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Chromatographic determination of sitagliptin and simvastatin in their pharmaceutical formulation

Mamdouh R.Rezk*, Sherif A.Abdel-Gawad

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St, ET-11562 Cairo, (EGYPT)

E-mail : mamdouhreda75@yahoo.com

ABSTRACT

Two methods namely, coupled TLC densitometry and high performance liquid chromatography, were used to determine sitagliptin and simvastatin simultaneously in their pharmaceutical dosage form. A TLC separation with densitometric detection of both drugs was achieved using benzene: n-butanol: triethylamine (9:2:0.5, by volumes] as a developing solvent. Furthermore, a high performance liquid chromatographic procedure with ultraviolet detection at 220 nm was developed for the separation and determination of the studied drugs using a C_{18} column. The mobile phase was composed of water: methanol: acetonitrile (1: 2: 2, by volumes). The final pH was adjusted to 4.6 ± 0.1 with *O*-phosphoric acid. The proposed methods were successfully applied for the determination of the studied drugs in pure forms, their mixtures and in pharmaceutical formulation containing them. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Sitagliptin;
Simvastatin;
TLC;
HPLC.

INTRODUCTION

Simvastatin [SIM], is butanoic acid, 2, 2-dimethyl-, 1, 2, 3, 7, 8, 8a-hexa hydro-3, 7-dimethyl-8-[2(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, Figure 1. It is a lipid-lowering agent that is HMG Co-A reductase inhibitor[1].

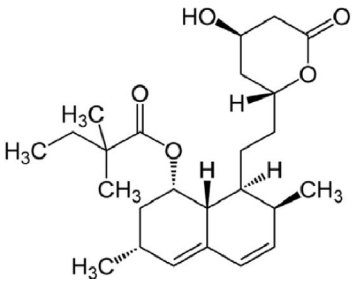

Figure 1 : Structural formula of simvastatin [SIM] M.W. [$C_{25}H_{38}O_5$ = 418.57]

Sitagliptin (STA) is [(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]butan -2-amine], Figure 2. It is an orally active and selective inhibitor of dipeptidyl peptidase-IV that is used for treatment of type II diabetes[2].

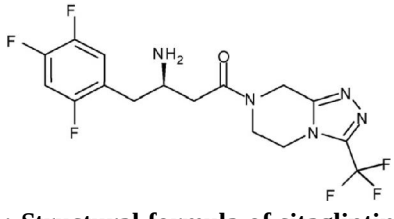

Figure 2 : Structural formula of sitagliptin [STA] M.W. [$C_{16}H_{15}F_6N_5O$ = 407.31]

Many techniques were reported as UV-Visible spectrophotometry[3,4], HPLC[5-10] for the determination of SIM alone, in presence of its metabolites or in

combination with other drugs. On the other hand, STA could be determined either alone or in presence of other drugs using different techniques like UV-Visible spectrophotometry[11] and HPLC[12-14].

Simultaneous determination of both drugs was achieved by the use of simultaneous equation spectrophotometric method[15] and chromatographic method[16].

In modern analytical laboratory there is always a need for simple and rapid method of analysis. The present work aimed to develop sensitive chromatographic methods for routine analysis and selective quantification of both STA and SIM in their pure forms or even in their pharmaceutical formulation. The methods described here include coupled TLC-densitometry and HPLC.

EXPERIMENTAL

Instrument

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.

TLC plates [20 cm x 20 cm, 0.25 mm] coated with silica gel 60 F_{254} [Merck, Germany] were used.

The chromatographic apparatus (Merck Hitachi interface D-7000, an isocratic pump (model L-7110), UV-visible detector (Model L-7420) and a Rheodyne injector (model 7161) equipped with 20-µL injector loop, La Chrom Merck Hitachi.

Stationary phase consisted of Xterra™ (250 x 4.6 mm, 5 µm) C_{18} column. The samples were injected by the aid of a 100 µL Hamilton® analytical syringe.

Materials and reagents

All chemicals and reagents were of analytical grade.

(a) Materials

Pure STA and SIM powder were kindly supplied by Merck Sharp & Dohme International, USA. The potency was found to be 100.50 ± 0.791% (*n*=6), for STA according to a reference HPLC method[13] and 100.87 ± 0.811% (n=6), for SIM according to the official HPLC method[1].

(b) Reagents

Benzene, *n*-butanol and phosphoric acid: Adwic, El-Nasr Pharm. Co. (Cairo, Egypt). Methanol and acetonitrile: HiPerSolv.®, HPLC-grade, E. Merck (Darmstadt, Germany). Triethyl amine L R: Laboratory Rasayan s.d. Fine-Chem Ltd. De-ionized water: Bidistilled from Aquatron Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire, UK).

(c) Pharmaceutical formulations

Juvisync™ tablet BN: G011008 claimed to contain 128.5 mg sitagliptin phosphate monohydrate equivalent to 100 mg sitagliptin free base and 20 mg simvastatin, manufactured by Merck Sharp & Dohme International, USA.

(d) Standard solutions

Standard stock solutions were prepared by dissolving STA and SIM, separately, in 70 % aqueous methanol into 50-mL volumetric flasks to obtain a final concentration of 1 mg/mL for the TLC and HPLC methods.

All calculations were done regarding the free base for STA.

Procedure

(a) TLC-densitometry

(A) Linearity

Stock standard solutions were prepared separately by dissolving 10 mg of STA or SIM in 10mL-volumetric flask with 70% aqueous methanol. Aliquots (1-20 µL) of each of STA or SIM (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, 0.25mm). 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 10 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; benzene + *n*-butanol + triethyl ammine (9: 2: 0.5, by volumes) by ascending chromatography through a distance of ~15 cm at room tem-

perature (25 °C). The developed plates were air-dried and the spots were detected under UV-lamp at 254 nm then scanned under the following instrumental conditions:

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

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

(b) HPLC method

(A) Linearity

Aliquots (0.1-1mL) from both SIM and STA standard solutions (each, 1 mg/mL) were accurately and separately transferred into a series of 10-mL volumetric flasks, the volume was then completed with the mobile phase to obtain a concentration range of 10-1000 µg/mL for each.

Samples were then chromatographed using Xterra™ C_{18} column (250 x 4.6 mm, 5 µm) as a stationary phase. The mobile phase was formed of water: methanol: acetonitrile (1:2:2, by volumes). The final pH of the mobile phase was adjusted to 4.6 ± 0.1 using phosphoric acid. The flow rate was 1mL/min, isocratically with UV detection at 220 nm. Peak area ratios were plotted against concentration to obtain calibration graphs then the regression equations were computed. The samples were filtered also through a 0.45-µm membrane filter, and were injected by the aid of a 100-µL Hamilton® analytical syringe. To reach good equilibrium, analysis was usually performed after passing ~ 50-60ml 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.

(c) Analysis of laboratory prepared mixtures of STA and SIM

Aliquots of STA and SIM were mixed to prepare different mixtures containing 10/1, 10/2 and 10/4 (w/w) of STA/SIM, respectively, and proceed as mentioned under each method. The concentration of each drug was calculated from the corresponding regression equation.

(d) Analysis of pharmaceutical formulation

Ten tablets were weighed and powdered to obtain the average weight per tablet. A mass of the powdered tablets, claimed to contain 100 mg of STA and 20 mg SIM was dissolved in small quantity of 70 % aqueous methanol. This mixture was sonicated for 15min. and diluted to mark with methanol. Aliquots were then removed and centrifuged at 5000 rpm for 20 min and then filtered through a 0.45 µm syringe filter. The solution was transferred to a volumetric flask and assayed as mentioned under each method. All determinations were done in triplicate.

(e) Method validation

The developed analytical methods were fully 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

Coupled TLC-densitometric method

A coupled TLC-densitometric method is described for the simultaneous determination of STA and SIM without prior separation. Different solvent systems were tried for the separation of both drugs. Satisfactory results were obtained by applying the experimental conditions mentioned before and using a mobile phase composed of benzene + *n*-butanol + triethylamine (9:2:0.5, by volumes), where R_f was 0.31 and 0.73 for STA and SIM, 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 254 nm over a range of 1-20 µg/spot, for both STA and SIM. The separated spots of the two drugs were scanned at 254 nm, figure 3. The regression equations were calculated and used for estimation of the concentration of the studied drugs in unknown samples.

HPLC method

A simple isocratic high-performance liquid chromatographic method was developed for the determination of STA and SIM in pure forms and in their mix-

Full Paper

tures. The mobile phase was chosen after several trials. The most suitable one was consisted of water: methanol: acetonitrile, (1:2:2, by volumes). The flow rate was 1mL/min., isocratically, at ambient temperature. By using the described chromatographic conditions, STA and SIM were well separated with average retention times of 3.3 min. and 8.8 min., for STA and SIM, respectively, Figure 4.

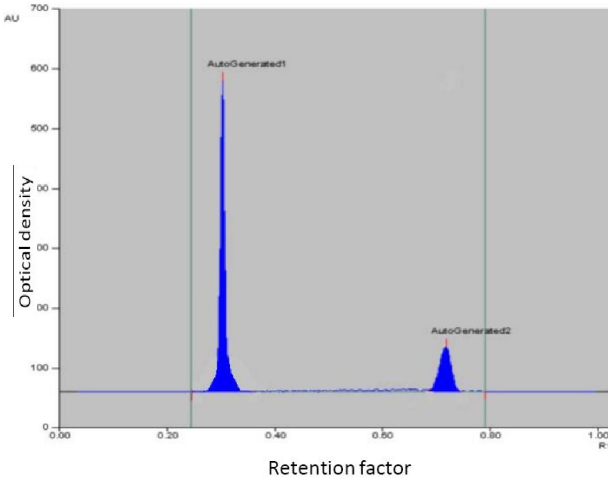

Figure 3 : A typical densitogram showing the separation of sitagliptin (R_f=0.31) and simvastatin (R_f=0.73)

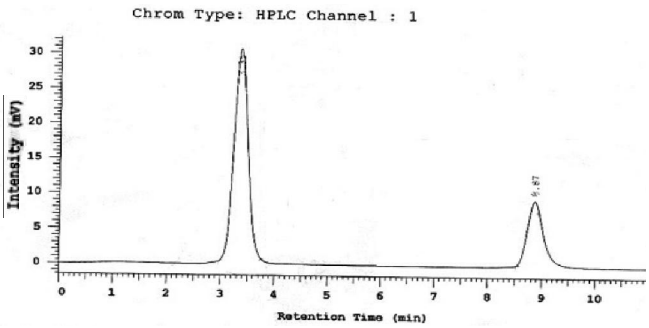

Figure 4 : A typical HPL chromatogram showing the separation of sitagliptin (3.3 min.) and simvastatin (8.8 min.)

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the external standard versus concentration. The regression equations were calculated and used for estimation of the concentration of the studied drugs in unknown samples.

(a) Analysis of laboratory prepared mixtures of STA and SIM

Different mixtures containing 10/1, 10/2 and 10/4 (w/w) of STA/SIM, respectively, were analyzed by the proposed methods and the results are shown in TABLE 1.

TABLE 1 : Determination of STA and SIM in laboratory-prepared mixtures by the proposed methods

Lab. mix. Ratio (STA/SIM)	Drug determined	TLC-Densitometric method	HPLC method
10/1	SIM (mean ± SD)	99.76 ± 1.182	98.83 ± 0.910
	STA (mean ± SD)	99.50 ± 0.805	100.25 ± 0.731
10/2	SIM (mean ± SD)	98.86 ± 0.886	99.25 ± 0.762
	STA (mean ± SD)	98.95 ± 0.754	101.18 ± 1.276
10/4	SIM (mean ± SD)	100.89 ± 1.252	101.19 ± 0.863
	STA (mean ± SD)	101.24 ± 0.945	101.17 ± 0.928

(b) Analysis of pharmaceutical formulation

Assay of STA and SIM in Juvisync™ tablet was done and satisfactory results were obtained, TABLE 2.

TABLE 2 : Determination of STA and SIM in their pharmaceutical formulation by the proposed methods

Preparation Juvisync™ tablet BN: G011008	TLC Densitometric method	HPLC method
STA (Mean ± SD)	101.28 ± 0.830	100.85 ± 0.635
SIM (Mean ± SD)	99.55 ± 0.704	98.96 ± 0.502

(c) Method validation

The developed analytical methods were fully 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.

TABLE 3 : Assay parameters and validation sheet for determination of STA and SIM by the proposed methods

Parameter	STA		SIM	
	TLC method	HPLC method	TLC method	HPLC method
Concentration Range	1-20µg band^{-1}	10-1000µg mL^{-1}	1-20µg band^{-1}	10-1000µg mL^{-1}
Slope	595.12	0.0242	272.60	0.0905
Intercept	-195.57	0.5459	-8.5657	0.4583
Mean	100.35	100.72	99.93	100.44
SD	0.825	0.974	0.892	0.832
Variance	0.681	0.949	0.796	0.692
CV^{+}	0.822	0.967	0.893	0.828
R	0.9987	0.9992	0.9996	0.9994

+ cv = coefficient of variation and r=correlation coefficient

Assay parameters and validation sheet for determination of STA and SIM by the proposed methods are listed in TABLE 3, while comparison of the results

obtained by the proposed methods and the reference one for STA and official method for SIM is shown in TABLE 4.

TABLE 4 : Statistical comparison for results obtained by the proposed methods and the reference one for STA and official method for SIM

Parameter	STA			SIM		
	TLC method	HPLC method	Reference Method[13]	TLC method	HPLC method	Official method[1]
Mean	100.35	100.72	100.50	99.93	100.44	100.87
SD	0.825	0.974	0.791	0.892	0.832	0.811
Variance	0.681	0.949	0.626	0.796	0.692	0.658
n	7	7	6	7	7	6
F-test	1.09 (4.95)*	1.52 (4.95)*		1.21 (4.95)*	1.05 (4.95)*	
Student's t-test	0.334 (2.201)*	0.449 (2.201)*		1.989 (2.201)*	0.942 (2.201)*	

***the values in the parentheses are the corresponding theoretical t-and F-values at p=0.05[17]**

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