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Development and validation of spectrophotometric methods for simultaneous determination of sitagliptin and simvastatin in binary mixture

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ARTICLE INFORMATION

ABSTRACT

Simple, selective and precise spectrophotometric methods were adopted for simultaneous determination of sitagliptin (SIT) and simvastatin (SIM) in new co-formulated pharmaceutical dosage form. In the first method, SIT was determined by measuring its zero order absorbance at 266.4 nm in the range of 40-360 µg/mL in the presence of up to 70% of SIM. While, the two cited drugs were determined simultaneously using third derivative method by measuring the sum of peak amplitudes (peak & valley) at 275.3-280.3 nm and 240.5-244.7 nm in the ranges of 40-360 µg/mL and 2-18 µg/mL for SIT and SIM, respectively. In the second method, the first derivative of ratio spectra method was applied by measuring the peak height at 255.9 and 275.2 nm using 18 µg/mL SIM as devisor over a concentration range of 40-360 µg/mL of SIT and at 228.3, 240.5 and 248 nm using 100 µg/mL of SIT as divisor over a concentration range 2-18 µg/mL SIM. In the third method the ratio subtraction spectrophotometric method was used, where SIM can be determined by dividing the spectra of the mixtures by the spectrum of SIT (40 µg/mL) followed by subtracting the constant absorbance value of the plateau, then finally multiply the produced spectrum by the spectrum of the devisor. Laboratory prepared mixtures were successfully tried for the three compositions of tablets (10, 20 and 40 mg of SIM) with 100 mg of SIT. The developed methods were validated as per International Conference of Harmonization guidelines.

1. Introduction

Sitagliptin (SIT), (*R*)-4-oxo-4-[3-(trifluoromethyl)-5, dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine, 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) (Figure 1). This increases active incretin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion [1,2].

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

Recently, U.S. Food and Drug Administration (FDA) [5] has approved a fixed-dose combination tablet consisting of sitagliptin and simvastatin. This is the first product to combine a type 2 diabetes drug with a cholesterol lowering drug in one tablet.

Many techniques like UV-visible spectrophotometry [6,7], HPLC [8-13] and flourimetry [14] have been reported for the

determination of SIT alone or in presence of the combination with other drugs. On the other hand, SIM could be determined either alone or in presence of its metabolites or in combination with other drugs using different techniques like UV-visible spectrophotometry [15-17], HPLC [18-24] and LC/MS/MS [25-28].

(a)

(b)
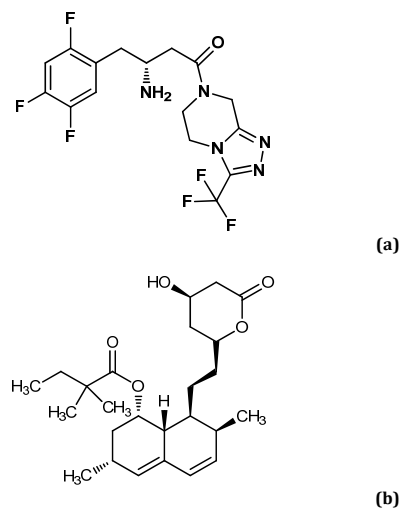
Figure 1. Chemical structure of (a) Sitagliptin ($C_{16}H_{15}F_6N_5O$) and (b) Simvastatin ($C_{25}H_{38}O_5$).

For the new co-formulated dosage form, a few methods were published for the determination of both drugs when present in combination. These methods comprise the use of simultaneous equation spectrophotometric method [29], RP-HPLC [30,31] and in human plasma by LC-MS/MS and its application to a human pharmacokinetic [32].

The goal of the present work is to develop validated, simple, accurate, precise, economic spectrophotometric methods due to its wide availability in most quality control laboratories for the simultaneous quantification of the combined diabetes with cholesterol lowering drug tablet.

2. Experimental

2.1. Instrumentation

A double beam UV-vis spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with matched quartz cell (1 cm path length) connected to IBM compatible computer and HP 680 inkjet printer (Hewlett Packard, USA). The bundled UVPC personal spectroscopy software version 3.7 was used; at a spectral bandwidth 2 nm and scanning speed of 2800 nm/min.

2.2. Chemicals and materials

Pure simvastatin and sitagliptin phosphate monohydrate were kindly supplied by Merck Sharp & Dohme International, USA. The marketed formulation studied was Juvisync™ tablets manufactured by Merck Sharp Dohme International, USA BN^O G011008 each tablet contains: 128.5 mg sitagliptin phosphate monohydrate equivalent to 100 mg sitagliptin free base and 20 mg simvastatin. Distilled water from "Aquatron" Automotive water Still A 4000 (Bibby Sterillin Ltd., Staffordshire, UK). Methanol from E. Merck, Darmstadt, Germany. Methanol spectroscopy grade is purchased from El-NASR Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo, Egypt.

2.3. Standard Solutions

Sitagliptin phosphate monohydrate standard solution (1 mg/mL) was prepared by dissolving 100 mg of the pure drug in 30 mL of 70% methanol into 100 mL measuring flask with continuous shaking for about 10 minutes. The volume was completed to the mark with the corresponding solvent. Simvastatin standard solution (0.1 mg/mL) was prepared by dissolving 10 mg of the pure base in the same solvent by the same manner to get the desired final concentration.

2.4. Procedures

2.4.1. Linearity

Aliquots of standard solutions of SIM (0.1 mg/mL) and SIT (1 mg/mL) equivalent to 20-180 μg and 0.4-3.6 mg in 70% methanol were accurately and separately transferred into a series of 10 mL volumetric flasks and the volume of each was completed to the mark with the same solvent.

For determination of SIT by the zero order method, the absorbencies of SIT were measured at 266.4 nm. For simultaneous determination of both drugs by the third derivative method, the peak amplitudes (peak & valley) were measured at 275.3-280.3 nm and 240.5-244.7 nm for SIT and SIM, respectively.

For simultaneous determination of both drugs by the first derivative of ratio spectra method, the peak heights were measured at 255.9 and 275.2 nm using 18 μg/mL SIM as devisor to determine SIT in a concentration range of 40-360 μg/mL and at 228.3, 240.5 and 248.0 nm (peak amplitude) using 100 μg/mL SIT as divisor to determine SIM in a concentration range 2-18 μg/mL SIM.

For determination of SIM by the ratio subtraction method, SIM can be determined by dividing the spectra of the mixtures of both drugs by the spectrum of SIT (40 μg/mL) followed by subtracting the constant absorbance value of the plateau and finally multiply the produced spectrum by the spectrum of the devisor.

2.4.2. Accuracy

Accuracy was assured by carrying out the previously mentioned procedures under linearity for the determination of different concentrations of pure SIT and SIM. The concentrations were calculated from the corresponding regression equations.

2.4.3. Precision

2.4.3.1. Intraday precision (Repeatability)

Three concentrations of each drug were analyzed three times intraday using the previously mentioned procedures. The percentage recoveries of each drug and its relative standard deviation were calculated using the suggested methods.

2.4.3.2. Intermediate precision

Three concentrations of each drug were analyzed on three successive days using the procedure stated under linearity. The percentage recoveries of each drug and its relative standard deviation were calculated using the suggested method.

2.4.4. Analysis of marketed formulation

Ten tablets (Juvisync™ tablets) were weighed and finely powdered. An amount of powdered equivalent to 64.25 mg SIT phosphate monohydrate and 10 mg SIM was transferred into a 100 mL round bottom flask; 30 mL 70% methanol were added and stirred for 30 minutes then filtered through 0.5 μm whatman paper into 100 mL measuring flask. The residue was washed with 2 x 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.

3. Results and discussion

A fixed-dose of sitagliptin and simvastatin in their new co-formulated pharmaceutical dosage form [5] is the first product to combine a type 2 diabetes drug with a cholesterol lowering drug in one tablet. The aim of this work is to develop simple and accurate methods for the simultaneous determination of the new combination of SIT and SIM in tablets. Molecular absorption spectroscopy has been extensively used for the determination of drugs in pharmaceutical preparation with a view to the development of analytical methods. The use of this technique for pharmaceutical analyses has the inherent constraint that most active drugs absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination.

The zero-order absorption spectra (D^0) of a mixture of SIT and SIM at the ratio of their presence in tablets showed overlapping (Figure 2) which allows the analysis of SIT in presence of SIM at 266.4 nm, but prevents the analysis of SIM. As SIT is soluble in water and slightly soluble in methanol while, SIM is insoluble in water and freely soluble in methanol trails were made to dissolve the mixture of the two drugs in methanol:water mixture. Different ratios of methanol and

water were tried and 70% were chosen; which fulfills complete solubility. A calibration curve is constructed relating the absorbance of zero order spectra of SIT at 266.4 nm to the corresponding concentrations where SIM shows no absorbance, the regression equation is computed.

$$A_{SIT} = 0.0033C + 0.0237 \quad r^2 = 0.9997 \quad (1)$$

where C is the concentration of SIT in μg/mL, A_{SIT} is the peak amplitude of the zero order spectrum of SIT at 266.4 nm, and r is the correlation coefficient.
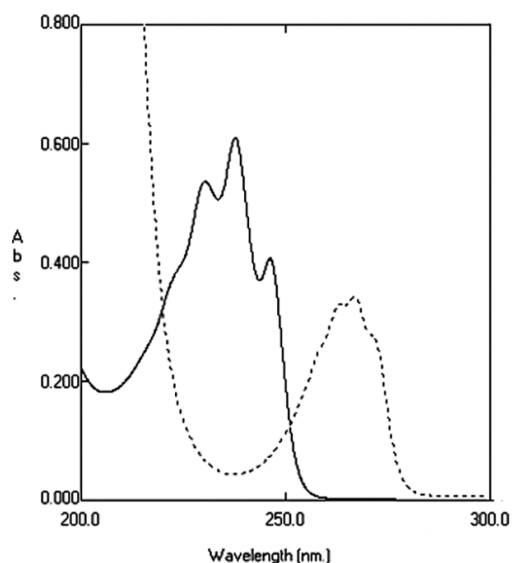

Figure 2. Zero order absorption spectra of 10 μg/mL simvastatin (—) and 100 μg/mL sitagliptin (……).

3.1. Derivative spectrophotometric method (³D)

For further improvement of the selectivity to resolve the overlap present between SIT and SIM in the mixture, a simple third derivative method (³D) [33-38] is applied. The method is based on measuring the sum of peak amplitudes (peak & valley) at 275.3-280.3 nm and 240.5-244.7 nm (Figure 3) for SIT and SIM, respectively.
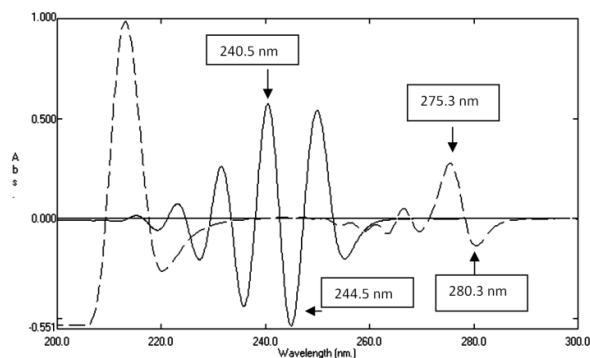

Figure 3. Third derivative spectra of 10 μg/mL simvastatin (—) and 100 μg/mL sitagliptin (——).

The main instrumental parameters that affect the shape of the derivative spectra such as the speed, the wavelength increment over which the derivative is obtained (Δλ) and degree of smoothing were optimized to give a well resolved peak. Linearity relationship was obtained between the peak amplitudes and the concentration over the range of 40-360

μg/mL and 2-18 μg/mL for SIT and SIM, respectively (Figure 4 and 5), from which the linear regression equation were computed and found to be:

$$^3D_{SIM} = 0.1156 - 0.0217 \quad r^2 = 0.9994 \text{ for SIM} \quad (2)$$

$$^3D_{SIT} = 0.0039 + 0.0279 \quad r^2 = 0.9995 \text{ for SIT} \quad (3)$$

where ³D is the sum of peak amplitudes (peak & valley) of the spectra, C is the corresponding concentration and r is the correlation coefficient.
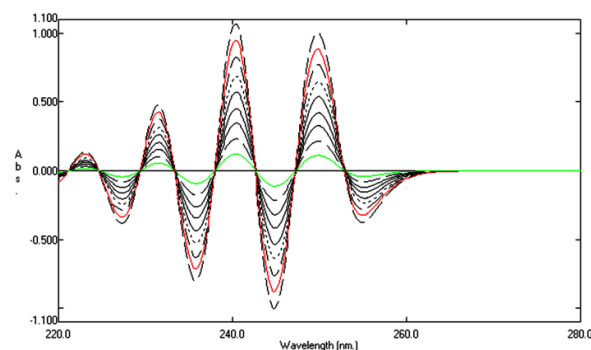

Figure 4. Third derivative spectra of simvastatin in the range of 2-18 μg/mL.

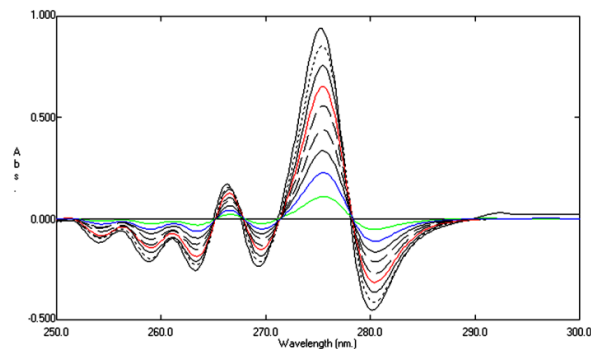**Figure 5.** Third derivative spectra of sitagliptin in the range of 40-360 μg/mL.

3.2. Ratio-spectra derivative spectrophotometric method (DD₁)

As can be seen in Figure 2, the absorption spectra of SIT and SIM; the maximum wavelengths of the two compounds are close to each other and their spectra overlap at 200-260 nm; which can't permits the determination of the cited drugs. Therefore, the simultaneous determination of SIT and SIM is impossible by classical spectrophotometry and it is necessary to use another method to solve this problem.

Salinas *et al.* [39] designed a spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals. Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interfere the assay. In this method the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is

obtained. The concentration of the other component is then determined from a calibration graph.

The main parameters that affect the shape of the ratio spectra which are wavelength, scanning speed, the concentration of the standard solution used as a divisor, the wavelength increment over which the derivative is obtained and the smoothing function are carefully tested. Accordingly, the first derivative of the ratio spectra presented in Figure 6 and 7 for sitagliptin and simvastatin in the different concentration may provide a good proof for this understanding. Different concentrations of divisor were also tried for SIT and SIM which give the best regarding average recovery percent when they were used for the prediction of SIT and SIM concentrations in bulk powder as well as in laboratory prepared mixtures.
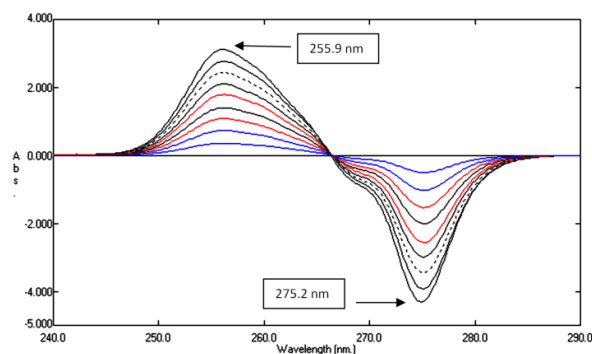

Figure 6. DD[1]-Spectra of sitagliptin in the range of 40-360 μg/mL using 18 μg/mL simvastatin as divisor.
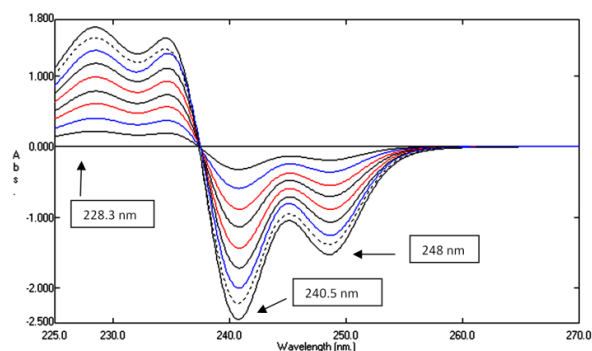

Figure 7. DD[1]-Spectra of simvastatin in the range of 2-18 μg/mL using 100 μg/mL sitagliptin as divisor.

The method was applied by measuring the peak height at 255.9 and 275.2 nm using 18 μg/mL SIM as devisor over a concentration range of 40-360 μg/mL of SIT (Figure 6) and at 228.3, 240.5 and 248.0 nm using 100 μg/mL of SIT as divisor over a concentration range of 2-18 μg/mL SIM (Figure 7). The linear regression equations are found to be:

$$P_{SIT} = 0.0086C + 0.053 \ r^2 = 0.9995 \tag{4}$$

$$P_{SIM} = 0.0120C - 0.0841 \ r^2 = 0.9994 \tag{5}$$

where, C is the concentration in μg/mL, P is the peak amplitude of the first derivative of the ratio spectrum curve and r is the correlation coefficient.

3.3. Ratio subtraction method

The ratio subtraction technique [40] depends on that, if you have a mixture of two drugs X and Y of overlapping spectra, you

can determine X by dividing the spectrum of the mixture by known concentration of Y as a divisor (Y'). The division will give a new curve that represents (X/Y) + Constant. If we subtract this constant, then multiply the new curve obtained after subtraction by Y' (the divisor), therefore we can obtain the curve of X again. This can be summarized as the following:

$$\frac{X+Y}{Y\prime} = \frac{X}{Y\prime} + \frac{y}{Y\prime} = \frac{X}{Y\prime} + \text{Constant} \tag{6}$$

$$\frac{X}{Y\prime} + \text{Constant} - \text{Constant} = \frac{X}{Y\prime} \tag{7}$$

$$\frac{X}{Y\prime} \cdot Y' = X \tag{8}$$

This constant can be determined directly from the curve by the straight line which is parallel to λ axis in this region. Practically, the ratio subtraction method starts by scanning zero- order spectra of the prepared standard solutions of SIM in 70% methanol (Figure 2), then the linearity is checked between absorbance at the selected wavelength at 237.5 nm and the corresponding concentration of SIM. The method depends on that, when a mixture of SIM (X) and SIT (Y); where the spectrum of (Y) is more extended (Figure 2), the determination of (X) could be done by scanning the zero order absorption spectra of the laboratory-prepared mixtures (SIM and SIT), dividing them by carefully chosen concentration (40 μg/mL) of standard SIT (Y = divisor) producing a new ratio spectra that represent (X/Y) + constant as shown in Figure 8 then subtraction of the absorbance values of these constants (Y/ Y') in plateau as shown in Figure 9, followed by multiplication of the obtained spectra by (Y') the divisor as shown in Figure 10.
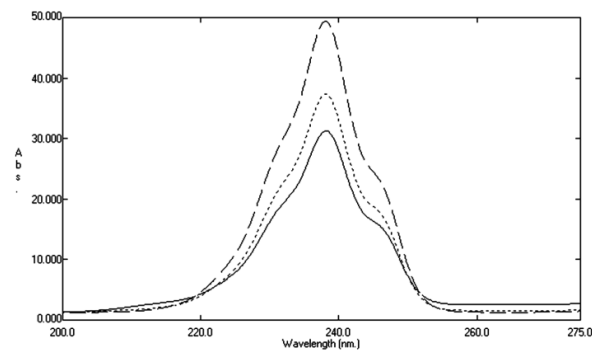
Figure 8. Absorption spectra of laboratory prepared mixtures of sitagliptin and simvastatin in the ratio of 100:10 (—), 60:12 (….) and 40:16 (— —).

Finally, the original spectra of SIM (X) could be obtained which are used for direct determination of SIM at 237.5 nm and calculation of the concentration from the corresponding regression equation. A linear correlation is obtained between the absorbance and the corresponding concentration of SIM at 237.5 nm. The regression equation is:

$$P_{SIM} = 0.059C + 0.0217 \ r^2 = 0.9999 \tag{9}$$

where C is the concentration of SIM in μg/mL, P_{SIM} is the peak amplitude of the zero order spectrum of SIM at 237.5 nm, and r is the correlation coefficient. On the other hand for determination of SIT alone a calibration curve is constructed relating the absorbance of zero order spectra of SIT at 266.4 nm to the corresponding concentrations where SIM shows no absorbance.

Table 1. Determination of simvastatin and sitagliptin phosphate monohydrate in laboratory prepared mixtures by the proposed methods.

Claimed Ratio (μg/mL)	Simvastatin									
	Ratio Subtraction		D³-Method		DD¹-Method					
	237.5 nm		240.5-244.7 nm		228.3 nm		240.5 nm		248 nm	
	Found*	Recovery	Found*	Recovery	Found*	Recovery	Found*	Recovery	Found*	Recovery
10:128.5	9.96	99.60	10.02	100.20	10.08	100.80	9.95	99.50	10.04	100.40
12:77.1	12.04	100.33	11.98	99.83	12.01	100.08	11.94	99.50	12.07	100.58
16:51.4	15.98	99.88	15.86	99.13	16.05	100.31	15.97	99.81	15.99	99.94
Mean ± S.D.	99.94±0.368		99.72±0.543		100.40±0.368		99.60±0.120		100.31±0.330	

* Average of three determinations.

Table 2. Determination of simvastatin and sitagliptin phosphate monohydrate in laboratory prepared mixtures by the proposed methods.

Claimed Ratio (μg/mL)	Sitagliptin							
	D³-Method		DD¹-Method				D⁰-Method	
	275.3-280.3 nm		255.9 nm		275.2 nm		266.4 nm	
	Found*	Recovery	Found*	Recovery	Found*	Recovery	Found*	Recovery
10:128.5	128.40	99.92	128.60	100.08	128.60	100.08	128.29	99.84
12:77.1	77.05	99.94	77.15	100.06	77.04	99.92	77.12	100.03
16:51.4	51.51	100.21	51.35	99.90	51.26	99.79	51.50	100.19
Mean ± S.D.	100.02±0.162		100.01±0.099		99.93±0.145		100.02±0.175	

* Average of three determinations.

Table 3. Determination of simvastatin in pharmaceutical formulation and application of standard addition technique.

| Pharmaceutical Formulation	Simvastatin											
	Taken (μg/mL)	Found * %±S.D.	Standard Addition Technique									
			Pure Added (μg/mL)	Pure Found * (μg/mL)					Recovery (%)				
				Ratio Subtr.	D³	DD¹			Ratio Subtr.	D³	DD¹		
						228.3 nm	240.5 nm	248 nm			228.3 nm	240.5 nm	248 nm
Juvisync™ tablets containing 20 mg Simvastatin and 128.5 mg Sitagliptin phosphate monohydrate equivalent to 100 mg Sitagliptin base BN⁰G011008.	10.00	101.32±0.924	2	1.99	2.01	2.02	1.99	1.98	99.50	100.50	101.00	99.50	99.00
			4	4.03	3.99	3.96	3.99	3.98	100.75	99.75	99.00	99.75	99.50
			6	6.02	5.98	5.97	5.97	5.97	100.33	99.50	99.67	99.50	99.50
			Mean						100.19	99.92	99.89	99.58	99.33
			S.D.						0.636	0.520	1.018	0.144	0.289
			R.S.D.						0.635	0.520	1.019	0.145	0.291

* Average of three determinations.
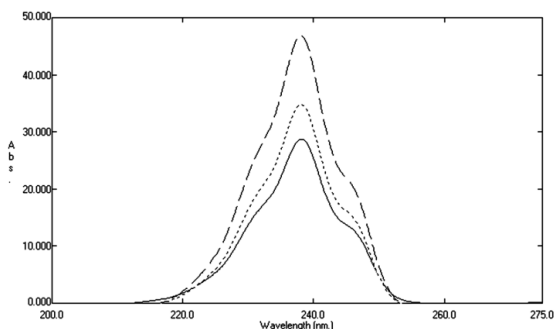
Figure 9. Absorption spectra of laboratory prepared mixtures of sitagliptin and simvastatin in the ratio of 100:10 (—), 60:12 (....) and 40:16 (——) after division on the spectrum of 40 μg/mL sitagliptin and subtraction of the constant value.

The selectivity of the proposed procedures is assessed by the analysis of laboratory prepared mixtures containing different ratios of the two drugs, where satisfactory results are obtained over the calibration ranges as shown in Tables 1 and 2. The proposed procedures are also applied for the determination of SIT and SIM in Juvisync™ tablets. The validity of the proposed procedures is further assessed by applying the standard addition technique (Table 3 and 4). Results obtained by the proposed procedures for the determination of pure samples of SIT and SIM are statistically compared to those obtained by the reference method [16] (D²-method for determination of simvastatin at 243.5 nm).

The results showed no significant differences between the proposed methods and the reported one as presented in Table 5 and 6; the observed good agreement between proposed method and the reference method, The high percentage recoveries (99.33-100.96) and low %R.S.D. (0.291-1.337)

values confirm the suitability of the proposed method for the routine determination of these components in the new combined formulation.
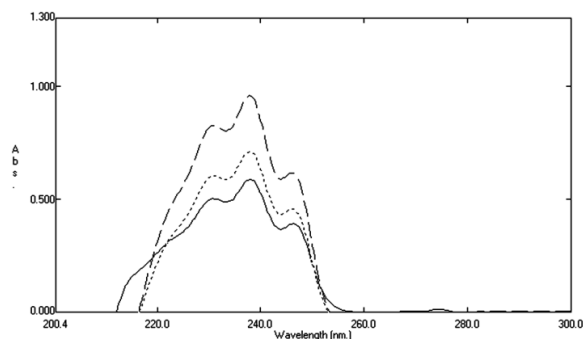
Figure 10. Absorption spectra of laboratory prepared mixtures of sitagliptin and simvastatin in the ratio of 100:10 (—), 60:12 (.....) and 40:16 (——) after division on the spectrum of 40 μg/mL sitagliptin and subtraction of the constant value then multiplication in the spectrum of 40 μg/mL sitagliptin.

3.4. Method validation

Validation was done according to ICH recommendations [41]. Linearity of the methods was evaluated by analyzing different concentrations of SIT and SIM ranging between 40-360 μg/mL and 2-18 μg/mL, respectively (Table 7 and 8). Each concentration was made in triplicate. The assay was performed according to the experimental conditions.

The percentage recovery was calculated for marketed formulation by standard addition of pure drugs at four known concentrations an excellent recovery were obtained at each level.

Table 4. Determination of sitagliptin in pharmaceutical formulation and application of standard addition technique.

Pharmaceutical Formulation	Sitagliptin										
	Taken (µg/mL)	Found* %±S.D.	Standard Addition Technique								
			Pure Added (µg/mL)	Pure Found * (µg/mL)				Recovery (%)			
				D^0	D^3	DD^1 255.9 nm	275.2 nm	D^0	D^3	DD^1 255.9 nm	275.2 nm
Juvisync™ tablets containing 20 mg Simvastatin and 128.5 mg Sitagliptin phosphate monohydrate equivalent to 100 mg Sitagliptin base BN⁰G011008.	64.25	99.18±0.896	40	40.65	40.73	39.75	40.51	101.63	101.83	99.38	101.28
			80	79.21	81.24	78.98	79.96	99.01	101.55	98.73	99.95
			120	121.01	119.40	121.11	119.84	100.84	99.50	100.93	99.87
			Mean					100.49	100.96	99.68	100.37
			S.D.					1.344	1.272	1.130	0.792
			R.S.D.					1.337	1.260	1.134	0.789

* Average of three determinations.

Table 5. Statistical comparison between the proposed methods for the determination of sitagliptin and a reference method.

Parameter	Sitagliptin				Reference Method *
	D^3	DD^1		D^0	
	275.3-280.3 nm	255.9 nm	275.2 nm	266.4 nm	
Mean±S.D.	100.25±0.526	100.39±0.650	100.42±0.547	99.96±0.381	100.12±0.489
R.S.D.	0.525	0.647	0.546	0.381	0.488
n	4	4	4	4	4
Variance	0.277	0.423	0.299	0.145	0.239
F-value ** (9.55)	1.159	1.770	1.251	1.648	-
Student's t-test ** (1.943)	0.362	0.663	0.813	0.516	-

* Reference colorimetric method for the determination of sitagliptin by condensation of its primary amino group with acetyl acetone and formaldehyde then measuring the produced color at 430 nm.
** Values in parenthesis are the theoretical values of t and F at p = 0.05.

Table 6. Statistical comparison between the proposed methods for the determination of simvastatin and a reference method.

Parameter	Simvastatin				Reference Method *
	D^3-Method	DD^1-Method			
	240.5-244.7 nm	228.3 nm	240.5 nm	248 nm	
Mean±S.D.	100.00±0.797	100.24±0.723	100.17±0.466	100.39±0.366	100.27±0.808
R.S.D.	0.797	0.721	0.465	0.365	0.806
n	4	4	4	4	3
Variance	0.635	0.523	0.217	0.134	0.653
F-value ** (9.55)	1.028	1.249	3.009	4.873	-
Student's t-test ** (1.943)	0.443	0.052	0.210	0.270	-

* D^2-method for determination of simvastatin at 243.5 nm.
** Values in parenthesis are the theoretical values of t and F at p = 0.05.

Table 7. Assay validation results of the proposed methods.

Parameter	Simvastatin			
	D^3-Method	DD^1-Method		
	240.5-244.7 nm	228.3 nm	240.5 nm	248 nm
Accuracy	100.00±0.797	100.24±0.723	100.17±0.466	100.39±0.366
Specificity and Selectivity	99.72±0.543	100.40±0.368	99.60±0.120	100.31±0.330
Precision Repeatability *	98.95±0.981	100.96±0.961	101.34±0.694	99.32±0.0.812
Intermediate Precision *	101.35±0.894	99.17±1.013	101.54±0.0.789	100.84±0.0.741
Robustness	99.68±0.541	100.95±0.514	101.07±0.941	101.24±0.514
Linearity, Slope	0.1156	0.0946	0.1404	0.0866
Linearity, Intercept	-0.0217	0.0412	0.0447	0.0357
Linearity, Correlation coefficient (r)	0.9997	0.9997	0.9998	0.9997
Range (µg/mL)	2-18	2-18	2-18	2-18
LOD ** (µg/mL)	1	1	1	1
LOQ ** (µg/mL)	2	2	2	2

* Intra-day and inter-day relative standard deviation of the average of three concentrations of the studied drug.
** LOD and LOQ are obtained experimentally.

Table 8. Assay validation results of the proposed methods.

Parameter	Sitagliptin			
	D^3-Method	DD^1-Method		D^0-Method
	275.3-280.3 nm	255.9 nm	275.2 nm	275.3-280.3 nm
Accuracy	100.25±0.526	100.39±0.650	100.42±0.547	99.96±0.381
Specificity and Selectivity	100.02±0.162	100.01±0.099	99.93±0.145	100.02±0.175
Precision, Repeatability *	99.51±0.1.123	99.42±0. 594	101.91±0.426	102.01±1.094
Intermediate Precision *	100.94±1.184	99.91±0.0.694	99.12±0.0.912	10159±1.278
Robustness	98.99±0.621	99.34±0.895	102.37±1.024	99.63±0.845
Linearity, Slope	0.0039	0.0086	0.012	0.0033
Linearity, Intercept	0.0279	0.053	0.0841	0.0237
Linearity, Correlation coefficient (r)	0.9997	0.9997	0.9998	0.9997
Range (µg/mL)	40-360	40-360	40-360	40-360
LOD ** (µg/mL)	20	20	20	20
LOQ ** (µg/mL)	40	40	40	40

* Intra-day and inter-day relative standard deviation of the average of three concentrations of the studied drug.
** LOD and LOQ are obtained experimentally.

Table 9. Results of accuracy for the simultaneous determination of simvastatin and sitagliptin by the proposed methods.

Claimed Simvastatin (µg/mL)	D³-Method		DD¹-Method					
	240.5-244.7 nm		228.3 nm		240.5 nm		248 nm	
	Found*	Recovery	Found*	Recovery	Found*	Recovery	Found*	Recovery
4	4.03	100.75	4.04	101.00	3.98	99.50	4.03	100.75
8	8.05	100.63	7.96	99.50	8.03	100.38	7.99	99.88
12	11.91	99.25	11.97	99.75	12.03	100.25	12.05	100.42
16	15.90	99.38	16.11	100.69	16.09	100.56	16.08	100.50
Mean ± S.D.	100.00±0.797		100.24±0.723		100.17±0.466		100.39±0.366	

* Average of three determinations.

Table 10. Results of accuracy for the simultaneous determination of simvastatin and sitagliptin by the proposed methods.

Claimed Sitagliptin (µg/mL)	D³-Method		DD¹-Method				D⁰-Method	
	275.3-280.3 nm		255.9 nm		275.2 nm		266.4 nm	
	Found*	Recovery	Found*	Recovery	Found*	Recovery	Found*	Recovery
80	79.85	99.81	80.96	101.20	80.79	80.79	79.69	99.61
160	161.51	100.94	160.84	100.53	161.12	161.12	159.64	99.78
240	240.91	100.38	239.15	99.65	240.68	240.68	239.87	99.95
320	319.58	99.87	320.58	100.18	319.15	319.15	321.56	100.49
Mean ± S.D.	100.25±0.526		100.39±0.650		100.42±.547		99.96±0.381	

* Average of three determinations.

The respective % Recovery and %R.S.D.s for the two drugs are shown in Table 3 and 4 where, the relative standard deviation is <1.5 in the assay of raw materials and tablets by the three proposed method.

Accuracy: The accuracy of the results was checked by applying the proposed methods for determination of different samples of SIT and SIM. The concentrations were obtained from the corresponding regression equations. From which the percentage recoveries were calculated with mean percentage recovery shown in Table 9 and 10. Accuracy of the methods was further assured by the use of the standard addition technique, it was performed by addition of known amounts of pure SIT (40, 80 and 120 µg/mL) and SIM (2, 4 and 6 µg/mL) to known concentrations of the pharmaceutical preparation (64.25 for SIT and 10 µg/mL for SIM) the resulting mixtures were assayed, and the results obtained were compared with the expected results (Table 3 and 4). The good recoveries of standard addition technique suggested good accuracy of the proposed methods.

Selectivity: The selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of SIT and SIM within the linearity range. Satisfactory results (Table 3 and 4); which prove that the proposed methods, in addition to its selectivity to the cited drug no interference from the presence of formulation matrix.

Robustness: Robustness which is the prove that the method is not affected by small deliberated change was tested i.e. by trying to apply the proposed methods using small variation of ratio of the solvent mixture used and the degree of the smoothing of the derivative curves and no effects was observed.

Repeatability: Three concentrations of SIT and SIM were analyzed three times intra-daily using the proposed methods. The percentage recoveries and relative standard deviation were calculated (Table 7 and 8).

Intermediate precision: The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. The percentage recoveries and relative standard deviation were calculated (Table 7 and 8).

3.5. Application of the method in tablets

The proposed methods were applied for the determination of SIT and SIM in their combined pharmaceutical formulation. Laboratory prepared mixtures were successfully tried for the three compositions of tablets (10, 20 and 40 mg of SIM) with 100 mg of SIT, the results are shown in Table 1. The high percentage recoveries (99.60-100.31%) and low %CV (0.099-0.543) values confirm the suitability of the proposed methods for the routine determination of these components in new combined formulation. Moreover, the proposed methods is proved to be much more sensitive than the published HPLC method [30] especially for SIM which is the lower concentration (linearity rang 2-18 µg/mL; while the reported method is 20-200 µg/mL).

4. Conclusion

The proposed methods are simple and do not require sophisticated technique or instrument. The methods also, offer a practical potential for the simultaneous determination of the cited drugs, without prior separation, especially with its advantages of acceptable sensitivity and high selectivity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision LOD and LOQ. The developed methods have several advantages, as it is robust, economical and much more sensitive than the recently published HPLC; in addition to its importance as very few methods were reported in the last few months for the first combination drug to treat type 2 diabetes and high cholesterol in one tablet.

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