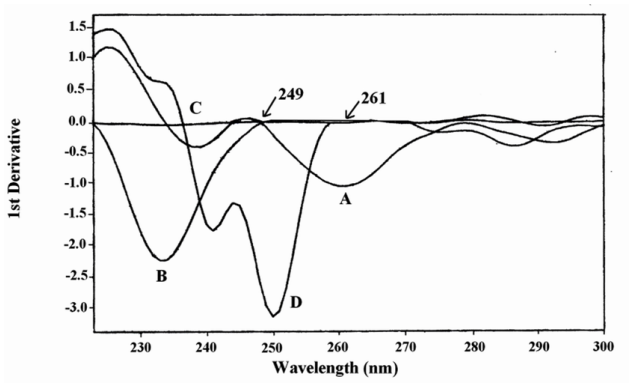

Figure 3. The First Derivative absorption spectra of: (A) - Ezetimibe Intact (10 µg/mL); (B) and (C) – Ezetimibe Alkaline Degradation Products (10 µg/mL); (D) - Simvastatin Intact (10 µg/mL).

The first derivative technique was able to enhance the resolution of overlapping absorption bands through the application of zero- crossing technique.

The first derivative spectrum of EZE displays a peak amplitude at 261 nm ($^1D_{261}$), while its degradation products and SIM have a zero-crossing point at 261 nm, so EZE could be determined by measuring the amplitude at 261 nm in presence of simvastatin and its degradation products without any prior separation procedures. On the other hand, the first derivative spectrum of SIM shows that SIM exhibits peak amplitude at 249 nm ($^1D_{249}$) where EZE and its degradation products spectra show a zero- reading value, so the amplitude at 249 nm could be used for calculating the concentration of SIM, in presence of EZE and its degradation products, Figure 3.

3.2. Methods validation

3.2.1. Linearity

The linearity of the proposed method was constructed by triplicate analysis of six standard solutions with different concentrations ranging 1-16 µg/mL of EZE and SIM. Linear regression analysis of EZE and SIM was driven (Table 1), with acceptable intercepts and good correlation coefficients, Figure 4 and 5. The LOD and LOQ were calculated from the slope (s) of the calibration plots and the standard deviation (SD) of the response. The values are given in Table 1.

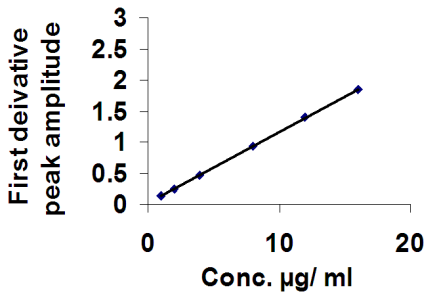

Figure 4. Linearity of peak amplitudes of first derivative absorption spectra at 261 nm to concentrations of EZE (1-16 µg/mL).

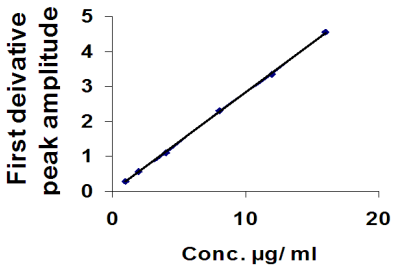

Figure 5. Linearity of peak amplitudes of first derivative absorption spectra at 247 nm to concentrations of SIM (1-16 µg/mL).

Table 2. Statistical comparison between the results of ezetimibe & simvastatin in drug substances applying first derivative spectrophotometric, reported and official methods.

	First Derivative Spectrophotometric Method			
Statistical Item	**Ezetimibe**	**Reported Method [30]**	**Simvastatin**	**Official Method [31]**
M[a] ± RSD %	99.61 ± 1.183	99.23 ± 1.074	100.47 ± 1.310	99.40 ± 1.148
SD	1.191	1.065	1.317	1.141
SE	0.533	0.477	0.589	0.510
Variance	1.418	1.135	1.733	1.302
t (2.31)[b]	0.538	-	1.369	-
F (6.39)[b]	1.244	-	1.331	-

[a] Average of 5 experiments.
[b] Tabulated values of t- test and F-ratio.

3.2.2. Accuracy

The validity of the proposed methods could be proved by analyzing authentic samples of the drug substances. The results obtained were in a good agreement with those obtained using reported and official methods [30,31]. Using student's *t* test and variance ratio *F* test statistically revealed no significant difference between the results of proposed, reported and official methods, (Table 2).

The recovery method was studied by analyzing samples of tablet formulations without the interference of tablet excipients (Butylated hydroxyanisole, Citric acid monohydrate, Croscarmellose sodium, Hypromellose, Lactose monohydrate, Magnesium stearate, Microcrystalline cellulose, Propyl gallate) [38]; also the standard addition technique was applied by adding a known amount of standard drugs to the drug products and the results were expressed as mean± R.S.D. %, (Table 1).

3.2.3. Precision

The precision of the suggested method was assessed by the repeatability and reproducibility of the first derivative spectrophotometric method. The intra- and inter-day variation expressed by mean ± R.S.D. % (Table 1), was determined by measuring triplicate analysis of three different concentrations (1.6, 8, 14.4 µg/mL) over a period of three days, (Table 1).

3.2.4. Specificity

The specificity of the proposed method was determined by preparing laboratory mixtures of EZE and SIM in presence of up to 100 % of EZE alkaline degradation products, (Table 1). The assay results were unaffected by the presence of degradates.

3.2.5. Robustness

Two sets of experiments were performed for the two drugs on UNICAM UV 300 with 1 cm matched quartz cells by two different analysts in different days with respect to the derivative amplitude as a response factor and no significant difference was obtained between the results in this study.

4. Conclusion

The present study described a validated derivative spectrophotometric method which can be used as a simultaneous assay of EZE and SIM in the presence of EZE degradation products and in drug products without interference from tablet excipients. The proposed methods are of great worth in quality control analysis of EZE and SIM owing to their improved simplicity, low-cost, their independence on expensive instruments, or critical analytical reagents, the reproducibility and the lack of complicated pretreatments before analysis.

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