**Regular Article**

**Application of New Spectrofluorometric Techniques for Determination of Atorvastatin and Ezetimibe in Combined Tablet Dosage Form**

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Two accurate, reliable, and highly sensitive spectrofluorometric methods were developed for simultaneous determination of the binary mixture of Atorvastatin and Ezetimibe without prior separation steps. The first method is based on double scan synchronous fluorescence spectrometry. Each of Atorvastatin and Ezetimibe can be determined independent of the other when scanned at \( \Delta \lambda = 100 \text{ nm} \) and \( 40 \text{ nm} \), respectively. The relative fluorescence intensity–concentration plots at two wavelengths, 272 (\( \Delta \lambda = 100 \text{ nm} \)) and 266 nm (\( \Delta \lambda = 40 \text{ nm} \)) were rectilinear over the range of 0.4–8 \( \mu \text{g/mL} \) (for Atorvastatin) and 0.6–8 \( \mu \text{g/mL} \) (for Ezetimibe), respectively. The second method is based on the technique of simultaneous equations (Vierodt’s method), in which two equations are solved simultaneously after using a single excitation wavelength of 273 nm and \( \lambda_{\text{Em1}} = 380 \text{ nm} \) of Atorvastatin and \( \lambda_{\text{Em2}} = 301 \text{ nm} \) of Ezetimibe. Under the optimum conditions, linear relationships were found between the relative fluorescence intensity and the concentrations of the investigated drugs in the range of 0.4–8 \( \mu \text{g/mL} \) (for Atorvastatin) 0.6–8 \( \mu \text{g/mL} \) (for Ezetimibe). The different experimental parameters affecting the fluorescence intensities of the two drugs were carefully studied and optimized. The proposed methods were successfully applied for the determination of the investigated drugs in pure form, dosage form and in synthetic mixtures with good recovery and the results obtained were favorably compared to those obtained with a reference method.

Key words Atorvastatin; Ezetimibe; synchronous fluorescence spectrometry; Vierodt’s method

Atorvastatin calcium (ATV), a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is a lipid regulating drug used to reduce low density lipoprotein (LDL)-cholesterol, apolipoprotein B and triglycerides, and to increase high density lipoprotein (HDL)-cholesterol in the treatment of hyperlipidaemias (Fig. 1a). It is also used for prophylaxis of cardiovascular events in patients with multiple risk factors including diabetes mellitus. ATV is a member of the drug class known as statins.1)

Ezetimibe (EZE) is an inhibitor of intestinal absorption of cholesterol and plant sterols (Fig. 1b). 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 the serum LDL-C.2) Combining the different mechanisms of action of these agents appears to provide substantial reductions in LDL-C, with additional favorable changes in total cholesterol, triglycerides, and HDL-C. Clinical studies have shown that co-administration of EZE plus ATV was significantly more effective at reducing LDL-C concentrations than EZE or ATV alone.3) There is an urgent need to develop analytical methods for the simultaneous analysis of EZE and ATV in pharmaceutical dosage forms due to the continuous increase in clinical use of these two agents as a combination. Literature survey reveals that several methods based on spectrophotometry,4–7) densitometry,8) HPLC9–12) and capillary electrophoresis13) were reported for the simultaneous determination of EZE and ATV in pharmaceutical preparations. These methods have disadvantages of high cost, low selectivity, use of organic solvents, complex sample preparation procedures and long analysis time. To the best of our knowledge, no report has been found for the simultaneous determination of EZE and ATV either by the double scan synchronous fluorescence spectroscopy or simultaneous equation method. Therefore, it was thought worthwhile to develop simple, precise, accurate fluorometric methods for simultaneous determination of the two drugs in coformulated tablets. In fluorometric methods, high sensitivity and selectivity are generally expected. However, problems of selectivity can occur in multicomponent analysis because of the overlap of the broadband spectra observed. Synchronous fluorescence spectroscopy (SFS) has been found to have several advantages such as simple spectra, high selectivity and low interference.14) Because of its sharp, narrow spectrum, SFS serves as a very simple, effective method of obtaining data for quantitative determination in a single measurement.15) On the other hand, the Simultaneous equations method, also known as Vierodt’s method, is applicable in the case of samples containing two...

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The proposed methods were found to be easier, simpler and more sensitive than most of the published spectrophotometric and chromatographic methods for the simultaneous determination of EZE and ATV in pharmaceutical preparations, whereas there is no need for using internal standard, prior separation step (which is time consuming and requires special instrumentation) or gradient elution, to adjust excitation and emission wavelengths. Also in most of these spectrophotometric methods, sophisticated mathematical treatment is needed to resolve the target compound without interference from the others in the mixture. The proposed methods are the simplest spectrofluorometric methods for the simultaneous determination EZE and ATV in pharmaceutical preparations. The present study describes the development of two new rapid, simple and sensitive fluorometric methods for the determination of the investigated drugs.

Experimental

Apparatus Fluorescence was measured on Kontron SFM25 (BIO-TEK Kontron, Switzerland) spectrofluorometer, equipped with a 150 W Xenon lamp and a photomultiplier detector. The spectrofluorometer was controlled by computer, using SFM25 software, the photomultiplier tube voltage was adjusted at 500 V and the optimum scan speed of 500 nm s⁻¹ was used. All measurements took place in a standard 10 mm path length quartz cell. The slit width of both monochromators was 5 nm and the synchronous spectra were recorded on an excitation scale. Buffer solutions were adjusted using a Jenway pH meter model 3510 coupled with a combined glass electrode.

Materials and Reagents All reagents and chemicals used were of analytical grade and bi-distilled water was used. Ezetimibe pure samples (certified to contain 99.91%) were kindly provided by Pfizer, Egypt. Atorvastatin pure samples (nominally containing 10.0 mg) were kindly provided by Glaxo SmithKline, Egypt. MeHvaine buffer of different pH values was prepared by mixing appropriate volumes of 0.1 M citric acid and 0.2 M Na₂HPO₄. Methanol, acetone, ethanol, dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF) were purchased from Sigma-Aldrich (Germany).

Standard Solutions Standard stock solutions of 1000 µg/mL (1 mg/mL) of each drug were prepared by dissolving appropriate amounts of the pure substances in DMF as selected solvent and were further diluted with DMF to produce appropriate working solutions before use. The standard solutions were stable for 10 d when kept in the refrigerator.

Procedures For SFS Synchronous spectra were obtained by scanning both monochromators simultaneously at constant wavelength differences of Δλ = 100 nm for ATV and Δλ = 40 nm for EZE. The synchronous fluorescence intensity measurements were made at the synchronous maxima of each compound at 272 and 266 nm for ATV and EZE, respectively.

For Vierodt’s Method The fluorescence intensity of ATV and EZE were measured at 380 and 301 nm using an excitation wavelength of 273 nm.

Method Validation

Linearity

For SFS Under the specified optimum conditions, the calibration curves of the investigated drugs were constructed by analyzing a series of concentrations of the standard solutions in the range shown in Table 1.

Table 1. Validation Parameters of the Proposed Spectrofluorometric Methods

| Parameters           | Atorvastatin | Ezetimibe |
|----------------------|--------------|-----------|
|                      | SFS method   | Vierodt’s method |
|                      | SFS method   | Vierodt’s method |
| Range (µg/mL)        | 0.4–8        | 0.4–8     | 0.6–8     | 0.6–6     |
| Slope                | 15.783       | 19.289    | 19.19     | 23.951    |
| Intercept            | 3.9879       | 4.9956    | 2.1647    | 5.4525    |
| Sₚ                   | 0.59         | 0.71      | 0.43      | 0.48      |
| Sₛ                   | 2.29         | 2.77      | 1.92      | 1.63      |
| Sᵥ/ₓ                 | 1.58         | 1.91      | 1.15      | 0.97      |
| LOD (µg/mL)          | 0.03         | 0.07      | 0.04      | 0.03      |
| LOQ (µg/mL)          | 0.08         | 0.2       | 0.12      | 0.12      |
| Correlation coefficient (r) | 0.9995      | 0.9995    | 0.9999    | 0.9999    |
| Accuracy (mean±S.D.) | 100.64±0.884 | 101.70±0.399 | 100.57±0.830 | 101.20±0.774 |
| Inter-day RSD%       | 0.549        | 0.772     | 1.176     | 0.595     |
| Intra-day RSD%       | 0.878        | 0.392     | 0.825     | 0.765     |
| %Error               | 0.51         | 0.23      | 0.48      | 0.44      |

Where: Sᵥ/ₓ standard deviation of the residuals, Sₚ standard deviation of the slope, Sₛ standard deviation of the intercept, %Error = %RSD ~/√n.

The fluorescence was measured on a Kontron SFM25 (BIO-TEK Kontron, Switzerland) spectrofluorometer, equipped with a 150 W Xenon lamp and a photomultiplier detector. The spectrofluorometer was controlled by computer, using SFM25 software, the photomultiplier tube voltage was adjusted at 500 V and the optimum scan speed of 500 nm s⁻¹ was used. All measurements took place in a standard 10 mm path length quartz cell. The slit width of both monochromators was 5 nm and the synchronous spectra were recorded on an excitation scale. Buffer solutions were adjusted using a Jenway pH meter model 3510 coupled with a combined glass electrode.

Materials and Reagents All reagents and chemicals used were of analytical grade and bi-distilled water was used throughout this study and is indicated by the word “water.” Atorvastatin pure samples (certified to contain 99.91%) were kindly provided by Pfizer, Egypt. Ezetimibe pure samples (certified to contain 99.91%) were kindly supplied by GlaxoSmithKline, Egypt. The pharmaceutical dosage form studied was Atoreza® tablets (nominally containing 10.0 mg) manufactured by Marcyrl Pharmaceutical Industries-El Obour City. McIlvaine buffer of different pH values was prepared by mixing appropriate volumes of 0.1 M citric acid and 0.2 M Na₂HPO₄. Methanol, acetone, ethanol, dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF) were purchased from Sigma-Aldrich (Germany).

Standard Solutions Standard stock solutions of 1000 µg/mL (1 mg/mL) of each drug were prepared by dissolving appropriate amounts of the pure substances in DMF as selected solvent and were further diluted with DMF to produce appropriate working solutions before use. The standard solutions were stable for 10 d when kept in the refrigerator.

Procedures For SFS Synchronous spectra were obtained by scanning both monochromators simultaneously at constant wavelength differences of Δλ = 100 nm for ATV and Δλ = 40 nm for EZE. The synchronous fluorescence intensity measurements were made at the synchronous maxima of each compound at 272 and 266 nm for ATV and EZE, respectively.

For Vierodt’s Method The fluorescence intensity of ATV and EZE were measured at 380 and 301 nm using an excitation wavelength of 273 nm.

Method Validation

Linearity

For SFS Under the specified optimum conditions, the calibration curves of the investigated drugs were constructed by analyzing a series of concentrations of the standard solutions in the range shown in Table 1.

Aliquots of ATV and EZE standard solutions in the concentration range shown in (Table 1) were transferred into a two separate series of 10 mL volumetric flasks and the solutions were diluted to volume with DMF and mixed well. A blank experiment was performed simultaneously. The values of the synchronous fluorescence intensities were recorded at the specified wavelengths. The relative fluorescence intensity of the synchronous spectra was plotted vs. the final concentration of the drugs (µg/mL) to get the calibration curves and the corresponding regression equations were derived.

For Vierodt’s Method Aliquots of ATV and EZE standard solutions equivalent to 4–80 µg and 6–60 µg, respectively were transferred into a two separate series of 10 mL volumetric flasks and the solutions were diluted to the volume with DMF and mixed well. The values of the fluorescence intensities...
were recorded at the specified wavelengths. A calibration curve was obtained for each drug at both \( \lambda \) by plotting relative fluorescence intensity (\( F \)) against concentration (\( C \)). The regression parameters were computed for each drug and used in solving the simultaneous equations.

**Accuracy**

The previously stated procedures were repeated for determination of different concentrations (0.7, 1, 2 \( \mu \)g/mL) of pure ATV and EZE samples. The concentrations were calculated each from its corresponding regression equation; the percentage recoveries were then calculated.

**Precision**

Three concentrations (0.7, 1, 2 \( \mu \)g/mL) of each drug were analyzed in triplicates on the same day (for repeatability studies) and on three successive days (for intermediate precision) using the previously mentioned procedures; the mean percentage recoveries and the relative standard deviations were then calculated.

**Specificity**

Aliquot volumes of ATV and EZE standard solutions in the ratios (1:1, 2:1, 4:1) were transferred into a series of 10mL volumetric flasks followed by dilution to volume with DMF, and mixed well. The recommended procedures were then performed.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

LOD and LOQ can be calculated using the following equation according to International Conference on Harmonisation (ICH) guidelines, \( \text{LOD} = 3.3 \times \text{S/N} \) and \( \text{LOQ} = 10 \times \text{S/N} \), where, \( N = \text{standard deviation of the intercept} \) and \( S = \text{slope of the corresponding calibration curve} \).

**Procedure for Commercial Tablets**

Twenty tablets were weighed and finely powdered in a mortar. A tablet powder equivalent to 10mg of ATV and equivalent to 10mg of EZE was accurately weighed and transferred to a 100mL volumetric flask and extracted with 50mL of methanol by ultrasonication for 30min. The extract was filtered into a 100mL volumetric flask. The conical flask was washed with few milliliter of methanol. The washings were passed into the same volumetric flask and completed to the mark with the same solvent. Aliquots covering the working concentration range were transferred into 10mL volumetric flasks. The recommended procedures were performed. The nominal content of the tablets were determined either from a previously plotted calibration curve or using the corresponding regression equation.

**Results and Discussion**

**Synchronous Fluorescence Spectroscopy (SFS)**

**Spectral Characteristics**

The SFS technique involves the simultaneous scanning of both the excitation and emission monochromators to allow their synchronization in such a way to maintain a well defined relationship between their wavelengths.

The normal excitation and emission spectra of ATV and EZE are shown in (Figs. 2, 3), where ATV shows excitation and emission maxima at 275nm and 380nm, respectively while EZE shows excitation and emission maxima at 268nm and 301nm, respectively. As the emission spectra of ATV and EZE were partially overlapped (Fig. 4) ATV and EZE could not be determined directly by normal fluorometric method. Thus conventional fluorometric determination suffers from overlapping of peaks of interest. Using SFS, the spectral band is narrowed and sharper peaks are obtained by applying the optimum wavelength offset (\( \Delta \lambda \)) between excitation and emission. Synchronous emission spectra of ATV and EZE were scanned by keeping a constant interval (\( \Delta \lambda=100 \text{nm} \) for ATV and \( \Delta \lambda=40 \text{nm} \) for EZE) between \( \lambda_{\text{emission}} \) and \( \lambda_{\text{excitation}} \) at 500 and 400nm, respectively for ATV, and \( \lambda_{\text{emission}} \) and \( \lambda_{\text{excitation}} \) at 440 and 400nm, respectively for EZE. Maximum peak was found to be 272nm as shown in (Fig. 5A) and 266nm as

![Fig. 2. Normal Excitation and Emission Fluorescence Spectra as Individual Component of 6 \( \mu \)g/mL ATV (\( \lambda_{\text{Ex}}=275 \text{nm}, \lambda_{\text{Em}}=380 \text{nm} \)](image)

![Fig. 3. Normal Excitation and Emission Fluorescence Spectra as Individual Component of 8 \( \mu \)g/mL EZE (\( \lambda_{\text{Ex}}=268 \text{nm}, \lambda_{\text{Em}}=301 \text{nm} \)](image)

![Fig. 4. Emission Fluorescence Spectra of (a) 8 \( \mu \)g/mL EZE and (b) 7 \( \mu \)g/mL ATV at 301 nm and 380nm, Respectively](image)
shown in (Fig. 5B) for ATV and EZE, respectively. When synchronous technique was applied, for each of (the binary mixture) ATV and EZE, using a 100 nm value for $\Delta \lambda$, only one single synchronous band at 272 nm was obtained, because the interval $\Delta \lambda$ can be found to match solely one pair of excitation and emission bands. Similarly, at $\Delta \lambda=40$ nm, only EZE yields a detectable signal that is independent of the presence of ATV.

Optimization of Experimental Variables

The different experimental parameters that affect the fluorescence intensity were carefully studied and optimized.

Selection of Optimum $\Delta \lambda$. In synchronous fluorimetry, the optimization of a constant interval between excitation and emission wavelength ($\Delta \lambda$ value) is of great importance in generating spectra have narrower band width than that of either the excitation or emission spectra. On resolving a mixture of fluorescent components, fluorescent scanning is greatly beneficial in simplifying the spectra and decreasing the extent of spectral overlaps. To entirely separate synchronous fluorescence peaks of ATV and EZE, the synchronous fluorescence spectra of the mixture were recorded using a wide range of different $\Delta \lambda$ values (20–100 nm). The best resolution of the mixture was achieved when the $\Delta \lambda$ of 100 nm for ATV and that of 40 nm for EZE were selected. The synchronous spectra with maximum fluorescence intensity at 272 nm and 266 nm were obtained when $\Delta \lambda$ was fixed at 100 nm and 40 nm.

Fig. 5. Synchronous Fluorescence Spectra of (A) ATV at Both $\Delta \lambda=40$ (a) and $\Delta \lambda=100$ nm (b), 8 $\mu$g/mL, (B) EZE at Both $\Delta \lambda=40$ (c) and $\Delta \lambda=100$ nm (d), 8 $\mu$g/mL.

Fig. 6. Synchronous Spectra of (A) 1 $\mu$g/mL ATV in DMF at Different $\Delta \lambda$, Obtaining the Best Spectrum at $\Delta \lambda=100$ nm (a) $\Delta \lambda=50$ nm, (b) $\Delta \lambda=70$ nm, (c) $\Delta \lambda=90$ nm, (d) $\Delta \lambda=100$ nm, (e) $\Delta \lambda=110$ nm, (f) $\Delta \lambda=120$ nm and (B) 1 $\mu$g/mL EZE in DMF at Different $\Delta \lambda$, Obtaining the Best Spectrum at $\Delta \lambda=40$ nm (a) $\Delta \lambda=20$ nm, (b) $\Delta \lambda=30$ nm, (c) $\Delta \lambda=40$ nm, (d) $\Delta \lambda=50$ nm, (e) $\Delta \lambda=60$ nm, (f) $\Delta \lambda=80$ nm, (g) $\Delta \lambda=110$ nm.
for ATV and EZE, respectively, as shown in Figs. 6(A) and (B).

The investigation confirmed that the synchronous spectrum of one analyte is not affected by the presence of the other and the mixture signal is the additive for ATV and EZE concentrations.

**Influence of pH** The influence of pH on the fluorescence intensity of the two drugs was studied using McIlvaine buffer covering the whole pH range (2.2–8). It was found that, using a buffer caused a significant progressive decrease in the fluorescence intensity. Therefore, to simplify the procedure no buffer was used throughout the study.

**Influence of Diluting Solvents** Dilution with different solvents including: water, methanol, acetone, ethanol, DMSO, and DMF was employed and excitation and emission spectra were recorded. Water caused the precipitation of EZE, while DMSO produced irreproducible readings with ATV, thus they could not be used as diluting solvents. It could be observed that DMF produced the highest synchronous fluorescence intensities compared with the other solvents as shown in Fig. 7. Thus DMF was chosen as the diluting solvent throughout the study.

**Luminescence Stability** Practical studies on the stability of the synchronous fluorescence intensity of the studied drugs showed that the instantaneously developed fluorescence intensity was stable for more than 2 h.

**Simultaneous Equations (Vierodt’s Method)** For analyzing a mixture containing two drugs (X and Y) each showing native fluorescence at the λ\textsubscript{Em} of the other (λ\textsubscript{Em1}=380 nm for ATV and λ\textsubscript{Em2}=301 nm for EZE), they can be applied simultaneous equations (Vierodt’s method).

Application of this technique for the determination of either component X or Y requires the following:

- The fluorescence emission spectrum of X should not severely overlap that of Y.
- X and Y should not interact with each other.
- Beer–Lambert’s Law should be valid for both X and Y at λ\textsubscript{Em1} and λ\textsubscript{Em2} over a reasonable concentration range.

Therefore, the fluorescence of the mixture at any wavelength is the summation of the fluorescence values of the components at this particular wavelength.

After studying the excitation and fluorescence emission spectra, 273 nm was selected as the excitation wavelength to produce the desirable degree of accuracy.\(^{25}\) The maximum emission wavelength of ATV and EZE were obtained at 380 and 301 nm, respectively (Fig. 8).

Let C\textsubscript{X} and C\textsubscript{Y} be the concentrations of (X and Y), respectively, in the diluted sample. Two equations are constructed based upon the fact that at λ\textsubscript{Em1} and λ\textsubscript{Em2} the fluorescence of the mixture is the sum of the individual fluorescence of (X and Y).

At λ\textsubscript{Em1}:

\[ F_1 = \alpha_1 b C_X + \beta_1 b C_Y \]  
(1)

At λ\textsubscript{Em2}:

\[ F_2 = \alpha_2 b C_X + \beta_2 b C_Y \]  
(2)

For measurements in 1 cm cells, b=1.

Rearrange Eq. 2

\[ C_Y = \frac{F_2 - (\alpha_2 C_X)}{\beta_2} \]

Substituting for \( C_Y \) in Eq. 1 and rearranging gives

\[ C_X = \frac{F_1 \beta_2 - F_2 \beta_1}{\alpha_1 \beta_2 - \alpha_2 \beta_1} \]

\[ \times 23.951 - F_2 \times 6.2308 \]

\[ = \frac{19.289 \times 23.951 - 0.9167 \times 6.2308}{19.289 \times 23.951 - 0.9167 \times 6.2308} \]  
(3)

and

\[ C_Y = \frac{F_2 \alpha_2 - F_1 \alpha_1}{\alpha_1 \beta_2 - \alpha_2 \beta_1} \]

\[ = \frac{19.289 \times 23.951 - 0.9167 \times 6.2308}{19.289 \times 23.951 - 0.9167 \times 6.2308} \]  
(4)

where \( F_1 \) and \( F_2 \) are the fluorescence of the mixture at 380 and 301 nm, respectively. \( C_X \) and \( C_Y \) are the concentrations of ATV and EZE in µg/L, respectively.

\[ \alpha_1 = \frac{F_{X1} - F_{Blank1}}{\text{Standard conc. of X}} \]

\[ \alpha_2 = \frac{F_{X2} - F_{Blank2}}{\text{Standard conc. of X}} \]

\[ \beta_1 = \frac{F_{Y1} - F_{Blank1}}{\text{Standard conc. of Y}} \]

\[ \beta_2 = \frac{F_{Y2} - F_{Blank2}}{\text{Standard conc. of Y}} \]

**Validation of the Methods** The validity of the methods was tested regarding: linearity and range, accuracy, repeatability, precision and specificity according to ICH recommendations.\(^{26}\) The regression plots showed a linear dependence of fluorescence values on drug concentration over the range cited in Table 1.

The validity of the methods was proved by statistical evalu-
### Table 2. Application of the Proposed Methods to the Determination of the Studied Drugs in Their Synthetic Mixtures

| Method          | Concentration taken (µg/mL) | Concentration found (µg/mL) | Recovery (%) |
|-----------------|-----------------------------|----------------------------|--------------|
|                 | Atorvastatin | Ezetimibe | Atorvastatin | Ezetimibe | Atorvastatin | Ezetimibe |
| (1) SFS method |              |            |              |            |              |           |
| Mix. 1          | 1            | 1          | 1.00         | 0.99      | 100.00       | 99.19     |
| Mix. 2          | 2            | 1          | 2.02         | 1.00      | 101.00       | 99.72     |
| Mix. 3          | 4            | 1          | 4.02         | 1.01      | 100.50       | 100.76    |
| Mean            |              |            |              |            | 100.50       | 99.89     |
| N               | 3            |            |              |            | 3            |           |
| V               | 0.25         |            |              | 0.64      |              |           |
| S.D.            | 0.50         |            |              | 0.80      |              |           |
| RSD (%)         | 0.50         |            |              | 0.80      |              |           |
| (2) Vierodt’s method |          |            |              |            |              |           |
| Mix. 1          | 1            | 1          | 1.00         | 1.01      | 99.61        | 100.57    |
| Mix. 2          | 2            | 1          | 2.03         | 1.00      | 101.56       | 99.87     |
| Mix. 3          | 4            | 1          | 4.05         | 1.00      | 101.30       | 100.29    |
| Mean            |              |            |              |            | 100.82       | 100.24    |
| N               | 3            |            |              |            | 3            |           |
| V               | 1.12         |            |              | 0.12      |              |           |
| S.D.            | 1.06         |            |              | 0.35      |              |           |
| RSD (%)         | 1.05         |            |              | 0.35      |              |           |

Each result is the average of three determinations.

### Table 3. Determination of the Studied Drugs in Atoreza® Tablets by the Proposed Methods and Application of the Standard Addition Technique

| Method          | Concentration added of each drug (µg/mL) | Concentration found (µg/mL) | Recovery (%) |
|-----------------|-------------------------------------------|----------------------------|--------------|
|                 | Atorvastatin | Ezetimibe | Atorvastatin | Ezetimibe | Atorvastatin | Ezetimibe |
| (1) SFS method  |                |            |              |            |              |           |
| Atoreza® + C16 tablets | 99.72±0.97 | 99.88±1.04 | 1           | 0.99      | 1.01         | 98.92      | 100.76    |
| Atorvastatin 10mg and Ezetimibe 10mg/tablet | 2 | 2.00 | 2.00 | 100.15 | 99.89     |
| B. No 1131996  | 3             |            | 2.98         | 3.01      | 99.29        | 100.46     |
| Mean           |              |            | 99.45        | 100.37    |              |            |
| N              | 3            |            | 3            | 3         |              |            |
| S.D.           | 0.63         |            | 0.63         | 0.44      |              | 0.44       |
| RSD (%)        | 0.63         |            | 0.63         | 0.44      |              | 0.44       |
| (2) Vierodt’s method |          |            |              |            |              |           |
| Atoreza® tablets | 99.45±1.13 | 99.82±1.23 | 1           | 1.01      | 1.00         | 100.99     | 100.15    |
| Atorvastatin 10mg and Ezetimibe 10mg/tablet | 2 | 2.00 | 2.04 | 99.79 | 101.86    |
| B. No 1131996  | 3             |            | 3.01         | 3.01      | 100.19       | 100.32     |
| Mean           |              |            | 100.32       | 100.78    |              |            |
| N              | 3            |            | 3            | 3         |              |            |
| S.D.           | 0.61         |            | 0.61         | 0.94      |              | 0.94       |
| RSD (%)        | 0.61         |            | 0.61         | 0.94      |              | 0.94       |

a) Average of three determinations.

### Table 4. Statistical Analysis of ATV and EZE by the Proposed Methods

| Items          | Atorvastatin | Ezetimibe |
|----------------|--------------|-----------|
|                | SFS          | Vierodt’s method | Reported method | SFS          | Vierodt’s method | Reported method |
| Mean           | 100.635      | 101.699   | 100.267         | 100.572      | 101.202         | 100.567         |
| N              | 3            | 3         | 3               | 3            | 3               | 3               |
| S.D.           | 0.8838       | 0.3986    | 0.6504          | 0.8300       | 0.7741          | 1.5190          |
| RSD%           | 0.8783       | 0.3919    | 0.6487          | 0.8253       | 0.7649          | 1.5104          |
| F-Test         | 1.847 (19)*  | 2.663 (19)*| 3.347 (19)*     | 3.849 (19)*  |
| Student’s t-test | 0.5815 (2.776)* | 3.253 (2.776)* | 0.0049 (2.776)* | 0.646 (2.776)* |

*The figures in parenthesis are the corresponding tabulated values at \( p=0.05 \).
ation of the regression lines, using the standard deviation of the residuals (S_{Y/X}), the standard deviation of the intercept (S_{\alpha}) and standard deviation of the slope (S_{\beta}). The results are abridged in Table 1. The small values of the figures point out to the low scattering of the points around the calibration curves and high precision. Accuracy was recorded as percent recovery±standard deviation (S.D.). Precision results were expressed as RSD% values. Separate determinations at different concentration levels were carried out for each drug to test for precision and accuracy. Low values of RSD% (<2%) suggest an excellent accuracy and precision of the methods, as shown in Table 1.

A set of laboratory prepared mixtures of the two investigated drugs in variable proportions were analyzed to evaluate the specificity of the suggested methods. The concentration of each drug was computed using the corresponding regression equation. The results indicate high accuracy of the proposed methods as shown in Table 2. The ICH guideline for determination of LOQ and LOD was followed. The estimation was based on the standard deviation of response. The standard deviation of y-intercepts of the regression lines can be used as the standard deviation of response. The values, shown in Table 1, indicate the adequate sensitivity of the methods.

The proposed methods were applied to the analysis of the two drugs in their commercial formulations. The experimental results are presented in Table 3. The obtained recoveries can be considered satisfactory.

Table 4 shows statistical comparison between the results obtained by applying the proposed methods and the reported spectroscopic method.

**Conclusion**

The present study proposes novel, simple, inexpensive, precise, accurate and highly sensitive methods for the simultaneous determination of binary mixture ATV and EZE in coformulated tablet Atoreza® without prior separation steps. In comparison to chromatographic techniques which need sophisticated instruments, expensive reagents and longer analysis time, the proposed methods require simpler instrument and procedures and cheaper reagents. The proposed methods do not require any sophisticated mathematical treatment for the absorption data, and they exhibit several advantages over other spectrophotometric methods for resolution of binary mixtures. ATV and EZE were well resolved and quantified without prior separation through simple mathematical treatment. The applicability of the developed methods was evaluated through the determination of the two drugs combination in several laboratory-prepared mixtures and in pharmaceutical tablets with good accuracy and precision. Therefore, the presented methodology is adequate for the routine quality control analysis of this fixed-dose combination.

**Conflict of Interest**

The authors declare no conflict of interest.

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