Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

DETERMINATION OF SIMVASTATIN AND EZATIMIBE IN COMBINED TABLET DOSAGE FORMS BY CONSTANT CENTER SPECTROPHOTOMETRIC METHOD

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Received: 02 July 2012, Revised and Accepted: 08 Aug 2012

ABSTRACT

A novel, simple, specific and accurate spectrophotometric method is developed and validated for determination of simvastatin (*SM)* and ezatimibe(*EZ*) namely; constant center spectrophotometric method(*CCSM)*.The proposed spectrophotometric method do not require any separation step. Linear correlation was obtained in range 2-16 µg/ml and 4-24 µg/ml for simvastatin and ezatimibe respectively, with mean recoveries 100.15± 1.281 for simvastatin and 100.12± 0.818 for ezatimibe. The proposed method was applied to pharmaceutical formulation and the results obtained were statistically compared with reported HPLC method**.** The statistical comparison showed that there is no significant difference between the proposed method and the reported HPLC method regarding both accuracy and precision. The method was validated according to ICH guidelines

Keywords: Simvastatin, Ezetimibe, Constant center, Constant calculation, Amplitude difference, Ratio difference, Constant multiplication

INTRODUCTION

Simvastatin(*SM*) (Fig. 1), a hypolipidemic drug belonging to the class of pharmaceuticals called statins is chemically designated as [(1S, 3R, 7R, 8S, 8aR)-8-[2-[(2R, 4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3, 7-dimethyl 1, 2, 3, 7, 8, 8ahexahydronaphthalen -1-yl]2, 2-dimethylbutanoate. It is used for the treatment of hypercholesterolemia[1]. Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMGCoA reductase, the rate limiting enzyme in cholesterol biosynthesis[2]. Different analytical methods have been reported for the determination of simvastatin, which include HPLC[3-6], HPLC-MS/MS[7], derivative spectrophotometry[8] and voltammetric techniques[9].

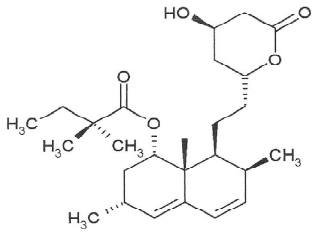

Fig. 1: Chemical structure of Simvastatin

Ezetimibe(*EZ*) (Fig. 2), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is designated as 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]- 4(S)-(4-hydroxyphenyl)-2-azetidinone. It prevents transport of cholesterol through the intestinal wall by selectively blocking the absorption of cholesterol from dietary and biliary sources. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and a subsequent reduction in serum LDL-C[10, 11]. Very few HPLC methods for the determination of ezetimibe are reported in literature[12-13].

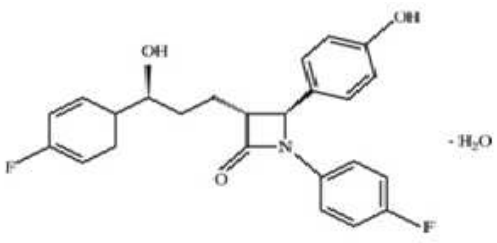

Fig. 2: Chemical Structure of Ezetimibe

Very few methods were reported for the simultaneous determination of these components in their pharmaceutical formulations[14-22].

The main problem of spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. Several spectrophotometric determination methods have been used for resolving mixtures of compounds with overlapping spectra. Derivative spectrophotometry offers a range which is more reliable with respect to utility and sensitivity than normal spectrophotometry[23], simultaneous equation and absorbance ratio(Q analysis) [24-26], Partial least squares regression (PLS) [27], principal component regression (PCR) [28], multi-wavelength linear regression analysis (MLR) [29], H-point standard addition method (HPSAM) for binary [30] and ternary[31] mixtures have been proposed. Salinas et al[32] proposed the derivative ratio spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Berzas Nevada et al [33] developed a new method for the analysis of ternary mixtures by derivative ratio spectra-zero crossing. In this method, the simultaneous determination of three compounds in ternary mixtures is realized by the measuring of the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra[34–37]. While, Dinc et al [26, 28, 37]proposed a the double divisor-ratio spectra derivative method which is based on the use of the coincident spectra of the derivative of the ratio spectra obtained by using a "double divisor" and measuring at either the maximum or minimum wavelengths

The aim of this work is to develop a novel method namely; constant center spectrophotometric method (*CCSM*) by using of smart original mathematical techniques utilizing the constants present in the ratio spectra which could be adapted to obtain the original spectra of both components in the binary mixture and analyze them at their λ_{max} with maximum accuracy and reproducibility. This method considered as a new approach of the ratio difference method [38] to enable constant value calculation. The new method was very simple, accurate, precise and did not require any sophisticated apparatus or computer programs.

THEORY

The constant center method consists of two steps complementary to each other namely constant calculation via amplitude difference method followed by constant multiplication.

The first step constant calculation via amplitude difference method depends on that, if you have a mixture of two drugs X and Y having overlapped spectra, you can determine X by dividing the spectrum of

the mixture by a known concentration of X as a divisor (X'). The division will give a new curve that represents $\frac{X+Y}{X'}$ i.e. $\frac{X}{X'}+\frac{Y}{X'}$, Where $\frac{X}{X'}$ is a constant. This can be summarized as follows

$$\frac{X+Y}{X'} = \frac{Y}{X'} + \frac{X}{X'} = \frac{Y}{X'} + cons\tan t$$

By selecting 2 wavelengths λ_1 and λ_2 on the obtained ratio curve of the mixture and subtract the values of the ratios at these two points $(\frac{Y}{X'})1$ and $(\frac{Y}{X'})2$, the constant $\frac{X}{X'}$ will be cancelled along with any other instrumental error or any interference from the interfering substance X, so component X will completely cancelled and the difference will represent component Y only; then;

$$P1 - P2 = (\frac{Y}{X'})1 + cons\ \tan t - \{(\frac{Y}{X'})2 + cons\ \tan t\}$$

$$p1 - p2 = (\frac{Y}{X'})1 - (\frac{Y}{X'})2$$

Where; P1 and P2 are the ratio amplitudes of the ratio spectrum at λ_1 and λ_2.

Amplitude difference method starts with computation of regression equation representing the linear relationship between the difference of ratio amplitudes of different concentration of pure Y at λ_1 and λ_2 using a certain concentration of X' as a divisor versus the corresponding ratio amplitude at one of the two selected wavelength λ_1 ; therefore

$\{(\frac{Y}{X'})1 - (\frac{Y}{X'})2\}$ = slope $(\frac{Y}{X'})1$ + intercept

Where, $\{(\frac{Y}{X'})1 - (\frac{Y}{X'})2\}$ is the difference of ratio spectra amplitudes at λ_1 and λ_2 and $(\frac{Y}{X'})1$ is the corresponding ratio amplitude at λ_1.

The postulated amplitude value $(\frac{Y}{X'})1$ (P postulated) that relating to component Y only in the mixture of X+Y can be calculated by using the previously computed regression equation using the difference of ratio amplitudes of the mixture at the two selected wavelengths

The constant value $\frac{X}{X'}$ can be calculated via amplitude difference method by subtracting the recorded amplitude of the ratio spectrum of the mixture $\frac{Y}{X'}+\frac{X}{X'}$ (Precorded) at (λ_1), and its postulated amplitude at the same wavelength (λ_1) {ΔP recorded – postulated}; then;

$$\frac{X}{X'} = \{\frac{Y}{X'} + \frac{X}{X'}\}1 - \{\frac{Y}{X'}\}1$$

i.e., C.V = [$P_{recorded}$] - [$P_{postulated}$]

$\frac{X}{X'}$

Where, C.V is the constant value $\frac{X}{X'}$, $P_{recorded}$ is recorded peak amplitude of the mixture at (λ_1) and $P_{postulated}$ is the postulated peak amplitude at (λ_1).

The zero order absorption spectrum of X (D^0) (original spectrum of X) could be obtained via the second step which called the constant multiplication step by multiplying the obtained constant value $\frac{X}{X'}$ by the divisor X'

$$X = \frac{X}{X'}\ x\ X'$$

The concentration of X can be calculated using its corresponding regression equation representing the linear relationship between the absorbance values of the zero order curves of X at its λ_{max} versus the corresponding concentration of X.

Similarly, The concentration of Y in the mixture can be determined by the same two steps [constant calculation followed by constant multiplication] using a known concentration of Y as a divisor (Y')

MATERIAL AND METHOD

Chemicals and Reagents

Standard simvastatin and ezetimibe with claimed purity of 100.41 and 100.01 for SM and EZ, respectively according to reported method[18].Were kindly donated by Global Napi Pharmaceuticals – Egypt. Inegy tablets, Batch No. NH49210 (10/10), NH24110 (10/20) and NH49212(10/40) of EZ and SM respectively, manufactured by Global Napi Pharmaceuticals – Egypt and were purchased from local market. methanol E. Merck, Darmstadt, FRG). All other chemicals were of analytical grade.

Apparatus

Spectrophotometric measurements were carried out on Shimadzu 1601 UVPC spectrophotometer, using 1.00 cm quartz cells. Scans were carried out in the range from 200–400 nm at 0.5 nm intervals.

Spectral characteristics

The absorption spectra of the two compounds were recorded over the range 200-400 nm.

Solution and Calibration

Stock solutions of EZ (4 mg/ml) and SM (4mg/ml) were prepared by dissolving the compounds in methanol then completing in 100 ml calibrated measuring flasks. Aliquots of the prepared stock solutions were further diluted with methanol to a final volume of 100 ml. The diluted solutions were used as the working solutions with concentrations; EZ (40 µg/ml) and SM (40µg/ml).

Linearity

Standard solutions containing 4-24 µg/ml EZ and 2-16 µg/ml SM, were prepared separately in methanol. The absorption spectra of the resulting solution were measured and stored in the computer. Construct two calibration curves relating the absorbance of the zero order spectra of SM at 238 nm versus the corresponding concentrations of SM and EZ at 233.5 nm versus the corresponding concentrations of EZ, the regression equations were computed. The stored absorption spectra of EZ and SM were divided by the absorption spectra of 8µg/ml SM and 4 µg/ml EZ, where the obtained ratio spectra were recorded. Construct Calibration curves by plotting the difference between the amplitudes of the obtained ratio spectra at [233.5 nm and 243.5 nm] and [238.5nm and 246.5 nm], versus amplitudes at 233.5 nm and 238.5 nm for EZ and SM respectively and the regression equations were computed.

Assay of Laboratory Prepared Mixtures

Into a series of 10-ml volumetric flasks, transfer accurately aliquots equivalent to (40 µg) and (40 µg -160µg) of EZ and SM respectively

from their stock working solutions EZ (40 µg/ml) and SM (40µg/ml) and complete to volume with methanol. The spectra of the prepared standard solutions were scanned and stored in the computer. The absorption spectra of different laboratory prepared mixtures were divided by the absorption spectra of 8 µg/ml standards SM and 4 µg/ml standard EZ. The ratio spectra of EZ and SM were recorded at [233.5nm and 243.5 nm] and [238.5nm and 246.5 nm], respectively. The postulated amplitudes at 233.5 nm and 238.5 nm were calculated using the corresponding regression equations and the constant values were obtained after subtraction the recorded amplitudes of the mixtures and its postulated amplitudes at the specified wavelength. Multiply the obtained constant values of SM and EZ for each mixture by the spectra of 8 µg/ml standard SM and 4 µg/ml standard EZ respectively, so the original spectra of SM and EZ were obtained. The concentrations of the drugs were calculated from the computed regression equations representing the absorbance of SM and EZ at 238 nm and 233.5 nm versus their concentrations respectively.

Application to pharmaceutical dosage forms

The contents twenty tablets were accurately weighed and powdered. An accurate weight of the mixed sample was transferred into a beaker, 50 ml ethanol were added with continuous magnetic stirring for about 10 minutes. The solution was filtered into a 100-ml volumetric flask, and the volume was completed with methanol. The proposed method was applied for the analysis of the pharmaceutical preparations solutions using the procedures mentioned under analysis of laboratory prepared mixtures for the proposed method and the concentrations of the cited drugs were calculated from the corresponding regression equations.

RESULTS AND DISCUSSION

In this work simple novel method; namely constant center spectrophotometric method (*CCSM)* was applied for resolving mixtures with spectral overlapping as simvastatin(SM) and ezatimibe (EZ). The absorption spectra of SM(X) and EZ(Y) show severe overlapping that prevents the use of direct spectrophotometry for the analysis of either EZ or SM without preliminary separation (Fig. 3).

The main task of this work was to apply the developed constant center spectrophotometric method for the determination of simvastatin and ezetimibe in their bulk powders and pharmaceutical dosage forms with satisfactory precision for good analytical practice (GAP). By applying the proposed method the original spectra of SM(X) and EZ(Y) will be obtained.

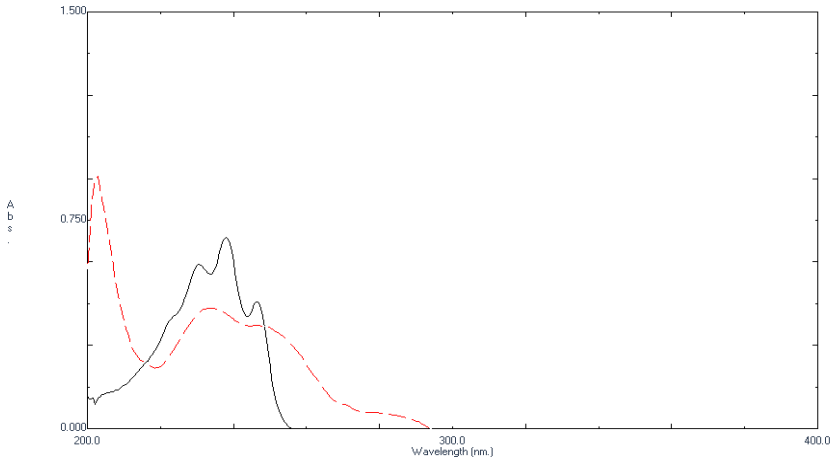

Fig. 3: Overlain spectra of 10µg/ml Ezetimibe (EZ) (- - -), 10µg/ml Simvastatin (SM)(—) in methanol

In the constant center method, the absorption spectrum of the mixture (EZ+ SM) is scanned and divided by the absorption spectrum of a known concentration of one of the components as a divisor, and the ratio spectrum is obtained represents $\frac{EZ}{SM'} + cons\tan t$ or $\frac{SM}{EZ'} + cons\tan t$. The selected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentrations 8 µg/ml SM and 4 µg/ml EZ gave the best results regarding average recovery percent when used for the analysis of SM and EZ concentrations in mixtures, respectively.

Ratio difference at two selected wavelength was applied to the ratio spectra of the cited drugs

$(\frac{EZ}{SM})1 - (\frac{EZ}{SM'})2$ Or $(\frac{SM}{EZ'})1 - (\frac{SM}{EZ'})2$, Where the interfering substance was cancelled and subsequently

show no interference. The only requirement for the selection of these two wavelengths is the contribution of the two components at these two selected wavelengths λ_1& λ_2 where the ratio spectrum of the interfering component shows the same value (constant) whereas the component of interest shows significant difference in these two ratio values at these two selected wavelengths with concentrations. The two selected wavelengths are (233.5nm and 243.5 nm) and (238.5nm and 246.5 nm) for EZ and SM respectively as shown in (Fig. 4, 5)

For the determination of SM in the binary mixture, the zero order spectrum of the mixture was scanned and the ratio spectrum of the mixture was obtained by using 8 µg /ml SM' as a divisor where practical amplitude at 233.5 nm were recorded $[\frac{EZ}{SM'} + \frac{SM}{SM'}]$ for each laboratory prepared mixture, while the postulated amplitude value of $[\frac{EZ}{SM'}]$ can be calculated by using the equation representing the linear relationship between the ratio difference of ratio spectra at 233.5nm and 243.5 nm (interfering component was cancelled) versus the corresponding ratio amplitudes at 233.5 nm

$$P1 - P2 = 0.1806 \frac{EZ}{SM'} - 0.0013 \quad (r2 = 0.9998) \quad (1)$$

Where; P1, P2 are the ratio amplitudes at 233.5 nm and 243.5nm of the ratio spectra of different concentration of EZ(4-24 µg / ml) using $\frac{EZ}{SM'}$ 8 µg/ml SM' as a divisor and is the corresponding ratio amplitudes of the ratio spectra at 233.5nm

The constant value was calculated by monitoring the effect on the amplitude of the ratio spectrum of EZ at 233.5 nm{ΔP recorded – postulated }, so the constant value was calculated by measuring the difference between the recorded amplitude and postulated amplitude at this wavelength.

$$\left[\frac{SM}{SM'}\right] = \left[\frac{EZ}{SM'} + \frac{SM}{SM'}\right] - \left[\frac{EZ}{SM'}\right]$$

$$C.V = [P_{recorded}] - [P_{postulated}]$$

Where; C.V is the constant value, $P_{recorded}$ is the recorded amplitude of the ratio spectrum of the laboratory prepared mixture using 8 µg/ml SM' as a divisor at 233.5 nm and $P_{postulated}$ is the calculated amplitude using the specified regression equation(1).

The original spectra of SM (X) in the mixture could be obtained by multiplying the obtained constant of $\frac{SM}{SM'}$ the laboratory mixture by SM (X') (the divisor), (Fig. 6) which is used for direct determination of SM at 238 nm and calculation of the concentration from the corresponding regression equation Table 1 **(**obtained by plotting the absorbance values of the zero order curves of SM at 238 nm against the corresponding concentrations).

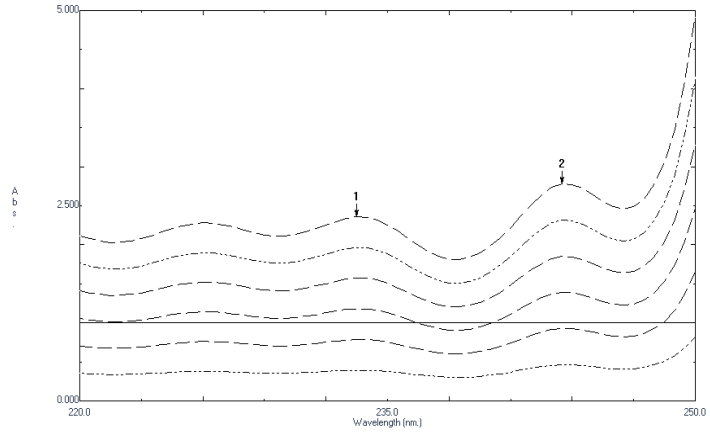

Fig. 4: Ratio spectra of Ezetimibe (EZ)(4-24µg/ml)(- - - -) and 8µg/ml of Simvastatin (SM) (—) using 8µg/ml of Simvastatin (SM') as a divisor showing the two selected wavelengths (233.5nm and 243.5 nm)

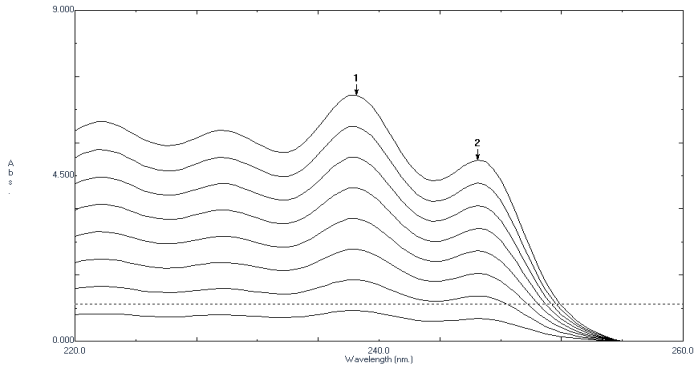

Fig. 5: Ratio spectra of SM (2-16µg/ml)(—) and 4µg/ml of EZ(……) using 4µg/ml of EZ' as a divisor showing the two selected wavelengths (238.5nm and 246.5 nm)

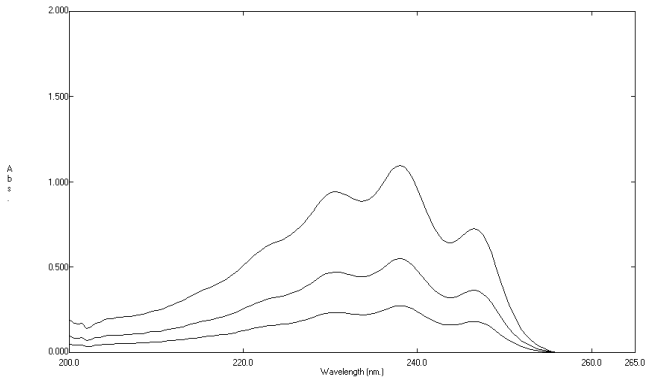

Fig. 6: Zero order absorption spectra of Simvastatin (SM) [4, 8, 16µg/ml] after multiplication by the spectrum of 8 µg/ml of SM'

Similarly EZ(Y) can be determined using 4 µg /ml EZ as a divisor at (238.5nm and 246.5 nm) versus 238.5 nm to calculate the constant value of EZ via amplitude difference step using regression equation (2)

$$P1 - P2 = 0.2631 \frac{SM}{EZ'} + 0.0018 \quad (r^2 = 0.9999) \quad (2)$$

Where; P1, P2 are the ratio amplitudes at 238.5 nm and 246.5nm respectively of the ratio spectra of different concentration of SM (2-16 µg /ml) using 4 µg /ml EZ' as a divisor and $\frac{SM}{EZ'}$ is the corresponding ratio amplitudes at 238.5nm

The original spectrum of EZ was obtained after multiplication of the calculated constant value by the 4 µg /ml EZ' as a divisor as shown in (Fig. 7)

Finally the EZ concentrations in the mixtures are calculated from the corresponding regression equation table 1 **(**obtained by plotting the absorbance values of the zero order curves of EZ at 233.5 nm against the corresponding concentrations)

The proposed method was successfully applied to the analysis of EZ and SM in their laboratory prepared mixtures and in tablet dosage forms. Table 2.

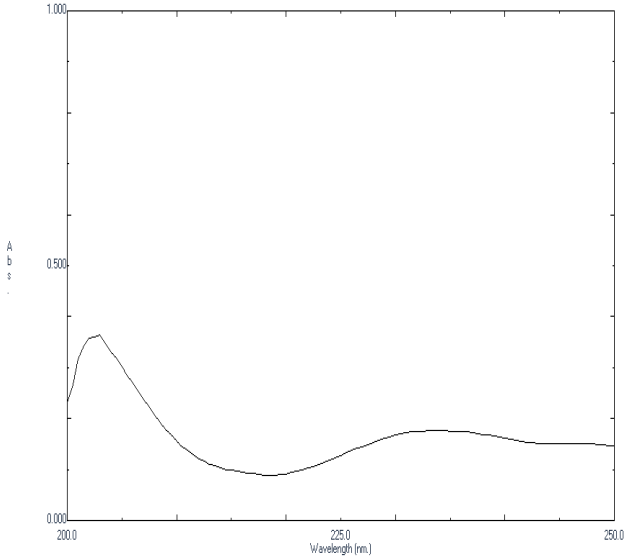

Fig. 7: Zero order absorption spectrum of Ezetimibe (EZ) (4 µg /ml) after multiplication by the spectrum of 4 µg /ml of Ezetimibe (EZ')

Table 1: Regression parameters and method validation sheet

Parameter	SM	EZ
	D^0 at 238 nm	**D^0 at 233.5 nm**
Linearity	2- 16µg/ml	4-24 µg/ml
Slope	0.0701	0.0425
Intercept	0.0023	0.0067
Correlation coefficient (r)	0.9995	0.9997
Mean + SD	100.15+ 1.281	100.12+0.818
Accuracy	100.02 + 0.856	99.87+ 0.542
RSD% [a]	0.174	0.143
RSD% [b]	0.286	0.227
Specificity*	100.08 + 0.267	99.85+ 0.673

RSD% [a,] RSD% [b] : the intraday and interday respectively (n= 3) relative standard deviation of concentrations SM (4, 8, 12 µg/ml) and EZ(6, 14, 20 µg/ml)

* Laboratory prepared mixtures

(n = 5) 5 sets each of 3 replicates

Table 2: Results of estimation of pharmaceutical dosage forms by the proposed method

Sample	SM D^0 at 238 nm Recovery + SD	EZ D^0 at 233.5 nm Recovery + SD
*Inegy tablet*s, BatchNo. *NH49210*	99.98 ± 0.528	100.35 ± 0.542
*Inegy tablet*s, Batch No.NH49110	100.14 ± 0.442	99.98 ± 0.372
*Inegy tablet*s, Batch No.N49112	100.05 ± 0.397	99.68 ± 0.429

L.p.laboratory prepared mixtures

* 5 sets each of 3 replicates.

Regarding accuracy and reproducibility, the proposed method (*CCSM*) showed maximum accuracy and reproducibility over the other reported methods that depends on cancelling the constant relating to the interfering substance as derivative ratio[18] and ratio difference[38] in the analysis of the binary mixtures since it was able to determine each component at its $\lambda_{max.}$

The main advantage of constant center method (*CCSM*) over the ratio subtraction coupled with extended ratio subtraction[38] or ratio subtraction method[39] that their application required the extension of one of the two components of the mixture, the first one was able to determine both components in the binary mixture at their λ_{max} whereas the last one limited to determine the less extended component only at its λ_{max}.It is also has advantage over derivative technique[23] since it avoid the critical measurement in this technique either at zero crossing or zero contribution of the interfering substance.

Regarding simplicity, the proposed method (*CCSM*) do not need specialized computer program to analyze the spectral data as in case of chemmometric technique which its application need matlab program and many spectral data derived from several mixtures containing different ratios of the two components.

Method validation

Validation was done according to ICH recommendations [40].

Linearity

The linearity of the methods was evaluated by analyzing six concentrations of SM and concentrations of EZ ranging between 2-16 µg/ml and 4-24 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.

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 EZ present in the pharmaceutical preparations to give accurate precise and linear results as shown in Table 1.

Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of SM and EZ. 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.

Precision

Repeatability

Three concentrations of SM (4, 8, 12 µg/ml) and EZ(6, 14, 20 µg/ml) were analyzed three times intraday using the proposed method. The relative standard deviations were calculated as shown in Table 1.

Reproducibility (intermediate precision)

The previous procedures were repeated interday on three different days for the analysis of the three chosen concentrations. The relative standard deviations were calculated as shown in Table 1.

Specificity

Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of SM and EZ within the linearity range. Satisfactory results were shown in Table 1

Stability

EZ and SM working solution showed no spectrophotometric changes up to 3 weeks when stored at 4 ?C.

Application of the proposed method for determination of EZ and SM in tablets

The proposed UV methods were applied for the determination of EZ and SM in their combined pharmaceutical formulation Inegy® tablets and the results are shown in table 2 and compared with that of the reported HPLC method[18]. The high percentage recoveries values confirm the suitability of the proposed methods for the routine determination of these components in combined formulation.

Statistical Analysis

Results obtained by the proposed method for the determination of pure samples of EZ and SM are statistically compared to those obtained by the reported HPLC method[18]. The results showed no significant differences between them as shown in Table 3.

Table 3: Statistical analysis of the results of analysis of pure powdered form

Parameter	Simvastatin (SM)		Ezetimibe (EZ)	
	D^0 at 238 nm	Reported method[18]	D^0 at 233.5 nm	Reported method[18]
Mean	100.15	100.41	100.12	100.01
SD	1.281	0.884	0.818	0.653
n	8	5	6	5
Variance	1.641	0.782	0.669	0.426
t-test	0.413 (2.201)		0.114 (2.262)	
F	2.098 (6.09)		1.574 (6.26)	

The figures in parenthesis are the corresponding theoretical values at P = 0.05.

As a final conclusion, The constant center spectrophotometric method is simple, sensitive and selective could be easily applied in quality control laboratories for the routine analysis of cited drugs in their available dosage forms as it shows equal accuracy and precision compared to the reported HPLC method[18], in contrast it is of lower cost. Furthermore, the proposed method can be applied for determination of both components in the binary mixtures at its λ_{max} even those mixtures with a severely overlapped spectra and their spectra show no extension for one of them. The proposed method is also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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