

Simultaneous determination of Simvastatin and Sitagliptin in tablets by new univariate spectrophotometric and multivariate factor based methods

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ABSTRACT

Five simple, sensitive and precise spectrophotometric and chemometric methods were used for simultaneous determination of Simvastatin (SM) and Sitagliptin (SIT) in their pure powdered forms and in the tablets. The proposed methods are the extended ratio subtraction method (EXRSM), ratio difference method (RDSM), mean centering of ratio spectra method (MCR) and chemometric methods, namely principal component regression (PCR) and partial least squares (PLS). In EXRSM; SM was determined at 237.5 nm, while SIT was determined at 267 nm, in RDSM; the difference in amplitudes at 237.5 and 245.5 nm was used for SM and 263.5 and 248.0 nm for SIT, while in MCR; SM and SIT were determined at 239.0 and 273.0 nm, respectively. PCR and PLS are factor based multivariate methods which utilize the whole spectra of SM and SIT. The developed methods were successfully applied for the determination of the studied drugs in their bulk powder, laboratory prepared mixtures and in tablets. All validation parameters of the developed methods were determined. The obtained results were statistically compared with each other along with a reported method.

1. Introduction

Simvastatin (SM) is a lipid-lowering agent that is derived synthetically from the fermentation products of *Aspergillus terreus*. After oral ingestion Simvastatin, an inactive lactone, is hydrolyzed to the corresponding ortho-hydroxy acid leading to the inhibition of 3-hydroxy 3-methyl glutaryl-coenzyme A (HMG-Co A) reductase which is responsible for catalysing the conversion of HMG-Co A to mevalonate, which is an early and rate limiting step in cholesterol biosynthesis [1,2]. SM (Figure 1) is chemically designated as butanoic acid, 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenylester (C₂₅H₃₈O₅).

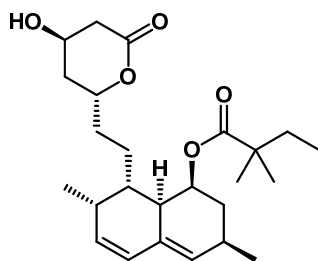


Figure 1. Chemical structure of Simvastatin.

Sitagliptin (SIT) is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor, which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases active incretin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion [3,4]. SIT (Figure 2) is chemically designated as (R)-

4-oxo-4-[3-(trifluoromethyl)-5,dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (C₁₆H₁₅F₆N₅O).

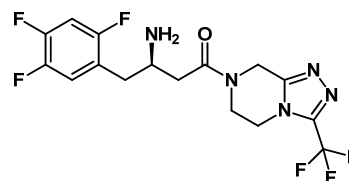


Figure 2. Chemical structure of Sitagliptin.

Recently, the FDA has approved a fixed-dose combination tablet consisting of simvastatin and sitagliptin. It is the first registered product in which a drug treating type 2 diabetes is present in combination with cholesterol lowering drug.

Many techniques like UV-Vis spectrophotometry [5,6] and HPLC [7-12] have been reported for the determination of SM alone, in presence of its metabolites or in combination with other drugs. On the other hand, SIT was determined either alone or in presence of other drugs using different techniques like UV-Vis spectrophotometry [13], HPLC [14-16], and fluorescence spectroscopy [17].

In the last few months, a few methods were published for the simultaneous determination of both drugs. These methods include the use of spectrophotometric methods [18,19] and chromatographic methods [20,21].

The aim of this work was to develop smart and simple methods for simultaneous determination of SM and SIT and to conduct a comparative study between univariate and multivariate methods in resolving spectrally overlapped bands. The univariate methods include two novel spectrophotometric

methods; namely extended ratio subtraction (EXRSM) and ratio difference (RDSM) and a well-established mean centering of ratio spectra (MCR). While, multivariate calibration methods include principal component regression (PCR) and partial least squares (PLS).

2. Theory

2.1. Extended ratio subtraction method (EXRSM)

Ratio subtraction method [22] is a well-established method. It depends on that, if you have a mixture of two drugs Z and Y having overlapped spectra, you can determine Z by dividing the spectrum of the mixture by a known concentration of Y as a divisor (Y'). The division will give a new curve that represents $\frac{Z}{Y'} + \text{constant}$. If we subtract this constant, then

multiply the new curve obtained after subtraction by Y' (the divisor), therefore we can obtain the zero order curve (D⁰) curve of Z again. This can be summarized as follows:

$$\frac{Z+Y}{Y'} = \frac{Z}{Y'} + \frac{Y}{Y'} = \frac{Z}{Y'} + \text{constant} \quad (1)$$

$$\frac{Z}{Y'} + \text{constant} - \text{constant} = \frac{Z}{Y'} \quad (2)$$

$$\frac{Z}{Y'} \times Y' = Z \quad (3)$$

For obtaining the second component (Y), an extension of the already developed method has been established as a new approach in which Y could be determined by dividing the obtained D⁰ spectrum of Z by a known concentration of Z as a divisor (Z') to get the constant $\frac{Z}{Z'}$. By dividing the spectrum of

the mixture by the same divisor (Z'). The division will give a new curve that represents $\frac{Z+Y}{Z'}$ i.e. $\frac{Z}{Z'} + \frac{Y}{Z'}$ where $\frac{Z}{Z'}$ is the

previously obtained constant. If we subtract this constant, then multiply the obtained curve by Z' (the divisor), therefore we can obtain a new curve of Y representing its D⁰ curve.

$$\frac{Y}{Z'} + \frac{Z}{Z'} - \frac{Z}{Z'} = \frac{Y}{Z'} \times Z' = Y \quad (4)$$

The concentration of Y was calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of Y at its λ_{max} against the corresponding concentrations).

2.2. Ratio difference spectrophotometric method (RDSM) [23]

A new method was also developed in which the amplitude difference between two points on the ratio spectra of a mixture is directly proportional to the concentration of the component of interest; independence of the interfering component is the basic principle of the ratio difference method.

This method depends on that, if you have a mixture of two drugs Z and Y having overlapped spectra, you can determine Z by dividing the spectrum of the mixture by a known

concentration of Y as a divisor (Y'). The division will give a new

curve that represents $\frac{Z+Y}{Y'}$ i.e. $\frac{Z}{Y'} + \frac{Y}{Y'}$, Where $\frac{Y}{Y'}$ is a constant. On the obtained ratio curve; by selecting 2 wavelengths λ_1 and λ_2 and subtract the amplitude at these two points, the constant $\frac{Y}{Y'}$ will be cancelled along with any other

instrumental error or any interference from the sample matrix. This can be summarized as follows:

$$\frac{Z+Y}{Y'} = \frac{Z}{Y'} + \frac{Y}{Y'} = \frac{Z}{Y'} + \text{constant} \quad (5)$$

Suppose the amplitudes at the two selected wavelength are P1 and P2 at λ_1 and λ_2 , respectively, then;

$$P1-P2 = \left(\frac{Z}{Y'}\right)_1 + \text{constant} - \left\{\left(\frac{Z}{Y'}\right)_2 + \text{constant}\right\} \quad (6)$$

$$P1-P2 = \left(\frac{Z}{Y'}\right)_1 - \left(\frac{Z}{Y'}\right)_2 \quad (7)$$

where; P1 is the Peak amplitudes of the ratio spectrum at λ_1 , P2 is the Peak amplitudes of the ratio spectrum at λ_2 . By using difference between P1 and P2 the interfering substance (Y) will be cancelled.

The only requirement for the two chosen wavelengths is that both components should have a spectral contribution at those wavelengths.

The concentration of Z is calculated using the regression equation representing the linear correlation between the differences of ratio spectra amplitudes at the two selected wavelengths to the corresponding concentrations of drug (Z). Similarly, Y could be determined by the same procedure using a known concentration of Z as a divisor.

2.3. Mean centering of ratio spectra method (MCR)

This is a well-established spectrophotometric method in which both binary and ternary mixtures could be determined without previous separation. In this method the ratio spectra are obtained after which the constant is removed by mean centering process [24-26]

3. Experimental

3.1. Apparatus

Spectrophotometer: SHIMADZU UV- 1601 PC, dual beam UV-Vis spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer and a HP-800 inkjet printer. Bundled UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm/min.

3.2. Software

All computations were performed in Matlab for Windows™ version 6.5 [27]. The PLS procedure was taken from PLS-Toolbox [28] for use with Matlab®6.5.

3.3. Chemicals and reagents

Pure samples: Pure simvastatin (SM) and sitagliptin

phosphate monohydrate (SIT) were kindly supplied by Merck Sharp & Dohme International, USA. Distilled water from "Aquatron" Automotive water Still A 4000 (bibby Sterillin Ltd., Staffordshire-UK). Methanol (E. Merck, Darmstadt-Germany).

Market samples: Juvisyne® tablets, labeled to contain 20 mg simvastatin and 128.5 mg sitagliptin phosphate monohydrate equivalent to 100 mg sitagliptin base per tablet (Batch No. G011008), manufactured by Merck Sharp & Dohme International, USA and were obtained from local market.

3.4. Standard solutions

Standard solutions of Simvastatin (SM) (0.1 mg/mL) and Sitagliptin phosphate monohydrate (SIT) (1 mg/mL) were prepared separately by dissolving 10 mg and 100 mg, respectively, of the pure powder in 30 mL of methanol:water (1:1, v:v) solvent mixture into 100 mL volumetric flask with continuous shaking for about 10 minutes. The volume was completed to the mark with the corresponding solvent.

3.5. Procedures

3.5.1. Spectral characteristic of SM and SIT

The zero-order (D^0) absorption spectra of 18, 36 $\mu\text{g/mL}$ SM and 360 $\mu\text{g/mL}$ of SIT were recorded against methanol as a blank over the range of 200-300 nm.

3.5.2. Construction of calibration curves

Aliquots equivalent to 20.0-180.0 μg of SM and 0.2-3.6 mg of SIT were accurately transferred from their respective standard solutions into two separate sets of 10 mL volumetric flasks then completed to volume with methanol. The prepared solutions were scanned from 200-300 nm and the scanned spectra were stored in the computer.

3.5.2.1. Ratio subtraction coupled with extended ratio subtraction methods (RS-EXRSM)

Standard solutions containing 2.0-18.0 $\mu\text{g/mL}$ SM and 20.0-120.0 $\mu\text{g/mL}$ SIT, were prepared separately in methanol. The absorption spectra of the resulting solution were measured. Construct calibration curve relating the absorbance of the zero order spectra of SM at 237.5 nm and SIT at 267.0 nm vs. the corresponding concentrations of SM and SIT, respectively, and the regression equations were computed.

3.5.2.2. Ratio difference method (RDMSM)

Standard solutions containing 2.0-18.0 $\mu\text{g/mL}$ SM and 20.0-120.0 $\mu\text{g/mL}$ SIT, were prepared separately in methanol. The absorption spectra of the prepared solutions were recorded and divided by the absorption spectra of 360 $\mu\text{g/mL}$ SIT and 18 $\mu\text{g/mL}$ SM, respectively. The calibration curves were constructed for SM and SIT by plotting the amplitudes difference between 237.5 and 245.5 nm for SM and between 248.0 and 263.5 nm for SIT versus the corresponding concentrations and the regression equations were computed.

3.5.2.3. Mean centering of ratio spectra method (MCR)

The scanned spectra of 2.0-18.0 $\mu\text{g/mL}$ of SM and 40.0-320.0 $\mu\text{g/mL}$ of SIT are exported to Matlab® for subsequent calculation. The spectra of SM were divided by the normalized spectrum of SIT, the obtained ratio spectra then mean centered. The same procedure was applied to SIT.

The calibration curves for SM and SIT are constructed by plotting the mean centered values at 239 and 273 nm for the two drugs, respectively, versus the corresponding concentration and the regression equations are computed.

3.5.3. Determination of SM and SIT in laboratory prepared mixtures

Into a series of 10 mL volumetric flasks aliquots of SM and SIT were accurately transferred from their corresponding standard solutions to prepare mixtures containing different ratios of the two drugs. The volumes were then completed with methanol. The spectra of the prepared solutions were recorded from 200-300 nm and stored in the computer. The procedures as under calibration were adopted, and then the concentration of each drug was calculated using the specified regression equation

3.5.3.1. Ratio subtraction coupled with extended ratio subtraction methods (RS-EXRSM)

The spectra of the laboratory prepared mixtures were divided by the spectrum of 360.0 $\mu\text{g/mL}$ of SIT, and then the absorbance in the plateau region at λ above 275 nm (the constants) was subtracted. The obtained curves were then multiplied by the spectrum of 360.0 $\mu\text{g/mL}$ standard SIT (the divisor). The obtained spectra were used for the determination of SM constants at the plateau region 230-240 nm were determined after dividing it using 18.0 $\mu\text{g/mL}$ standard SM as a divisor. Then, the absorbance of the previously obtained constants was subtracted. The obtained curves were then multiplied by the spectrum of 18.0 $\mu\text{g/mL}$ standard SM (the divisor). The obtained curves were then used for the determination of SIT at 267 nm using the corresponding regression equation.

3.5.3.2. Ratio difference spectrophotometric method (RDMSM)

The absorption spectra of different laboratory prepared mixtures were divided by the absorption spectra of 360.0 $\mu\text{g/mL}$ SIT and 18.0 $\mu\text{g/mL}$ standard SM. The ratio spectra were then recorded at 237.5 and 245.5 nm, 248.0 nm and 263.5 nm, for SM and SIT, respectively. The concentrations of the drugs were calculated from the computed regression equations.

3.5.3.3. Mean centering of ratio spectra (MCR)

The procedure as under calibration was adopted, and then the concentration of each drug was calculated using the specified regression equation.

3.5.3.4. Chemometric methods

Experimental design was used for the construction of the calibration and validation sets [29]. A five-level, two-factor calibration design was used in which 1.0-5.0 mL and 0.6-1.4 mL aliquots of SM and SIT standard solutions, respectively, were accurately transferred, combined and diluted to 10 mL with methanol. The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-300 nm and transferred to Matlab® for subsequent calculations.

3.5.4. Application of the proposed methods to the analysis of SM and SIT in pharmaceutical formulations

Ten tablets (Juvisyne® tablets) were accurately weighed, crushed, mixed well and finely powdered. A weight equivalent to 10 mg SIM and 64.25 mg SIT phosphate monohydrate was transferred into a 250 mL beaker; 30 mL 70% methanol were added and stirred for ~30 minutes then filtered into 100 mL measuring flask. The residue was washed with ~20 mL 70% methanol, and then the volume was completed to the mark with the same solvent and mixed well. One mL of the resulted solution was transferred to 10 mL measuring flask then the volume was completed to the mark using the same solvent and mixed well. The general procedure was followed as mentioned

before and the concentration of drug was calculated from the corresponding regression equation.

4. Results and discussion

The main task of this work was to establish simple, sensitive and accurate analytical methods for the simultaneous determination of SM and SIT in their bulk powders and pharmaceutical dosage form with satisfactory precision for good analytical practice (GAP). As well, to construct a statistical comparison between the ability of the proposed methods in determining the two drugs.

The overlapped absorption spectra of SM and SIT (Figure 3) hinder their determination by direct spectrophotometry especially in the presence of high concentration of SM as in the available pharmaceutical preparation.

The choice of the divisors is a critical step, the selected divisors should compromise between minimal noise and maximum sensitivity. Standard spectra of 360 µg/mL SIT and 18 µg/mL SM were the best tested divisors which gave the best results regarding average recovery percent and standard deviation for the determination of SM and SIT, respectively.

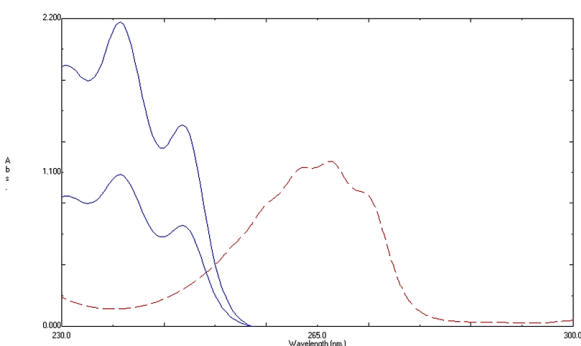


Figure 3. The zero order absorption spectra of 18, 36 µg/mL SM (—) and 360 µg/mL SIT (---) in methanol.

4.1. Univariate calibration

4.1.1. Extended ratio subtraction method (EXRSM)

The ratio subtraction method was applied to solve the mixture of SM (Z) and SIT (Y) of overlapping spectra by dividing the spectrum of the mixture by a standard spectrum of 360 µg/mL SIT (Y') as a divisor. The division will give a new

curve that represents $\frac{Z}{Y'} + \text{constant}$, Figure 4.

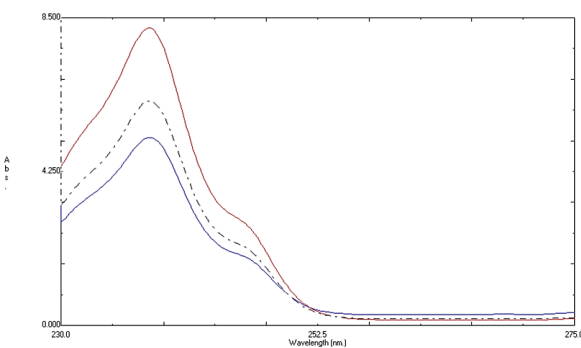


Figure 4. Division spectra of laboratory prepared mixture of Simvastatin (SM) 10, 12, 16 µg/mL and Sitagliptin (SIT) 100, 60, 40 µg/mL, respectively using 360 µg/mL of SIT as a divisor.

If we subtract this constants in plateau above 275 nm, then

multiply the new curve obtained after subtraction by Y' (the divisor), therefore we can obtain the original zero order spectrum of SM (Z) in the mixture, Figure 5. The obtained curves were used for direct determination of SM at 237.5 nm and calculation of the concentration SM in the mixture from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of SIT at 237.5 nm against the corresponding concentrations).

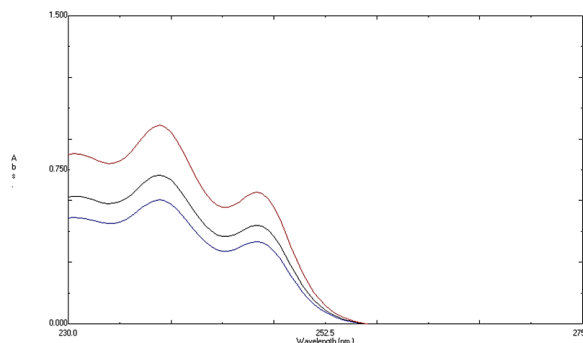


Figure 5. Zero order absorption spectra Simvastatin (SM) 10, 12, 16 µg/mL after subtraction of the constants and multiplication by the spectrum of 360 µg/mL of SIT.

The determination of SIT (Y) could be done by the extended ratio subtraction by dividing these obtained spectrum of SM by carefully chosen standard spectrum of SM (18 µg/mL) producing ratio spectrum represent the constant SM/SM in plateau (230-240 nm), Figure 6. The previously scanned zero order absorption spectrum of the laboratory-prepared mixture (SM and SIT), dividing by the standard SM (Z') as a divisor producing a new ratio spectrum that represent SIT/SM + constant, then by subtraction of the obtained constant SM/SM followed by multiplication of the obtained spectrum by the standard SM (Z') (the divisor). Finally, the original spectrum of (SIT) Y could be obtained which are used for direct determination of SIT at 267 nm, Figure 7 and calculation of the concentration SIT in the mixture from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of SIT at 267 nm against the corresponding concentrations).

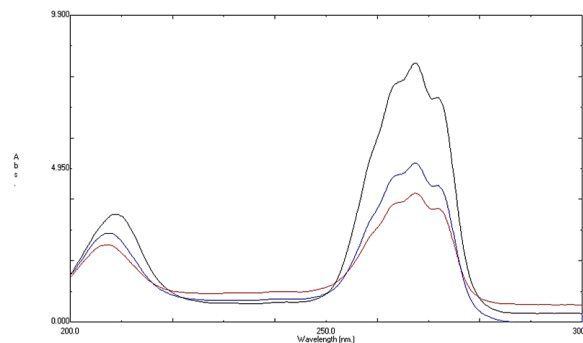


Figure 6. Division spectra of laboratory prepared mixture of Simvastatin (SM) 10, 12, 16 µg/mL and Sitagliptin (SIT) 100, 60, 40 µg/mL, respectively using 18 µg/mL of SM as a divisor.

The EXRSM has the advantage that the two drugs in the mixture could be determined at their λ_{max} in contrary to the previously established ratio subtraction method [22] in which only the spectrally un-extended drug could be determined. This method is valid for the analysis of binary and ternary mixtures with extended spectra.

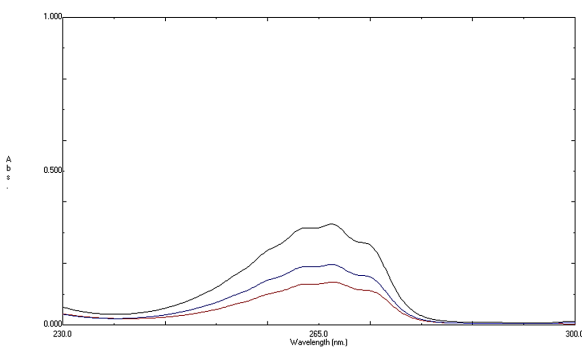


Figure 7. Zero order absorption spectrum of Sitagliptin (SIT) 40, 60, 100 µg/mL after subtraction of the constants and multiplication by the spectrum of 18 µg/mL of SM.

4.1.2. Ratio difference spectrophotometric method (RDSM)

The absorption spectra of SM (Z) and SIT (Y) show severe overlapping that prevents the use of direct spectrophotometry for the analysis of either SIT or SM without preliminary separation, [Figure 3](#). The absorption spectrum of the mixture is scanned and divided by the standard absorption spectrum of one of its components, and the ratio spectrum is then obtained which represents

$$\frac{SM}{SIT'} + \text{constant} \text{ or } \frac{SIT}{SM'} + \text{constant} \quad (9)$$

This was applied to solve the problem of the overlapped absorption spectra of the cited drugs using the difference in the amplitudes of the ratio spectra

$$\left(\frac{SM}{SIT'}\right)_1 - \left(\frac{SM}{SIT'}\right)_2 \text{ or } \left(\frac{SIT'}{SM}\right)_1 - \left(\frac{SIT'}{SM}\right)_2 \quad (10)$$

where, the interfering substance was cancelled and subsequently shows no interference. The method was suitable for the simultaneous determination of SM and SIT. The interfering substance must have spectral contribution at the two selected wavelengths and the ratio value at the selected wavelengths should be with significant value to minimize the error.

The amplitudes at 237.5 and 245.5 nm were selected for determination of SM (Z) using ratio spectrum of the mixture and SIT (360 µg/mL) as a divisor, [Figure 8](#). Similarly, the two selected wavelengths for the estimation of SIT (Y) using SM (18 µg/mL) as a divisor were 248 nm and 263.5 nm, [Figure 9](#).

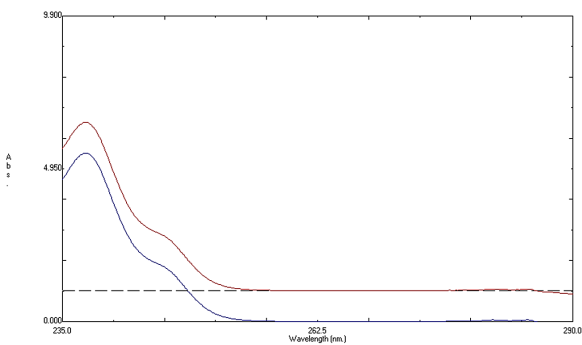


Figure 8. The ratio spectra of 10 µg/mL SM (—), 360 µg/mL SIT (---) and a mixture containing 10 µg/mL SM and 360 µg/mL SIT (—) using a divisor of 360 µg/mL SIT in methanol.

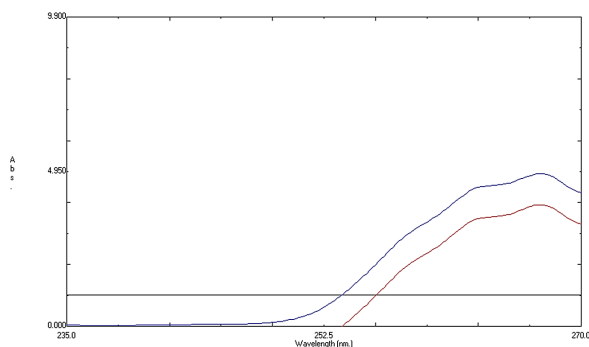


Figure 9. The ratio spectra of 60 µg/mL SIT (—), 18 µg/mL SM (---) and a mixture containing 60 µg/mL SIT and 18 µg/mL SM (—) using a divisor of 18 µg/mL SM in methanol.

4.1.3. Mean centering of the ratio spectra method (MCR)

As shown in [Figure 3](#), the absorption spectra of SM and SIT show spectral overlap. So, the absorption spectra of the standard solutions of the SM with different concentrations were recorded in the wavelength range of 200-300 nm and divided by the normalized spectrum of SIT and the obtained ratio spectra were mean centered ([Figure 10](#)). The concentration of SM was determined by measuring the amplitude at 239 nm corresponding to maxima.

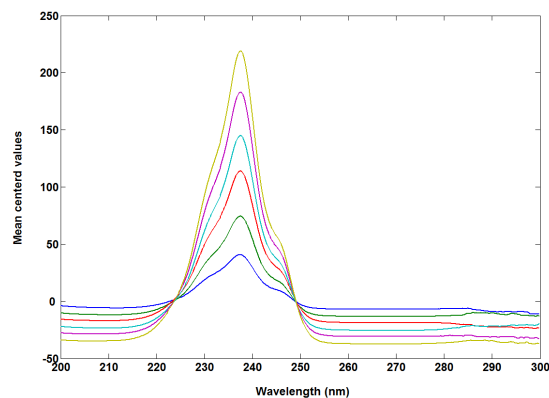


Figure 10. Mean centered ratio spectra of 2-18 µg/mL SM using normalized SIT as a divisor.

The absorption spectra of the standard solutions of SIT were recorded in the wavelength range of 200-300 nm and divided by the normalized spectrum of SM and the obtained ratio spectra were then mean centered ([Figure 11](#)). The concentration of SIT was determined by measuring the amplitude at 273 nm corresponding to maxima. The effect of divisor concentration on the analytical parameters such as slope, intercept and correlation coefficient of the calibration graphs was also tested. Different concentrations of divisor were used but it was observed that changing the concentration had no significant effect in their linear calibration range and the calculated analytical parameters. Therefore, a normalized spectrum of each of SM and SIT was used as divisor spectrum in the proposed method.

4.2. Multivariate calibration methods

Multivariate calibration methods are very useful in spectral analysis as the simultaneous inclusion of many spectral wavelengths instead of using a single wavelength greatly improves the precision and predictive ability of these methods [30].

Table 1. Validation and regression parameters of determination of Simvastatin (SM) and Sitagliptin (SIT) by the proposed methods*.

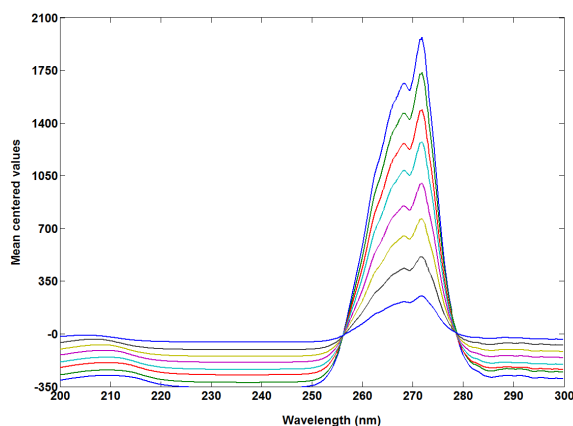
Parameter	EXRSM		RDSM		MCR		Chemometric methods			
	SM	SIT	SM	SIT	SM	SIT	PCR		PLS	
							SM	SIT	SM	SIT
Range ($\mu\text{g/mL}$)	2-18	40-240	2-18	20-120	2-18	40-320	8-16	40-120	8-16	40-120
Slope	0.0601	0.0035	0.3599	0.0369	13.088	9.1388	0.9833	0.9759	0.9833	0.9756
SE of slope	0.1321	2.9954	0.0326	0.07363	0.00087	0.00093	0.0174	0.0201	0.01741	0.0198
Intercept	0.0059	0.0030	0.0846	0.0093	6.8961	48.861	0.075	0.8454	0.0752	0.8453
SE of intercept	0.0901	1.4753	0.1343	0.2123	0.1297	1.1751	0.2169	1.8021	0.2167	1.777
R	0.9998	0.9997	0.9995	0.9999	0.9993	0.9997	0.9991	0.9996	0.9990	0.9994
Accuracy	100.03	100.13	99.98	100.07	99.67	99.34	99.99	101.11	99.97	101.10
	± 0.222	± 0.135	± 0.468	± 0.743	± 0.525	± 0.874	± 0.435	± 0.682	± 0.654	± 0.678
Precision	0.564	0.432	0.687	0.598	0.984	0.785	0.763	0.853	0.541	0.874
LOD	0.0765	0.432	0.0987	0.498	0.0398	0.7419	0.4377	4.245	0.437	4.19
LOQ	0.232	1.309	0.299	1.509	0.1207	2.2469	1.3265	12.863	1.326	12.697
RMSEP	-	-	-	-	-	-	0.18342	1.8021	0.18335	1.8188

* EXRSM: Extended ratio subtraction method, RDSM: Ratio difference spectrophotometric method, MCR: Mean centering ratio spectrophotometric method, PCR: Principal component regression, PLS: Partial least squares.

Table 2. Determination of simvastatin (SM) and sitagliptin (SIT) in laboratory prepared mixtures and tablets by the proposed methods and the results obtained by applying standard addition technique*.

Sample	EXRSM		RDSM		MCR		Chemometric methods			
	SM	SIT	SM	SIT	SM	SIT	PCR		PLS	
							SM	SIT	SM	SIT
Lab. mixture	99.98	99.99	100.01	99.97	100.34	99.98	101.67	99.34	101.66	99.29
	± 0.325	± 0.215	± 0.216	± 0.633	± 0.654	± 0.987	± 0.934	± 0.657	± 0.560	± 0.654
Juvisync tablet	100.26	100.28	99.90	100.48	99.65	99.49	99.90	101.17	99.91	101.04
labeled to contain 20 mg SM and 128.5 mg SIT phosphate equivalent to 100 mg sitagliptin base/tablet (B.N. G011008)	± 0.493	± 0.404	± 0.087	± 0.299	± 0.277	± 0.263	± 0.089	± 0.364	± 0.328	± 0.280
Standard addition	100.05	99.27	100.34	100.03	98.34	98.99	100.23	98.99	100.21	99.01
	± 0.376	± 0.588	± 0.717	± 0.647	± 0.798	± 0.923	± 0.343	± 0.608	± 0.344	± 0.723

* EXRSM: Extended ratio subtraction method, RDSM: Ratio difference spectrophotometric method, MCR: Mean centering ratio spectrophotometric method, PCR: Principal component regression, PLS: Partial least squares, B.N.: Batch number.

**Figure 11.** Mean centered ratio spectra of 40-320 $\mu\text{g/mL}$ SIT using normalized SM as a divisor.

For spectral resolution of SM and SIT, two different regression models were constructed and used for the determination of SM and SIT in their pure forms, laboratory prepared mixtures and in pharmaceutical preparation. These multivariate methods are PCR and PLS.

4.2.1. Experimental design of the calibration and validation sets

Brereton [29] constructed multilevel-multifactor design in which, the levels (L) are the concentrations used and the number of experiments is L^2 . For the calibration and validation sets, different laboratory prepared mixtures of SM and SIT were prepared. The concentration range for SM and SIT are 8-16 and 40-120 $\mu\text{g/mL}$, respectively. The spectra of the prepared mixtures were recorded in the range of 200-300 nm

and the spectral data acquisition was taken with 0.1 nm intervals, thus producing 501 data points per spectrum, thus the produced spectral data matrix has 25 rows representing different samples and 501 columns representing wavelengths (25 \times 501). Seventeen samples were chosen and used for calibration and eight were used for external validation

4.2.2. PCR and PLS models

In order to apply PCR and PLS to the data, the raw data of the calibration samples were mean centered [31] as a preprocessing step and random subsets was applied as an internal cross validation method [32]. To choose the optimum number of significant latent variables, F statistics [33] was applied.

Table 3. Statistical analysis of the proposed methods and the reported spectroscopic methods [6,12] for simvastatin (SM) and sitagliptin (SIT) in their pure powdered forms and their dosage form*.

Parameter	EXRSM		RDSM		MCR		Chemometric methods			
	SM	SIT	SM	SIT	SM	SIT	PCR		PLS	
	SM	SIT	SM	SIT	SM	SIT	SM	SIT	SM	SIT
<i>Pure powders</i>										
Mean	100.09	100.22	100.46	99.98	100.33	99.69	98.97	98.68	98.97	98.64
Variance	2.411	1.750	2.062	0.399	1.386	2.183	1.010	2.145	1.011	2.063
n	8	8	8	8	8	8	8	8	8	8
t-test	0.7878 (2.228)	0.744 (2.2009)	0.735 (2.2009)	0.901 (2.228)	0.131 (2.2009)	0.5506 (2.2009)	2.080 (2.2009)	2.1409 (2.2009)	2.078 (2.2009)	2.189 (2.2009)
F-test	4.6127 (4.876)	3.109 (4.876)	3.944 (4.876)	1.409 (5.050)	2.653 (4.876)	3.879 (4.876)	1.933 (4.876)	3.813 (4.876)	1.932 (4.876)	3.666 (4.876)
<i>Dosage form</i>										
Mean	100.26	100.28	99.90	100.48	99.65	99.49	99.90	101.17	99.71	101.04
Variance	0.243	0.163	0.007	0.089	0.077	0.069	0.008	0.132	0.108	0.078
n	5	5	5	5	5	5	5	5	5	5
t-test (2.571)	2.122	1.472	1.481	2.191	0.677	0.876	1.495	2.139	0.278	1.945
F-test (6.388)	5.926	2.703	5.434	4.932	1.871	6.362	5.148	3.334	2.625	5.625

* EXRSM: Extended ratio subtraction method, RDSM: Ratio difference spectrophotometric method, MCR: Mean centering ratio spectrophotometric method, PCR: Principal component regression, PLS: Partial least squares, n: Number of experiments.

Table 4. One way ANOVA testing for the proposed methods used for the determination of simvastatin (SM) and sitagliptin (SIT).

Source of variation	SS	df	MS	F	p-value	F-crit
SM						
Between Groups	18.24850152	5	3.6497003	2.008816	0.097609	2.443429
Within Groups	74.49051575	41	1.8168418			
Total	92.73901727	46				
SIT						
Between Groups	18.96108818	5	3.7922176	2.331831	0.060948	2.462548
Within Groups	61.79875336	38	1.626283			
Total	80.75984155	43				

* SS: Sum of squares, df: Degree of freedom, MS: Means of squares, F: Calculated F value, F-crit: Tabulated F value, p-value: Probability.

After the PCR and PLS models have been constructed, it was found that the optimum number of LVs described by the developed models was two factors for PCR and PLS.

Calibration graphs were constructed by plotting the predicted concentrations for each compound by each of the developed models versus the true concentrations. The statistical parameters of the linear relationship between the calculated and the true concentration of SM and SIT in the calibration set are represented in Table 1.

In order to assess the predictive ability of each of the developed models, it was applied on an external validation set for determination of the two components. The recoveries, mean recoveries, standard deviation, relative standard deviation and RMSEP values are summarized in Table 2. It is clear from the obtained results that the two models are of equal efficacy and described by three factors; both models were successfully applied for the determination of SM and SIT in pharmaceutical dosage form.

The proposed univariate and multivariate methods were successfully applied for the determination of SM and SIT in laboratory prepared mixtures and in tablets with good recovery as shown in Table 3. The results obtained by those methods statistically compared by each other and by those obtained upon applying the reported methods [6,12] and no significant difference has been observed regarding to both accuracy and precision, Table 4.

4.3. Method validation

Validation was done according to ICH recommendations [34].

4.3.1. Linearity

The linearity of the methods was evaluated by analyzing 6 concentrations of SM and concentrations of SIT between 2-18 µg/mL and 20-360 µg/mL respectively. Each concentration was repeated three times. The assay was performed according to the experimental conditions previously mentioned. The linear

equations were summarized in Table 1.

4.3.2. Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of SM and SIT. The concentrations were obtained from the corresponding regression equations. From which the percentage recoveries suggested good accuracy of the proposed methods were calculated with mean percentage recovery shown in (Table 1).

4.3.3. Range

The calibration range was established through considerations of the practical range necessary according to adherence to Beer's law and the concentration of SM and SIT present in the pharmaceutical preparations to give accurate precise and linear results (Table 1).

4.3.4. Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of SM and SIT within the linearity range. Satisfactory results were shown in Table 3.

4.3.5. Precision

4.3.5.1. Repeatability

Three concentrations of SM (6, 12, 18 µg/mL) and SIT (40, 60, 120 µg/mL) were analyzed three times intra-daily using the proposed methods. The relative standard deviations were calculated (Table 1).

4.3.5.2. Intermediate precision

The previous procedures were repeated inter-daily on three different days for the analysis of the three chosen

concentrations. The relative standard deviations were calculated (Table 1).

4.3.6. Application of the method in assay of tablets

The proposed UV methods were applied for the determination of SM and SIT in their combined pharmaceutical formulation and the results are shown in (Table 2). The good recoveries confirm the suitability of the proposed methods for the routine determination of these components in combined formulation.

4.3.7. Statistical Analysis

Results obtained by the proposed procedures for the determination of SM and SIT in pure form and in pharmaceutical dosage form are statistically compared to those obtained by the reported methods [6,12]. The results showed no significant differences between them (Table 4).

5. Conclusion

It could be concluded that the proposed procedures are simple, do not require sophisticated techniques or instruments. They are also sensitive and selective and could be used for routine analysis of SM and SIT in their available dosage form without prior separation. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

References

- [1]. URL: <http://en.wikipedia.org/wiki/simvastatin>
- [2]. URL: <http://www.drugbank.ca/drugs/DB00641>
- [3]. URL: <http://en.wikipedia.org/wiki/sitagliptin>
- [4]. URL: <http://www.drugbank.ca/drugs/DB01261>
- [5]. Singla, V.; Bhaskar, R.; Bhaskar, R. *Rasayan J. Chem.* **2010**, *3(3)*, 507-513.
- [6]. Lei, W.; Mandana, A. *J. Pharmaceut. Biomed.* **1999**, *2*, 202-211.
- [7]. Anantha, K. D.; Sujana, D. P.; Vijayasree, V.; Seshagiri, R. *J. E-J. Chem.* **2009**, *2*, 541-544.
- [8]. Stephen, R. B.; Rajamanickam, V.; Joseph, M. F. P.; Ashik, E. A. L.; Rajveer, C.; Kumaraswamy, D. *J. Adv. Pharm. Res.* **2010**, *1*, 133-139.
- [9]. Nilesh, J.; Ruchi, J.; Hemant, S.; Deepak, K. *J. Asian J. Res. Chem.* **2008**, *1*, 29-31.
- [10]. Nagaraju, P.; Vishnu, V. Z. *J. Glob. Pharm. Technol.* **2010**, *4*, 113-117.
- [11]. Naotaka, U.; Kazuhide, I.; Shunsuke, H. *J. Chromatogr. B* **1997**, *694*, 211-217.
- [12]. Carlucci, G.; Mazzeo, P.; Biordi, L.; Bologna, M. *J. Pharmaceut. Biomed.* **1992**, *10*, 693-697.
- [13]. Sekaran, B. C.; Rani, P. A. *Eclat. Quim. Sao Paulo* **2010**, *35*, 45-53.
- [14]. Xiang-Fei, J.; De-Wei, S.; Ye, C.; Xin-Gang, L.; Xiao-Meng, W.; Tian-Yan, Z.; Wei, L. *J. Chin. Pharm. Sci.* **2010**, *20*, 63-69.
- [15]. Shyamala, M.; Mohideen, S.; Satyanarayana, T.; Narasimha, R.; Suresh, K. P.; Swetha K. *Am. J. Pharm. Tech. Res.* **2011**, *1*, 93-101.
- [16]. El-Bagary, R.; El-kady, E.; Ayoub, B. *Talanta* **2011**, *85*, 673-680.
- [17]. El-Bagary, R.; El-kady, E.; Ayoub, B. *J. Int. Biomed. Sci.* **2011**, *7*, 62-69.
- [18]. Ankur, K.; Sheetal, S. *Int. J. Pharm.* **2012**, *2*, 609-612.
- [19]. Abdel-Gawad, S.; El-Sherif, Z. *Eur. J. Chem.* **2012**, *3(4)*, 447-454.
- [20]. Burugula, L.; Mullangi, R.; Pilli, N. R.; Makula, A.; Lodagala, D. S.; Kandhagatla, R. *Biomed. Chromatogr.* **2012**, *5(2)*, 102-111.
- [21]. Gebremariam, K.; Gowris, S. *Inv. Rapid Pharm. Anal. Qual. Assur.* **2012**, *6*, 621-631.
- [22]. El-Bardicy, M. G.; Lotfy, H. M.; El-Sayed, M. A.; El-Tarras, M. F. *J. AOAC Int.* **2008**, *91*, 299-310.
- [23]. Lotfy, H. M.; Hegazy, M. A. *Spectrochim Acta A* **2012**, *96*, 259-270.
- [24]. Bahram, M. *Anal. Chim. Acta* **2007**, *603*, 13-19.
- [25]. Afkhami, A.; Bahram, M. *Anal. Chim. Acta* **2004**, *526*, 211-218.
- [26]. Lotfy, H. M.; Aboul-Alamein, A. M.; Hegazy, M. A. *J. AOAC Int.* **2010**, *39*, 1844-1855.
- [27]. Matlab ver. 5.3., Mathworks Inc., Natick, Massachusetts, United States, 1999.
- [28]. Wise B. M.; Gallagher N. B.; Version 2.1.1, PLS Toolbox, Eigenvector Research, Inc. Manson, USA, 2001.
- [29]. Brereton R. *Analyst* **1997**, *122*, 1521-1529.
- [30]. Chemo, Y.; Gong, X. *Anal. Chim. Acta* **1997**, *354*, 163-171.
- [31]. Pharm-Gia, T.; Duong, Q. P. *Math. Comput. Model* **1989**, *12*, 1613-1621.
- [32]. Brereton, R. *Analyst* **2000**, *125*, 2125-2154.
- [33]. Haaland, D. M.; Thomas, E. V. *Anal. Chem.* **1988**, *60*, 1193-1202.

- [34]. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, 62, US FDA Federal Register, 1997.