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Chromatographic determination of ezitimibe and simvastatin in their pharmaceutical formulation

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ABSTRACT

Two accurate and precise methods were developed for the Simultaneous determination of Ezitimibe and Simvastatin in their pharmaceutical formulations. A high performance liquid chromatographic method with ultraviolet detection at 240 nm was developed for the separation and determination of the studied drugs using a C_{18} column. The mobile phase was composed of 0.1 M ammonium acetate buffer (pH 4.5): acetonitrile: methanol [35: 55: 10, by volumes]. A TLC separation with densitometric detection of both drugs was achieved using toluene: n-butanol: triethylamine (8.5:2.5:0.5, by volumes) as a developing solvent. The proposed methods were validated according to the ICH guidelines. The developed methods were found to be accurate, precise and suitable for the quantitative determination of both the drugs in their pure form, mixtures and in pharmaceutical formulations containing them.

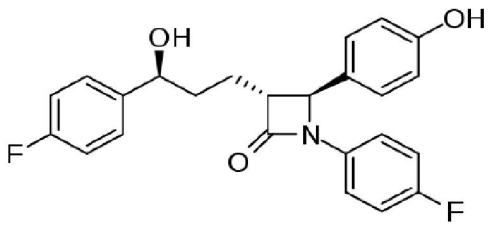
KEYWORDS

Ezitimibe;
Simvastatin;
HPLC;
TLC-densitometery.

INTRODUCTION

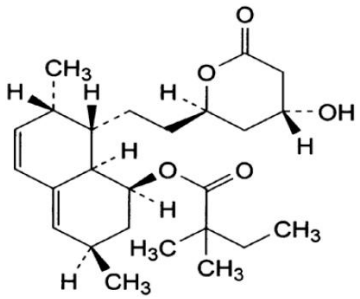
Ezitimibe [EZ], is (3*R*,4*S*)-1-(*p*-fluorophenyl)-3-[(3*S*)-3-(*p*-fluorophenyl)-3-hydroxypropyl]-4-(*p*-hydroxyphenyl)-2-azetidinone[1]. It is the first lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of fat-soluble nutrients[2], figure 1.

Simvastatin [SM], is (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydro naphthalen-1-yl 2,2-dimethylbutanoate, (figure 2), is



M.W. [$C_{24}H_{21}F_2NO_3$ = 409.4]

Figure 1 : Structural formula of ezitimibe [EZ]



M.W. [$C_{25}H_{38}O_5$ = 418.6]

Figure 2 : Structural formula of simvastatin [SM]

a lipid-lowering agent that is HMG Co-A reductase inhibitor[3].

A combination of SM and EZ has a complementary action with each other. It is significantly more effective than SM or EZ alone for reducing plasma concentration of cholesterol[4]. The literature survey showed few methods for quantitative estimation of EZ and SM in body fluids and in pharmaceutical formulations. Several methods were developed for determination of EZ and SM with other combinations in pharmaceutical dosage form by spectrophotometry[5] and chromatographic methods[6-11]. There is no pharmacopoeial method for determination of EZ alone or in combination with SM in pharmaceutical dosage forms.

In modern analytical laboratory, there is always a need for simple, rapid and validated methods for simultaneous determination of drug combinations that could be used for routine analysis. The present work aimed to develop simple and rapid instrumental methods for the quantification of EZ and SM in bulk form, in their mixtures or in their pharmaceutical formulations. These methods are chromatographic ones; *namely*, HPLC and TLC with densitometric detection.

EXPERIMENTAL

Instruments

A liquid chromatography consisted of Shimadzu class-vp V6.12 SP4, a model 1050 solvent delivery system, and a UV- visible spectrophotometric detector. The separation was performed on a Supelcosil LC extra column (150 mm × 4.6 mm; 5 μm).

TLC-plates [20 cm x10 cm, 0.25mm] coated with silica gel 60 F254 [Merck, Germany] were used.

Camag TLC scanner 3 S/N 130319 with WinCATS software and Camag Linomat 5 auto sampler [Muttenz, Switzerland] with Camag micro syringe [100 μL] were used.

Materials and reagents

All chemicals and reagents were of analytical grade.

(A) Materials

Reference EZ and SM standards were kindly supplied by Hikma Pharma Egypt, 6th October City (Cairo, Egypt). The potency was found to be 99.85 ± 0.56% (*n*=6), for EZ according to a reference HPLC method[9] and 100.24 ± 0.39% (n=6), for SM according to the official HPLC method[3].

(B) Pharmaceutical formulations

LIPTRIN® 10/10, labeled to contain10mg of EZE and 10 mg SIM, BN: 90478A, Chemipharm Co, Egypt.

ZOCOZET® 10/20, labeled to contain10mg of EZE and 20 mg SIM, BN: 1030099, Macyrl Pharmacutical Industries, Egypt.

ALKOR PLUS®10/40, labeled to contain10mg of EZE and 40 mg SIM, BN: 005, Hikma Pharma, Egypt.

(C) Standard solutions

Standard stock solutions were prepared by dissolving EZ and SM, separately in methanol into 50-mL volumetric flasks to obtain a final concentration of 0.2 mg/mL for the HPLC method and 1 mg/mL of each drug for the TLC densitometric one.

(D) Reagents

Ammonium acetate, *n*-butanol, and toluene: Adwic, El-Nasr Pharm. Co. (Cairo, Egypt). Methanol and acetonitrile: HiPerSolv.®, HPLC-grade, E. Merck (Darmstadt, Germany). Triethylamine LR: Laboratory Rasayan s.d. Fine-Chem Ltd. De-ionized water: Bidistilled from "Aquatron" Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire, UK).

Procedure

(A) Liquid chromatographic method

Linearity

Aliquots (0.1-1 mL) from both EZ and SM standard stock solutions (each, 0.2 mg/mL) were transferred separately into series 10-mL volumetric flasks. The volumes were then completed with the mobile phase to obtain a concentration range of 2-20 μg/mL for each. The prepared solutions were then analyzed using the following chromatographic conditions.

The stationary phase was a Supelcosil LC extra C_{18} column (150 mm × 4.6 mm; 5 μm). Mobile phase was consisted of 0.1M ammonium acetate + acetonitrile + methanol (35: 55: 10, by volumes). The final pH was adjusted to 4.5. The mobile phase was prepared daily, filtered through a 0.45-μm Millipore membrane and was degassed for ~15 minutes in an ultrasonic bath prior to their use. Flow rate was 1.2 mL/min. isocratically

at ambient temperature (~25 ± 1°C) with a UV-detection at 240 nm. The samples were filtered also through a 0.45-μm membrane filter, and were injected by the aid of a 50-μL Hamilton® analytical syringe. To reach good equilibrium, the analysis was usually performed after passing ~ 50-60 ml of the mobile phase, just for conditioning and pre-washing of the stationary phase. Regression equations were constructed and used for estimating the concentration of both drugs in pure samples, laboratory prepared mixtures and in pharmaceutical formulations.

(B) TLC-Densitometery

Linearity

Stock standard solutions were prepared separately by dissolving 10 mg of EZ or SM in 10 mL-volumetric flask with methanol. Aliquots (1-14 μL) of EZ or (1-18μL) of SM (each 1 mg/mL) were applied on thin layer silica plates. The specified chromatographic conditions were adopted, and calibration curves were constructed by plotting the areas under peaks (AUP) versus drug concentration and the corresponding regression equations were computed.

Analysis was performed on pre-coated thin layer chromatographic plates, silica gel 60 F_{254} (20 cm x 20 cm, 0.25 mm). Samples were applied on the plates in the form of bands by Camag Linomat 5 auto sampler utilizing a 100-μL Hamilton micro-syringe. The band length was 4 mm and dosage speed was 150 nL/sec. Bands were applied 12 mm apart from each other. The air-dried plates, were developed in a chromatographic tank, pre-saturated, for at least one hour, with the developing mobile phase toluene + *n*-butanol + triethyl ammine (8.5: 2.5: 0.5, by volumes) by ascending chromatography through a distance of ~15 cm at room temperature (25 °C). The developed plates were air-dried and the spots were detected under UV-lamp at 240 nm then scanned under the following instrumental conditions:

- Source of radiation: Deuterium lamp
- Scan mode: Absorbance mode
- Slit dimension: 3 mm x 0.45 mm
- Scanning speed: 20 mm/ sec.

Regression equations were constructed and used for estimating the concentration of both drugs in laboratory prepared mixtures and in pharmaceutical formulations.

(C) Analysis of pharmaceutical formulations

At least 10 tablets were weighed and powdered to obtain the average weight per tablet. A mass of the powdered tablets, claimed to contain 10 mg of EZ and (10, 20 or 40 mg) SM was dissolved in small quantity of methanol. This mixture was sonicated for 15 min. and diluted to mark with methanol. Aliquots were then removed and centrifuged at 5000rpm for 20 min and then filtered through a 0.45 μm syringe filter. The solution was transferred to a volumetric flask and made up to a sufficient volume with the solvent to get a concentration of (1mg/mL). All determinations were done in triplicate. The same procedure was used to estimate the concentration of the drug in three different strengths of EZ and SM in their tablets.

(D) Method validation

The developed analytical methods were validated according to ICH guidelines. Comparison of the results obtained by the proposed methods and the reference ones and statistical analysis of data was done.

RESULTS AND DISCUSSION

Liquid chromatographic method

A simple isocratic high-performance liquid chromatographic method was developed for the determination of EZ and SM in pure forms and in their mixtures. The mobile phase was chosen after several trials. The most suitable one was consisted of 0.1M acetate buffer (pH 4.5): acetonitrile: methanol (35: 55: 10, by volumes). The flow rate was 1.2 mL/min. By using the described chromatographic conditions, EZ and SM were well separated with average retention times of 3.19 min. and 5.54 min., for EZ and SM, respectively, Figure 3.

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the external standard versus concentration. The linearity ranges and analytical data for the calibration graphs are listed in TABLE 1.

The precision of the method was evaluated and the relative standard deviation values from inter-day analysis were found to be 0.109% and 0.07% for EZ and SM respectively, and from intra-day 0.99% and 0.2% for EZ and SM, respectively. Results for HPLC analysis of laboratory-prepared mixtures with different

proportions of the two drugs are given in TABLE 2.

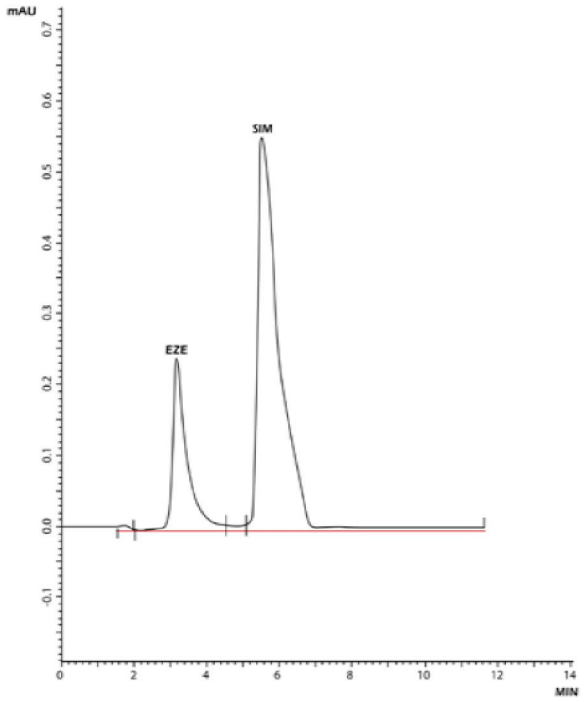


Figure 3 : Liquid chromatographic separation of EZ (3.19 min) from SM (5.54 min), experimental conditions

TLC-densitometery method

A TLC-densitometric method is described for the simultaneous determination of SM and EZ without prior separation. Different solvent systems were tried for the separation of both drugs. Satisfactory results were obtained by using a mobile phase composed of toluene + *n*-butanol + triethylamine (8.5:2.5:0.5, by volumes), where *R*f was 0.7 and 0.6 for EZ and SM, respectively. The separation allows the determination of both drugs without interference from each other. The linearity was confirmed by plotting the measured peak area *versus* the corresponding concentrations at 240 nm over a range of 1–14 µg/spot, for EZ and over a range of 1–18 µg/ spot, for SM where a linear response was obtained. The regression equations were listed in TABLE 1.

The separated spots of the two drugs were scanned at 240 nm, figure 4.

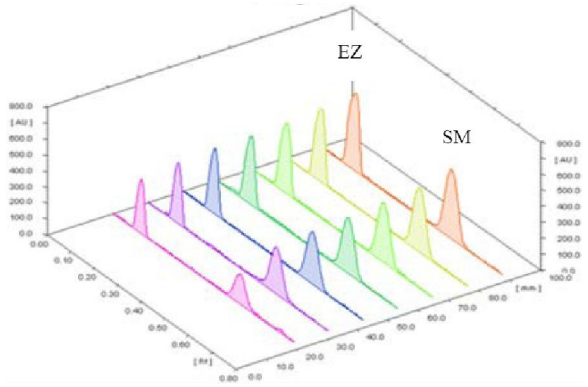


Figure 4 : Three dimensional TLC densitogram showing the separation of EZ from SM, see experimental conditions

To assess the specificity, accuracy and selectivity of the TLC-D method for assay of both drugs without interference from one another, six synthetic mixtures of EZ and SM at various concentrations within the linear-

TABLE 1 : Assay parameters and validation sheet for determination of EZ and SM.

	HPLC		TLC	
	EZ	SM	EZ	SM
Range	2-20 µg/mL	2-20 µg/mL	1-14 µg/spot	1-18 µg/spot
Slope	0.4395	0.4253	0.102	0.796
Intercept	0.1292	0.03345	0.891	6.76
Correlation coefficient	0.999	0.999	0.999	0.999

TABLE 2 : Determination of SM and EZ in laboratory prepared mixtures by the proposed methods

Lab. Mix.(EZ/SM) Component	HPLC method	TLC method
EZ	100.30 ± 0.56	98.99 ±1.02
SM	100.00 ± 0.36	99.20 ± 0.97

TABLE 3 : Assay validation parameters of the proposed methods for the determination of EZ and SM.

Parameter	HPLC method		TLC method	
	EZ	SM	EZ	SM
Accuracy	100.23 ± 0.54	100.16 ± 0.12	99.76 ± 0.34	100.08 ± 1.30
Specificity	100.07 ± 0.68	100.02 ± 0.76	99.38 ± 1.53	100.80 ± 1.14
Repeatability	99.96 ± 0.24	100.11 ± 0.73	99.96 ± 0.93	101.14 ± 1.72
Intermediate precision	99.98 ± 0.99	100.03 ± 0.39	99.89 ± 0.94	100.23 ± 1.04
Linearity range	20-200 µg/mL	20-200 µg/mL	1-14 µg/spot	1-18 µg/spot
Slope	0.4412	0.3515	0.102	19.893
Intercept	0.1308	0.1986	0.0891	41.22
Correlation Coefficient	0.999	0.999	0.999	0.999

ity range were prepared and analyzed. Satisfactory results were obtained and shown in TABLE 2. TABLE 3 shows the assay validation parameters of the proposed methods for the determination of EZ and SM.

Analysis of pharmaceutical formulations

The suggested methods were successfully applied for the determination of EZ and SM in their pharmaceutical formulations, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique, and the precision was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results.

CONCLUSION

The proposed HPLC method is rapid, accurate and precise for the simultaneous determination of EZ and SM in tablet dosage forms and can be used for routine quality control of these drugs in their mixtures and in pharmaceutical formulations. The HPLC method is a versatile method and may offer advantages over the derivative method for the selective determination of the two intact drugs in a variety of matrices. The TLC-D method has some advantages over HPLC such as low cost, large sample capacity and minimal volume use of solvent. With these two methods, we can gain the advantages of speed, lower cost, and environmental protection without sacrificing accuracy.

REFERENCES

[1] S.C.Sweetman; Martindale: The Complete Dug Reference, 35th Edition, The Pharmaceutical Press: London, 1234 **(2007)**.

[2] T.Kosoglou, P.Statkevich, A.O.Johnson-Levonas, J.F.Paolini, A.J.Bergman, K.B.Alton; Clin.Pharmacokinetics, **44**, 467-494 **(2005)**.

[3] The British Pharmacopoeia, Her Majesty's Stationary Office, London, UK, **(2010)**.

[4] M.H.Davidson, D.Maccubbin, M.Stepanavage, J.Strony, T.Musliner; Am.J.Cardiol., **97(2)**, 223-228 **(2006)**.

[5] S.Balaji, A.Sunitha; Pak.J.Pharm.Sci., **23**, 375 **(2010)**.

[6] B.G.Chaudhari, N.M.Patel, P.B.Shah; J.AOAC Int., **90**, 1242 **(2007)**.

[7] R.P.Dixit, C.R.Barhate, S.G.Padhye, C.L.Viswanathan, M.S.Nagarsenker; Indian J.Pharm.Sci., **72**, 204 **(2010)**.

[8] M.Hefnawy, M.Al-Omar, S.Julkhuf; J.Pharm. Biomed.Anal., **50**, 527 **(2009)**.

[9] H.M.Lotfy, A.M.Aboul Alamein, M.A.Hegazy; J.AOAC Int., **93**, 1844 **(2011)**.

[10] P.R.Oliveira, T.Barth, V.Todeschini, S.L.Dalmora; J.AOAC Int., **90**, 1566 **(2007)**.

[11] C.Yardimci, N.Ozaltin; J.Chromatogr.Sci., **48**, 95 **(2010)**.