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Simultaneous Estimation and Validation of Simvastatin and Ezetimibe by HPTLC in Pure and Pharmaceutical Dosage Forms

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A simple, rapid, sensitive high performance thin layer chromatographic method has been developed and validated for simultaneous estimation of simvastatin and ezetimibe in pure and pharmaceutical dosage form. It was performed on TLC plate precoated with silica gel $60F_{254}$ as a stationary phase using mobile phase composing of ethyl acetate:chloroform (80:20) and the detection was carried out in absorbance/ reflectance mode at 220 nm showing R_f value 0.76 for simvastatin and 0.89 for ezetimibe. The percentage estimation of labeled claims of simvastatin and ezetimibe from commercial tablet was found to be 99.62, 99.34 by height and 99.40, 99.48 by area, respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. Linearity was observed between 600 and 1400 µg/mL for simvastatin and ezetimibe. The recoveries of drugs by standard addition method were found in the range of 99.73 and 99.59 for both the drugs. The proposed method is precise, accurate and can be used for routine analysis of simvastatin and ezetimibe in tablets.

Key Words: HPTLC, Simvastatin, Ezetimibe.

INTRODUCTION

Simvastatin[1-4] (SIM) is a hypolipidemic drug for oral administration. Chemically it is 2,2-dimethyl butanoic acid (lS,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl] ethyl]-1-naphthalenyl ester. Its empirical formula is $C_{25}H_{38}O_5$ and molecular weight is 418.56. SIM is HMG-CoA reductase inhibitor synthetic analog of lovastatin.

Ezetimibe[1-4] (EZM) is a novel and selective cholesterol absorption inhibitor drug for oral administration, chemically it is (3R.4S)-1-(4-

fluorophenyl)-3-[(3S)-3-(4-fluoro phenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone. Its empirical formula is $C_{24}H_{21}F_2NO_3$ and its molecular weight is 409.42. EZM is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol, resulting in lowered plasma total cholesterol and LDL-C levels.

The combination of SIM 10 mg and EZM 10 mg provides 52 % reduction in LDL-C. The combination of SIM plus EZM promises an additional margin of safety.

The literature survey[5-10] indicates that SIM and EZM have been determined individually by using UV-spectrophotometry, high performance liquid chromatography in pharmaceutical and biological fluids preparations. No method has been reported for estimation of SIM and EZM simultaneously. In the present investigation an attempt was made to develop a simple and economical validated HPTLC with greater precision, accuracy and sensitivity for the simultaneous estimation of SIM and EZM in pure and tablet dosage forms.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade silicagel $60F_{254}$ precoated aluminum plates with thickness 200 µm, E-Merck, Germany were used as a stationary phase the instrument used was CAMAG-HPTLC system comprising of CAMAG LINOMAT-N automatic Sample applicator, CAMAG TLC SCANNER III with CAT S 4 software, CAMAG-UV cabinet and CAMAG twin trough glass chamber with stainless steel lids. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Pure standards of SIM and EZM were obtained as gift samples from AEON Pharmaceuticals.

Preparation of standard solution: An accurately weighed quantity of 250 mg of SIM (RS) and EZM (RS) were dissolved in methanol make up to 25 mL to obtain a stock solution of 10000 µg/mL of SIM and EZM.

Mixed standard solution: Solution containing SIM and EZM each of 600 µg/mL was prepared and mixed to get the mixed standard solution.

Chromatographic conditions: Optimized standard chromatographic conditions required where, stationary phase comprising of TLC aluminum foiled plates precoated with silica gel $60F_{254}$ with thickness of 200 µm ethyl acetate, chloroform in the ratio of 80:20 v/v solution was used as a mobile phase and the chamber was saturated for 10 min. Sample was applied at a constant rate of 0.16 µL/s having scan speed 10 mm/s with 16 mm band width the samples were separated by ascending technique. The chamber was maintained at 20 ± 5ºC temperature and 50-60 % relative humidity. The scanning was carried out by absorbance/reflectance mode with slit dimension 5 × 0.45 mm. The detection was carried out at 220 nm.

Calibration curve: SIM and EZM solutions ranging from 600 to 1400 μg/mL were applied on TLC plate by μL syringe with the help of automatic sample applicator. The plates were developed, dried and densiometrically scanned at 220 nm. Peak height and area were recorded for each concentration and curves (concentration/peak height/area) were constructed.

System suitability test: The system suitability test was performed by repeated application of each 10 μL of mixed standard solution and development of chromatogram. The mean, standard deviation and coefficient of variance of peak area and peak height were calculated.

Standard laboratory mixtures: Different laboratory mixtures were prepared in the same manner as that of standard solution to get the final concentration of about 600 μg/mL of 81 M and EZM. 10 μL of mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on TLC plates in the form of 16 mm band. The plates were then developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 220 nm.

Assay procedure: 20 Tablets (Simlo-EZ labeled to contain each 10 mg of SIM and EZM) were weighed, powdered an accurately weighed quantity of powder equivalent to 10 mg (130 mg powder drug) of SIM and EZM was transferred to 10 mL volumetric flask. The contents were dissolved in methanol and volume made up to the mark. The contents were mixed well using ultrasonicator and filtered through Whatmann filter paper no. 42. This was used as a sample solution after preparation of the sample the same procedure was followed as under laboratory mixture.

The contents of the drugs in average weight of tablet were calculated as follows:

$$\text{Labelled claim (\%)} = \frac{W_E}{W_A} \times 100$$

where W_E = weight of drug estimated (μg), W_A = weight of drug applied (μg) on the basis of labeled claim.

Validation of proposed method: The proposed method validated for the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. Accurately known amounts of standard drugs were added to known amount of pre analyzed tablet powder and was analyzed by the proposed method to ascertain if there are positive or negative interferences from excipients present in formulation. The per cent recovery was calculated using following the formula.

$$\text{Recovery (\%)} = \frac{(A - B)}{C} \times 100$$

where A = total drug estimated in mg; B = amount of drug contributed by tablet powder (as per proposed method); C = amount of pure drug added.

Precision: Replicate estimations of drugs in sample were carried out by the proposed method and SD/RSD value was calculated as a measure of precision.

Ruggedness: Ruggedness was tested under different conditions, *i.e.*, analyzing the samples on different days and by different analysis.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity, *viz.*, ethyl acetate, chloroform, toluene and diethyl ether and their mixtures in different proportions were tried as a mobile phase for development of chromatogram. The mobile phase was found to be more suitable was ethyl acetate:chloroform 80:20 v/v. It gave the good resolution of two components reasonably good with R_f values of 0.76 of SIM and 0.89 EZM. The 220 nm wavelengths were selected for densiometric evaluation of chromatogram as both drugs have sufficient and high absorbance and showing better sensitivity.

The per cent estimations of drugs in the laboratory mixture with the ± SD were found to be 99.82, 100.08 by peak height and 100.07, 101.06 peak area for both the drugs and per cent drug estimation in marketed formulation shows 99.62, 99.40 by peak height and 99.34, 99.48 by peak areas for both drugs, respectively and are given in Table-1.

TABLE-1
ESTIMATION OF SIM AND EZM

Sample	Statistics	Estimation of labeled claim* (%)			
		SIM		EZM	
		By height	By area	By height	By area
Standard laboratory mixture	Mean	99.8200	100.0800	100.0200	101.0600
	± SD	0.3113	0.3563	0.3301	0.4950
	CV	0.3120	03560	0.3299	0.4895
Marketed preparation	Mean	99.6200	99.4000	99.3400	99.4800
	± SD	0.1581	0.2705	0.2702	0.1303
	CV	0.1587	0.2710	0.2719	0.1310

*Mean of five values.

The concentration response plots of drugs show linearity over the concentration range of 600-1400 µg/mL for SIM and EZM with coefficient of correlation values 0.9943, 0.9962 by peak height and 0.9959, 0.9987 by peak area for both drugs, respectively and are given in Table-2.

TABLE-2
LINEARITY STUDIES

Drug	Linearity range (μg)	Coefficient of correlation		Slope		Y-intercept	
		By height	By area	By height	By area	By height	By area
SIM	600-1400	0.9943	0.9959	11.213	11.906	-297	-300
EZM	600-1400	0.9962	0.9987	21.062	21.855	-301	-299

The accuracy of the method was evaluated by per cent recovery and by standard addition method for both drugs by peak height and peak area. The results of the method is within the limit of 98-102 % and shows that the method is free from the influence of excipients and are given in Table-3.

TABLE-3
RECOVERY STUDIES

Sample	Statistics	Recovery* (%)			
		SIM		EZM	
		By height	By area	By height	By area
Standard laboratory mixture	Mean	98.6800	99.7300	98.6200	99.3400
	± SD	0.1303	0.1303	0.1923	0.7820
	CV	0.1321	0.1309	0.1950	0.7872

*Mean of five values.

TABLE-4
RUGGEDNESS STUDY OF SIM AND EZM

Sample	Statistics	Labeled claim* (%)			
		SIM		EZM	
		By height	By area	By height	By area
Different days	Mean	99.4800	99.5800	99.1400	99.1700
	± SD	0.1303	0.3271	0.2073	0.1766
	CV	0.1310	0.3284	0.2091	0.1781
Different analysts	Mean	99.3400	99.3600	99.8600	99.3400
	± SD	0.3042	0.3015	0.2764	0.2660
	CV	0.3062	0.3035	0.2782	0.2677

*Mean of five values.

The replicate estimation of both drugs in the same batch of the tablet analyzed by the proposed methods yielded quite concurrent result indicat-

ing the reliability of the method. The values of SD and RSD and coefficient of correlation are within the prescribed limit of 2 % showing high precision of the method.

The ruggedness studies were performed for the estimation of % label claim of SIM and EZM. The proposed method was reproducible under different conditions like different days and by different analysts and the results are given in Table-4.

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REFERENCES

1. The Merk Index, edn. 13, p. 693, 1529 (2001).
2. The United States Pharmacopoeia 25 National Formulary 20 Asian Edition, p. 1571 (2002).
3. H.P. Rang, M.M. Dale, J.M. Ritter, P.K. Moore, Pharmacology, edn. 5, pp. 310-312 (2003).
4. J.G. Hardman, L.E. Limbird, Alfred Goodman Gilman Goodman Gilman's The Pharmacological Bases of Therapeutics, edn. 10, pp. 994-995 (2001).
5. R. Sistla, V.S.S.K. Tata, Y.V. Kashyap, D. Chandrasekar and P.V. Diwan, *J. Pharm. Biomed. Anal.*, **39**, 517 (2005).
6. L. Wang and M. Asgharnejad, *J. Pharm. Biomed. Anal.*, **21**, 1243 (2000).
7. M.K. Srinivasu, A.N. Raju and G.O. Reddy, *J. Pharm. Biomed. Anal.*, **29**, 717 (2002).
8. D.G. Sankar and D.V.S.P. Kumar, *Asian J. Chem.*, **18**, 823 (2006).
9. D.G. Sankar, D.V.S.P. Kumar, M.V. Krishna and P.V.M. Latha, *Asian J. Chem.*, **17**, 2812 (2005).
10. P.D. Sethi, HPTLC Quantitative Analysis of Pharmaceutical Formulations, edn. 1, pp. 1-68 (1996).

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