Journal of Chromatography
Separation Techniques

Abdelwahab et al., J Chromat Separation Techniq 2012, 3:3
DOI: 10.4172/2157-7064.1000126

Research Article Open Access

TLC- Densitometric Method for Determination of some Cholesterol Lowering Drugs in Different Combinations

Nada S Abdelwahab[1]*, Badr A EL-Zeiny[2] and Salwa I Tohamy[1]

[1]*Beni-suif University, Faculty of pharmacy, Department of Analytical Chemistry, Beni-Suef, Egypt*
[2]*Cairo University, Faculty of pharmacy, Department of Analytical Chemistry, Cairo-Egypt*

Abstract

Sensitive, selective, precise and economic TLC-Densitometric method has been developed for determination of two binary mixtures containing the antihyperlipidemic Ezetimibe (EZ) in its combination with Atorvastatin calcium (AT) [mixture I] and with Simvastatin (SIM) [mixture II].

In the developed TLC-Densitometric method EZ, AT and SIM were quantitatively separated on $60F_{254}$ silica gel plates using ethyl acetate: hexane: glacial acetic acid (5.5:4.5:0.1 by volume) as a developing system with UV detection at 254 nm. Factors affecting the chromatographic separation have been studied, moreover the method has been validated as per ICH guidelines and it has been successfully applied for determination of the studied drugs in their different dosage forms without interference from excepients. Results obtained by the developed TLC-Densitometric method were statistically compared with those obtained by the reported spectrophotometric method and no significant difference was found between them.

Keywords: TLC-densitometry; Ezetimibe; Atorvastatin; Simvastatin

Introduction

Ezetimibe (EZ) is (3R, 4S)-1-(4-flurophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone [1]. It is the first in a new class of cholesterol absorption inhibitors that blocks the intestinal absorption of dietary and biliary cholesterol, without affecting the uptake of triglycerides or fat soluble vitamins. It reduces total cholesterol, LDL, triglycerides and increases HDL in patients with hypercholesterolemia [2-7]. It is used for treatment of hypercholesterolemia and homozygous sitosterolemia [8]. Atorvastatin calcium (AT) is [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1-Hpyrrole-1-heptanoic acid, calcium salt trihydrate [1]. It is a member of a class known as statins and it is a specific inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-coA) reductase, the enzyme that catalyzes the conversion of HMG- coA to mevalonate, which is the rate limiting step in biosynthesis of cholesterol [9-13]. It is used for lowering blood cholesterol and preventing strokes thought anti-inflammatory and other mechanisms [14]. Simvastatin (SIM) is 2,2dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-1naphthylenylester-[1S(1α,3α,7β,8β(2s*,4s*),-8aβ)] butanioc acid [1]. It is a member of the statins group and like Atorvastatin it acts as a specific inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-coA) reductase [15]. It is hydrolyzed after oral administration in the liver to its active form, the β-hydroxy acid [16]. And it is used for lowering blood cholesterol. It is frequently prescribed for the treatment of hypercholesterolemia and was shown to significantly decrease the mortality associated with coronary heart diseases [17]. Combinations of either EZ/AT or EZ/SIM have the advantages of greater therapeutic effects than either drug alone [21-24]. These combinations have significant effects on reducing LDL cholesterol level in the blood compared to using each of these drugs individually.

Reviewing the literature in hand, none of the recommended pharmacopeias has been determined EZ and AT or EZ and SIM mixtures. EZ and AT have been determined by some techniques including HPLC [18], HPTLC-Densitometric [19] and spectrophotometric methods [20]. On the other hand EZ and SIM have been determined in their association by HPLC [21-23], HPTLC-Densitometric [24] and spectrophotometric methods [25].

Only one method [26] has been published for determination of EZ/AT and EZ/SIM combinations which depend on using first derivative of ratio spectra spectrophotometric method (1DD) for EZ /AT and EZ / SIM mixtures and measuring each of the two mixtures in separate steps using different instrumental conditions. From the previous literature review, no TLC-Densitometric method has been developed for simultaneous determination of the studied mixtures and no methods have been used for their determination without preliminary separation steps.

This work aims to develop selective, sensitive and accurate TLC-Densitometric method for simultaneous determination of the three studied drugs using the same solvent system, scanning wavelength and the same run hence it is time and cost effective and it can be used as alternative method to the high cost RP-HPLC method in quality control laboratories. The developed TLC-Desitometric method has the advantage of being more selective than the published spectrophotometric and HPTLC methods because it is able to separate the three components without interference from each other or from tablets excipients, moreover it does not need any sophisticated apparatus or high cost solvents compared to the published RP-HPLC methods.

***Corresponding author:** Nada S Abdelwahab, Beni-suif University, Faculty of pharmacy, Department of Analytical Chemistry, Beni-Suef, Egypt, Tel: +201117236884; Fax: +208212317950; E-mail: nadasayed2003@yahoo.com

Received April 14, 2012; **Accepted** May 21, 2012; **Published** May 23, 2012

Citation: Abdelwahab NS, EL-Zeiny BA, Tohamy SI (2012) TLC- Densitometric Method for Determination of some Cholesterol Lowering Drugs in Different Combinations. J Chromat Separation Techniq 3:126. doi: 10.4172/2157-7064.1000126

Experimental Instruments

a) CAMAG TLC scanner 3 S/N 130319 with win CATS software

The following requirements are taken into consideration:

-Source of radiation: deuterium lamp

-Scan mode: absorbance mode

-Slit dimension: 3 mm × 0.45 mm

-Scanning speed: 20 mm s^{-1}, and

-Output: chromatogram and integrated peak area

b) Linomat 5 autosampler (Switzerland)

c) CAMAG microsyringe (100 µL)

d) Precoated silica gel aluminum plates 60 F_{254}, ALLUGRAM® SIL G/UV 254 (Macherey-Nagel, Germany) 20 × 20 cm with 0.2 mm thickness

e) Sonix TV ss-series ultrasonicator (USA)

Samples

Standard samples

1. Pure EZ was kindly supplied by Egyptian Co. for Chemicals and Pharmaceuticals, ADWIA CO, 10th of Ramadan City, Egypt.
2. Pure AT was kindly supplied by Marcyrl Pharmaceutical Industries, El- Obour City, Egypt.
3. Pure SIM was kindly supplied by Chemipharm Pharmaceutical Industries, 6th October City, Egypt.

Pharmaceutical dosage forms

1) Atoreza® tablets (10/10) (B.N.1031061) labeled to contain EZ equivalent to 10 mg and atorvastatin calcium equivalent to 10 mg of atorvastatin, were manufactured by Marcyrl Pharmaceutical Industries, El Obour City, Egypt.

2) Zocozet ® tablets (10/10) (B.N.1031118) labeled to contain 10 mg each of EZ and SIM, were manufactured by Marcyrl Pharmaceutical Industries, El Obour City, Egypt.

3) Lipitrin® tablets (10/10) (B.N.90478A) labeled to contain 10 mg each of EZ and SIM, were manufactured by Chemipharm Pharmaceutical Industries 6th- October City, Egypt.

4) Lipitrin® tablets (10/20) (B.N.80330B) labeled to contain 10 and 20 mg each of EZ and SIM, respectively, were manufactured by Chemipharm Pharmaceutical Industries 6th- October City, Egypt.

5) Alkorplus® tablets(10/20) (B.N.008) labeled to contain 10 and 20 mg each of EZ and SIM, respectively, were manufactured by Hikma Pharm for Pharmaceutical Industries 6th October City, Egypt.

6) Alkorplus® tablets(10/40) (B.N.012) labeled to contain 10 and 40 mg each of EZ and SIM, respectively, were manufactured by Hikma Pharm for Pharmaceutical Industries 6th October City, Egypt.

Chemicals and Solvents

All chemicals and solvents used throughout this work were of analytical grade and were used without purification. Ethyl acetate, hexane, glacial acetic acid and methanol (El-Nasr Pharmaceutical Chemicals Co. Abu- Zabaal, Cairo, Egypt)

Standard solutions

Standard stock solutions of EZ, AT and SIM: Standard stock solutions of EZ, AT and SIM were prepared in methanol in the concentration of 1 mg/mL

Standard working solutions of EZ, AT and SIM: Standard working solutions of EZ, AT and SIM were prepared in methanol in the concentration of 0.1 mg/mL

Procedure

Chromatographic conditions

TLC-Densitometric method was performed using pre-coated 60 F_{254} silica TLC aluminium plates (20×10 cm). The plates were pre-washed with methanol and activated at 100°C for 15 minutes prior to samples application. Samples were applied in the form of bands (4 mm length, 8.9 mm spacing and 15 mm from the bottom edge of the plate). Linear ascending development was performed in a chromatographic tank previously saturated with ethyl acetate: hexane: glacial acetic acid (55:45:1 by volume) for one hour at room temperature to a distance of about 80 mm. The developed plates were air dried and then scanned at 254 nm.

Construction of the calibration curves

Accurate volumes (4-40 µl),(4-31 µl) and (5-29 µl) of EZ, AT and SIM, respectively were accurately transferred from their standard working solutions (0.1 mg/mL), applied in triplicates on the prewashed TLC plates in the form of bands and the procedure under the chromatographic conditions was followed. The area under peak was then recorded and the calibration curve for each drug was constructed by plotting the mean integrated peak area versus the corresponding concentration.

Application to pharmaceutical formulations

The content of ten tablets of Atoreza® (10/10), Zocozet® (10/10), Lipitrin® (10/10 and 10/20) and Alkorplus® (10/20 and 10/40) tablets were separately weighed and then finely powdered. Accurate amounts each of the powdered tablets equivalent to 1 mg EZ (and the corresponding concentration of either AT or SIM) were separately weighed, dissolved in 75 mL methanol and sonicated for about 15 minutes. The prepared solutions were then filtered, transferred quantitatively to four separate 100 mL volumetric flasks and the volume was then completed to the mark with methanol. Appropriate dilutions of the prepared solutions were made to prepare their working solutions (0.1 mg/mL) and the developed method was then followed.

Results and Discussion

Ezetimibe is cholesterol lowering drug that is co-formulated with both AT and SIM which are used in cases of high cholesterol level. Hence, they play an important role in the treatment of some serious diseases such as heart disease [26]. Instrumental planar chromatography with precise application of the samples, and computer controlled evaluation and quantification of the developed chromatograms has been considered as reliable for quality control and quantitative drug

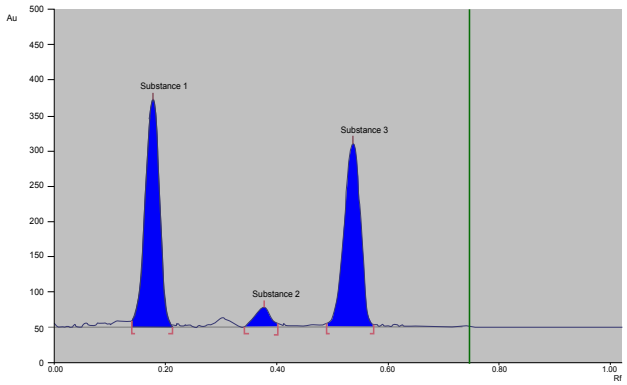

Figure 1: Thin layer chromatograms of (1) AT, (2) SIM and (3) EZ using ethyl acetate : hexane: glacial acetic acid (5.5:4.5:0.1, by volume). (Two dimensional).

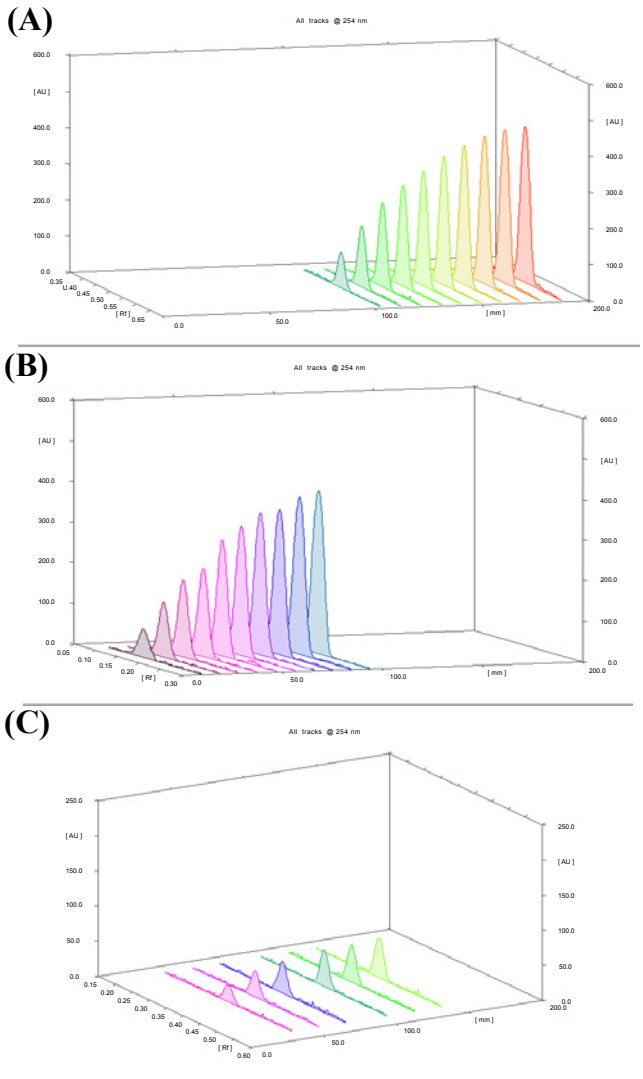

Figure 2: TLC-Densitograms of different concentrations of (a) EZ, (b) AT and (c) SIM.

testing [27].

The main task of this work is to establish sensitive and selective TLC-Densitometric method for determination of EZ in its binary mixture with either AT or SIM in their bulk powder and in their combined pharmaceutical dosage forms using one and the same developing system and scanning wavelength with satisfactory precision for Good Analytical Practice (GAP).

Method optimization

Experimental conditions such as developing system composition, band dimensions, scanning wavelength and slit 000dimension were optimized in order to provide accurate, precise and reproducible chromatographic separation. The first step is to test all the published TLC-Densitometric developing systems [19,24] [in the first method the mobile phase was composed of chloroform: benzene: methanol: acetic acid (6:3:1:0.1 by volume) and the detection of the developed spots was carried out at 250 nm while in the second method the mobile phase was composed of n- hexane: acetone (6:4 v/v) and the detection of the developed spots was carried out at 234 nm]. Unfortunately, none of them was able to separate all the EZ/AT and EZ/SIM mixtures together using one developing system.

Different developing systems of different compositions were tried such as chloroform: methanol [(7:3 v/v) and (5:5 v/v)], chloroform: ethyl acetate [(7:3 v/v) and (5:5 v/v)], ethyl acetate: hexane (5.5:4.5 v/v) and ethyl acetate: hexane: glacial acetic acid (5.5:4.5:0.1 by volume). Using the first and second systems resulted in very bad resolution between the studied drugs. Replacing chloroform with hexane in the third one slightly enhanced the resolution but with tailed asymmetric peaks. On the other hand addition of glacial acetic acid in the last system enhanced both the chromatographic resolution and the peaks symmetry. So developing system consisted of ethyl acetate: hexane: glacial acetic acid (5.5:4.5:0.1 by volume) was used as the developing system.

Different band dimensions were tested in order to obtain sharp and symmetric peaks. The optimum band width chosen was 4 mm and inter-space between bands was 8.9 mm. Moreover Different scanning wavelengths were tried such as 210, 220 and 254 nm where scanning at 254 nm gave the best sensitivity for all the separated components. After method optimization the R_f values of AT, SIM and EZ are 0.2, 0.4 and 0.59 respectively as shown in Figure 1. The slit dimensions of scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slit dimensions were tried, where 3 mm × 0.45 mm proved to be the slit dimension of choice which provides highest sensitivity.

Method validation

Validation was performed according to ICH guidelines [28].

Linearity and range: Linearity of the proposed method was evaluated and it was evident in the range of 0.4-4 µg/band, 0.4-3.1 µg/band and 0.5-2.9 µg/band for EZ, AT and SIM, respectively Figure 2. The regression equations for EZ, AT and SIM were calculated and found to be:

$Y_1 = 0.2740C_1 + 0.3190$ $r_1 = 0.9996$, for EZ

$Y_2 = 0.4090C_2 + 0.1060$ $r_2 = 0.9996$, for AT

$Y_3 = 0.0271C_3 + 0.0951$ $r_3 = 0.9995$, for SIM

Where Y_1, Y_2 and Y_3 are integrated peak area $\times 10^{-4}$, C_1, C_2 and C_3 are the corresponding concentrations of EZ, AT and SIM in µg/band, respectively, r_1, r_2 and r_3 are the corresponding correlation coefficients.

	TLC-Densitometric method		
Parameters	**EZ**	**AT**	**SIM**
Linearity range	0.4-4 µg/band	0.4-3.1 µg/band	0.5-2.9 µg/band
Slope	00.274	0.4090	0.0271
SE of slope	0.0026	0.0042	0 .0003
Intercept	0.3190	0.1060	0.0951
SE of intercept	0.0064	0.0077	0.0005
Correlation coefficient	0.9996	0.9996	0.9995
Accuracy	99.74%	99.13%	99.97%
Precision			
Repeatability	1.705	1.233	1.405
Intermediate	1.722	1.535	1.854
			precision

Table 1: Regression and analytical parameters of the proposed method for determination of EZ, AT and SIM

Parameters	TLC-Densitometric Method		Reported Method [26]	
	EZ	AT	EZ	AT
Atoreza ®[a] (B.N. 1031061)	101.05± 1.302	99.86± 1.695	101.93± 0.749	99.78± 1.049
Standarad Addition[b]	100.52± 1.810	100.26± 2.072		
F-test c	3.022 (9.272)	2.584 (6.591)		
Student's t-test [c]	1.165 (2.446)	0.125 (2.364)		

[a]Average of 5 determinations.

[b]Average of 3determinations.

[c]The values in the parenthesis are corresponding theoretical value at p= 0.05.[degree of freedom in dosage form =6 for EZ and 7 for AT].

[26] Spectrophotometric determination of EZ and ATR using [1]DD at 289.5 and 288 nm for EZ and AT, respectively and methanol as solvent.

Table 2: Determination of the EZ and AT in Atoreza® tablets by the proposed method and statistical comparison with the reported method.

Parameters	TLC- Densitometric Method		Reported Method [26]	
	EZ	SIM	EZ	SIM
Zocozet®[a] (B.N.1031118)	98.29%± 2.856	101.25%± 1.553	101.1%± 1.341	100.86%± 1.470
F-test (6.388)[b]	4.534	1.115		
Student's t-test (2.306)[b]	1.279	0.603		
Lipitrin®[a] (B.N.90478A)	101.78± 1.281	100.59± 1.967	102.68± 0.858	102.74 0.960
F-test (6.388)[b]	2.227	4.193		
Student's t-test (2.306)[b]	1.304	2.193		
Alkorplus®[a] (B.N.008)	100.6± 1.516	102.46± 2.123	102.08± 0.725	101.82± 1.273
F-test (6.388)[b]	4.364	2.778		
Student's t-test (2.306)[b]	1.968	0.583		

[a]Average of 5 determinations

[b]The values in the parenthesis are corresponding theoretical value at

p= 0.05 [degree of freedom in dosage form =8].

[26] Spectrophotometric determination of EZ and SIM using [1]DD at 299.5 and 242.5 nm for EZ and AT, respectively and methanol asa solvent.

Table 3: Results of analysis of EZ and SIM in different dosage forms by proposed method and results of statistical comparison with the reported one.

Good linearity is evident from the high value of correlation coefficient and low value of intercept as shown in Table 1.

Accuracy: Accuracy of the method was checked by applying the proposed method for determination of different blind samples of pure EZ, AT and SIM. The concentrations were calculated from the corresponding regression equations and the results are presented in Table 1. Accuracy of the method was further assured by applying the standard addition technique on different pharmaceutical dosage forms where good recoveries were obtained revealing no interference from excipients, Tables 5,6.

Precision

A) Repeatability: Three concentrations of EZ, AT and SIM (0.6, 1.2 and 1.8 µg/band) were analyzed three times intra-daily using the proposed method. Good % RSD was obtained, confirming the repeatability of the method as shown in Table 1.

B) Intermediate precision: The previous procedure was repeated inter-daily on three different days for the analysis of the three chosen concentrations. Acceptable % RSD was obtained and given in Table 1.

Specificity: Specificity of the method was tested by how accurately and specifically the analytes of interest are determined in the presence of other components (e.g.: co-formulated drugs, excipients, impurities, degradation products, etc). This is evident from TLC-Densitograms in Figure 1.

The good recovery percentages obtained by applying the proposed method on pharmaceutical dosage forms, Tables 2,3 also proved the specificity of the proposed method as shown in Figure 3.

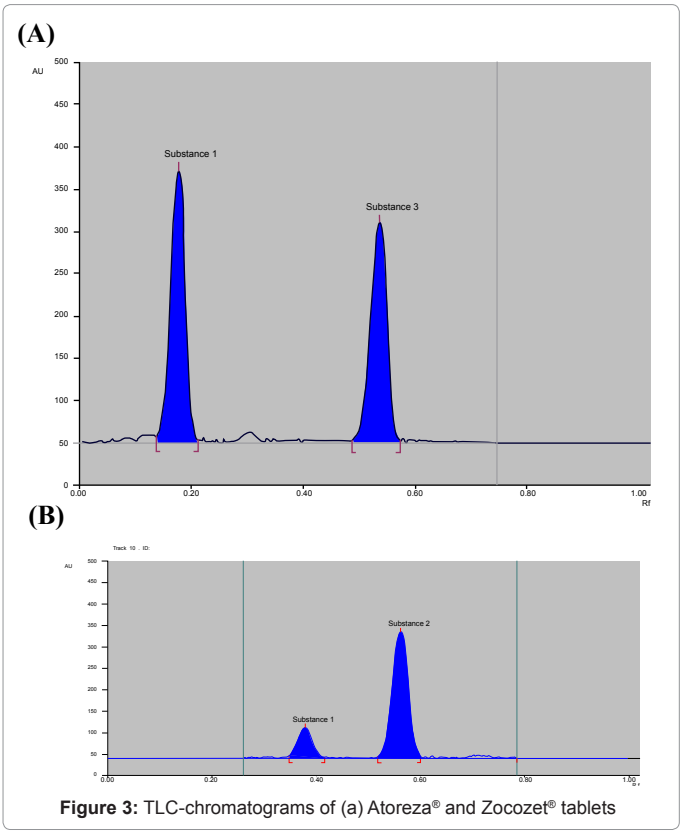

Figure 3: TLC-chromatograms of (a) Atoreza® and Zocozet® tablets

Parameters	EZ	SIM	AT
Symmetry factor	1	1.25	1
Rrsolution (Rs)	3	2.72	7
Selectivity (α)		1.75	1.42

Table 4: System suitability testing parameters of TLC-Densitometric method.

	EZ			AT				
Parameters	Recovery [%]	Pure added [µg/band]	Pure Found[b] [µg/band]	Recovery [%]	Recovery [%]	Pure added [µg/band]	Pure Found[b] [µg/band]	Recovery [%]
Atoreza ®[a] (B.N. 1031061)		6	6.08	101.33		6	6.08	101.40
		8	8.21	102.62		8	7.77	97.16
		10	9.95	99.58		10	10.13	101.33
		12	1.18	98.55		12	12.14	101.17
Mean± SD	101.05± 1.302			100.52± 1.81	99.86± 1.695			100.26± 2.072

[a]Average of 5 determinations

[b]Average of 3 determinations

Table 5: Determination of EZ and AT Atoreza® tablets by the proposed method and application of standard addition techniq

	EZ			AT				
Parameters	Recovery [%]	Pure added [µg/band]	Pure Found[b] [µg/band]	Recovery [%]	Recovery [%]	Pure added [µg/band]	Pure Found[b] [µg/band]	Recovery [%]
Zocozet®[a] ((B.N. 1031118)		6	6.13	102.23		6	6.09	101.58
		8	7.85	98.23		8	8.01	100.12
		10	10.03	100.30		10	9.97	99.70
Mean±SD	98.29± 2.856			100.25± 2.00	101.25± 1.553			100.46± 0.986
Lipitrin®[a] (B.N.90478A)		6	6.16	102.66		6	6.02	100.42
		8	8.22	102.75		8	7.92	99.06
Mean± SD	101.78± 1.281			102.70± 0.063	100.59± 1.967			99.74± 0.961
Alkorplus®[a] (B.N. 008)		4	3.99	99.75		6	6.12	102
		6	6.04	100.66		8	8.23	102.87
		8	8.12	101.50		10	10.22	102.2
Mean± SD	100.6± 1.516			100.63± 0.875	102.46± 2.123			102.35± 0.455

[a]Average of 5 determinations

[b]Average of 3 determinations

Table 6: Determination of EZ and SIM in different tablets by the proposed method and application of standard addition technique.

Robustness: The robustness meaning is the capacity of the method to remain unchanged upon intended small change in method parameters e.g.: changing PH ± 0.1, changing mobile phase composition, changing saturation time ± 5 min and changing the scaling wavelength ± 1 nm. The low value of % RSD shows that the method is robust and that deliberate small changes in the studied factors do not lead to significant changes in R_f values, area or symmetry of the peaks.

System suitability: In order to validate the suggested TLC-Densitometric method, an overall system suitability testing was done to determine if the operating system are preformed properly. Parameters including resolution (R_s), peak symmetry, and selectivity (α) were calculated where good results were obtained and the peak information is given in Table 4.

After method optimization and validation it has been applied for the determination of EZ, AT and SIM in different pharmaceutical dosage forms where acceptable percentage recoveries have been obtained and shown in Tables 2,3. Furthermore, its validity was assessed by applying the standard addition technique which showed that tablet excipients did not interfere (Table 5,6).

On the other hand, statistical comparison of the results obtained by the developed method with those obtained by the reported spectrophotometric one [26] using F and Student's t-tests showed no significant difference. The developed method has the advantages of being economic, reproducible, and accurate and can be easily applied in quality control laboratories.

Conclusion

In the present work sensitive and selective TLC-Densitometric method for the determination of EZ, AT and SIM in their pure form and in different dosage forms has been developed and validated.

The developed TLC-Densitometric method is considered superior to the reported spectrophotometric methods of being more selective and sensitive. On the other hand it can be used as alternative method to the published HPLC methods in laboratories lacking the facilities of HPLC. The developed TLC-Densitometric method is time effective since several samples can be run simultaneously using a small quantity of the developing system, which lowers the analysis time and cost. Finally we can conclude that the suggested method can be used in routine analysis of the studied drugs without any preliminary separation step.

References

1. O'Neil MJ (2001) The merck index: An encyclopedia of Chemicals, Drugs and Biologicals. (13th Edn), Merck, Rahway, NJ.
2. Sharma M, Mhaske DV, Mahadik M, Kadam SS, Dhaneshwar SR (2008) UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation. Ind J Pharm Sci 70: 258-260.
3. Uçaktürk E, Ozaltin N, Kaya B (2009) Quantitative analysis of ezetimibe in human plasma by gas chromatography-mass spectrometry. J Sep Sci 32: 1868-1874.
4. Sistla R, Tata VS, Kashyap YV, Chandrasekar D, Diwan PV (2005) Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. J Pharm. Biomed Sci 39: 517-522.
5. Dalmora SL, Oliveira PR, Barth T, Todeschini V (2008) Development and validation of a stability-indicating micellar electrokinetic chromatographic method for the determination of ezetimibe in pharmaceutical formulations. J Anal Sci 24: 499-503.
6. Barhate CR, Mohanraj K (2011) What is the degradation product of ezetimibe? J Pharm Biomed Anal 55: 1237-1238.
7. Gajjar AK, Shah VD (2011) Isolation and structure elucidation of major alkaline degradant of Ezetimibe. J Pharm Biomed Anal 55: 225-229.
8. Basha SJ, Naveed SA, Tiwari NK, Shashikumar D, Muzeeb S, et al. (2007) Concurrent determination of ezetimibe and its phase-I and II metabolites by HPLC with UV detection: Quantitative application to various in vitro metabolic stability studies and for qualitative estimation in bile. J Chromatogr B Analyt Technol Biomed Life Sci 853: 88-96.
9. Jani AJ, Dosandi B, Rathnam S, Mehta AA (2010) Liquid Chromatographic-MS/MS Determination of Atorvastatin and Metabolites in Human Plasma. Eursian J Anal Chem 5: 46-52.
10. Liu D, Jiang J, Zhou H, Hu P (2008) Quantitative Determination of Atorvastatin and Para-hydroxy Atorvastatin in Human Plasma by LC–MS–MS. J Chromatogr Sci 46: 862-866.
11. Stanisz B, Kania L (2006) Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase. Acta pol Pharm 63: 471-476.
12. Shirkhedkar AA, Surana SJ (2010) Development and validation of a reversed-phase high-performance thin-layer chromatography-densitometric method for determination of Atorvastatin calcium in bulk drug and tablets. J AOAC Int 93: 798-803.
13. Guillén D, Cofán F, Ros E, Millán O, Cofán M, et al. (2009) Determination of Atorvastatin and its metabolite Ortho-hydroxyatorvastatin in human plasma by on-line anion-exchange solid-phase extraction and liquid chromatography tandem mass spectrometry. Anal Bioanal Chem 394: 1687-1696.
14. Skorda D, Kontoyannis CG (2007) Identification and quantitative determination of atorvastatin calcium polymorph in tablets using FT-Raman spectroscopy. Talanta 74: 1066-1070.
15. Yang H, Feng Y, Luan Y (2003) Determination of Simvastatin in human plasma by liquid chromatography–mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 785: 369-375.
16. Wang L, Asgharnejad M (2000) Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form. J Pharm Biomed Anal 21: 1243-1248.
17. Patel BN, Sharma N, Sanyal M, Shrivastav PS (2008) Simultaneous determination of simvastatin and simvastatin acid in human plasma by LC-MS/MS without polarity switch: application to a bioequivalence study, J Sep Sci 31: 301-313.
18. Chaudhari BG, Patel NM, Shah PB, Patel LJ, Patel VP (2007) Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of atorvastatin and ezetimibe from their combination drug products. J AOAC Int 90: 1539-1546.
19. Chaudhari BG, Patel NM, Shah PB, Modi KP (2006) Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe. Ind J Pharm Sci 68: 793-796.
20. Sonawane SS, Shirkhedkar AA, Fursule RA, Surana SJ (2007) Simultaneous spectrophotometric estimation of atorvastatin calcium and ezetimibe in tablets. Ind J pharm Sci 69: 683-684.
21. Hefnawy M, Al-Omar M, Julkhuf S (2009) Rapid and sensitive simultaneous determination of ezetimibe and simvastatin from their combination drug products by monolithic silica high-performance liquid chromatographic column. J Pharm Biomed Anal 50: 527-534.
22. Oliveira PR, Barth T, Todeschini V, Dalmora SL (2007) Simultaneous liquid chromatographic determination of ezetimibe and simvastatin in pharmaceutical products. J AOAC Int 90: 1566-1572.
23. Dixit PR, Barhate CR, Padhye SG, Viswanthan CL, Nagaresenker MS (2010) Stability Indicating RP-HPLC Method for Simultaneous Determination of Simvastatin and Ezetimibe from Tablet Dosage Form. Ind J Pharm Sci 72: 204-210.
24. Dixit PR, Barhate CR, Nagaresenker MS, Stability indicating HPTLC method for simultaneous determination of ezetimibe and simvastatin. Chromatographia 67: 101-107.
25. Moussa BA, Mohamed MF, Youssef NF (2010) Derivative spectrophotometric method for simultaneous determination of ezetimibe and simvastatin in combined tablets. Eur J chem. 1: 348-351.
26. Maher HM, Youssef RM, Hassan EM, El-Kimary EI, Barary MA (2011) Enhanced spectrophotometric determination of two antihyperlipidemic mixtures containing ezetimibe in pharmaceutical preparations. Drug Test Anal 3: 97-105.
27. Traitler H (1991) Proceeding of the sixth international symposium on instrumental planar chromatography, Institute for chromatography, Bad Duerkheim.
28. International Conference on Harmonization (ICH) (1997) Q2B, Validation of Analytical Procedures, Methodology. (Volume 62), US FDA Federal Register.