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**ORIGINAL ARTICLE / ÖZGÜN MAKALE**

# SPECTROPHOTOMETRIC CO-ESTIMATION OF ATORVASTATIN AND EZETIMIBE IN A BINARY MIXTURE USING PRINCIPLE COMPONENT REGRESSION AND PARTIAL LEAST SQUARES REGRESSION MODELS

## *TEMEL BİLEŞEN REGRESYONU VE KISMİ EN KÜÇÜK KARELER REGRESYONU MODELLERİ KULLANILARAK ATORVASTATİN VE EZETİMİBİN İKİLİ KARIŞIMLARDA EŞZAMANLI TAYİNİ*

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## ABSTRACT

**Objective:** *Fixed-dose formulations, such as combination of atorvastatin (AT) and ezetimibe (EZ), present analytical challenges due to overlapping spectral features in UV-Vis spectroscopy. Although traditional chromatographic methods are effective in these cases, they are not cost-efficient, and the required instrumentation is not easily accessible. This study aims to develop a simpler, cost-effective spectrophotometry-based approach for the simultaneous quantification of AT and EZ in fixed-dose formulations.*
**Material and Method:** *Principal component regression (PCR) and partial least squares (PLS) regression models were used in combination with UV-Vis spectroscopy. A calibration set of binary mixtures was prepared in the working range of 4–36 µg/ml for both drugs. PCR and PLS models were constructed using three latent variables. The developed methods were evaluated for accuracy, precision, and selectivity using laboratory-prepared samples. The applicability of the methods was demonstrated by analyzing commercial tablet samples.*
**Result and Discussion:** *Both models achieved high recovery rates (98–102.3%) and low relative standard deviations (<2.0%), confirming their accuracy and precision. Standard addition studies confirmed the selectivity of the methods. Then, the proposed PCR and PLS methods successfully quantified AT and EZ in commercial film-coated tablets without extensive sample preparation, offering a simple, cost-effective, and efficient alternative to conventional techniques.*
**Keywords:** *Atorvastatin, chemometrics, ezetimibe, PCR, PLS, spectrophotometry*

## ÖZ

**Amaç:** *Atorvastatin (AT) ve ezetimibin (EZ) kombinasyonu gibi sabit dozlu formülasyonlardaki etken maddelerin spektral örtüşmeleri, UV-GB spektroskopi temelli analiz yöntemlerinin geliştirilmesini zorlaştırır. Geleneksel kromatografik yöntemler bu tür durumlarda etkili olsa da, maliyet açısından verimli değildir ve gerekli cihazlara erişim kolay olmayabilir. Bu çalışma, sabit dozlu formülasyonlarda AT ve EZ'in eş zamanlı miktar tayini için daha basit ve ekonomik bir spektrofotometri temelli yaklaşım geliştirmeyi amaçlamaktadır.*
**Gereç ve Yöntem:** *Bu çalışmada, temel bileşen regresyonu (PCR) ve kısmi en küçük kareler regresyonu (PLS) modelleri, UV-GB spektroskopisi ile birlikte kullanılmıştır. Her iki ilaç için de 4–*

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*36 µg/ml çalışma aralığında ikili karışımlardan oluşan bir kalibrasyon seti hazırlanmıştır. PCR ve PLS modelleri üç gizli değişken kullanılarak oluşturulmuştur. Geliştirilen yöntemler, laboratuvarda hazırlanan örnekler kullanılarak doğruluk, kesinlik ve seçicilik açısından değerlendirilmiştir. Yöntemlerin uygulanabilirliği, ticari tablet örnekleri analiz edilerek gösterilmiştir.*

**Sonuç ve Tartışma:** *Her iki modelle yüksek geri kazanım oranları (%98–102.3) ve düşük bağıl standart sapma değerleri (<%2.0) elde edilmiş, yöntemlerin doğruluk ve kesinliklerini doğrulamıştır. Yöntemlerin seçiciliği, standart ekleme çalışmaları ile teyit etmiştir. Kapsamlı numune hazırlama gerektirmeden, PCR ve PLS yöntemleri kullanılarak film kaplı tabletlerdeki AT ve EZ'in eş zamanlı miktar tayinleri başarıyla gerçekleştirilmiştir. Geliştirilen yöntemler, geleneksel tekniklere kıyasla basit, ekonomik ve verimli alternatifler sunmuştur.*

**Anahtar Kelimeler:** *Atorvastatin, ezetimib, kemometri, PCR, PLS, spektrofotometri*

## INTRODUCTION

Fixed-dose formulations are commonly used in cardiovascular diseases due to their advantages such as improved patient adherence, better clinical outcomes, and reduced health-care costs [1,2]. However, they present significant challenges for analytical chemists due to the complexity. The pharmaceutical industry relies heavily on chromatographic techniques to address this challenge, but these techniques require long periods of method development, sophisticated instrumentation, trained personnel, and higher costs [3]. Consequently, developing reliable, accurate, precise yet simple, low-cost and environmentally sustainable analytical methods for fixed-dose formulations is an important task.

UV-Vis spectroscopy offers simple, straightforward, and cost-effective analytical procedures for quality control and pharmaceutical research. However, it often becomes inadequate when the analytes exhibit overlapping spectral features. Since univariate spectroscopic methods cannot resolve spectral interferences, the use of chemometric and multivariate calibration techniques becomes necessary to analyze the overlapping spectra of active pharmaceutical ingredients in fixed-dose formulations [3-5]. In particular, principal component regression (PCR) and partial least squares (PLS) regression techniques provide effective solutions by extracting latent variables and modelling the relationship between analyte concentrations and the spectral data with overlapping features [6-8].

Atorvastatin (AT) and ezetimibe (EZ), two widely prescribed lipid-lowering agents, are frequently combined in fixed-dose formulations to enhance therapeutic efficacy, improve patient adherence, and reduce side effects [9]. AT is a statin that inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby decreasing hepatic production of low-density lipoprotein cholesterol. EZ, an inhibitor of intestinal cholesterol absorption, is usually combined with a statin such as AT [10]. However, the spectrophotometric analysis of this combination presents a challenge because their absorbance spectra overlap almost completely in the region 200-300 nm. Indeed, a literature survey reveals that the quantitative estimation of AT and EZ has been mostly studied by using chromatographic techniques [11-24]. Conversely, some studies have proposed UV-Vis spectroscopy combined with simple mathematical algorithms as an alternative for analyzing AT and EZ. Techniques such as Vierordt's method [25], H-point standard additions method [26], difference absorbance method, [27,28], Q-absorbance method [29], ratio spectra method [28], first derivative spectroscopy [26,30], and ratio spectra derivative spectrophotometry [26,31] have provided relatively simple and cost-effective approaches. However, these techniques rely on the accurate wavelength selection, are sensitive to noise, and have limited applicability in multi-component mixtures [32-34]. In contrast, multivariate calibration methods, such as PCR and PLS uses full spectral data, reduce collinearity, and improve signal-to-noise ratios [35-36].

The objective of this study was to demonstrate the applicability of PCR and PLS models combined with UV-Vis spectrophotometry as reliable alternatives to conventional methods for the co-estimation of AT and EZ in fixed-dose formulations. PCR and PLS regression models were developed, and their performance was evaluated through a series of validation studies, including accuracy, precision, selectivity, and robustness. Finally, the proposed methods were successfully applied to the analysis of film-coated tablet samples, demonstrating their applicability in pharmaceutical analysis.

## MATERIAL AND METHOD

### Instruments and Software

UV-Vis spectra of the samples were recorded using a Shimadzu UV-2550 double-beam spectrophotometer (Kyoto, Japan), equipped with UVProbe Software (Shimadzu, Kyoto, Japan). Measurements were conducted using a quartz cuvette with a 1 cm optical path length, and the slit width was set to 2 nm. Spectra were recorded between 210–340 nm, with a spectral resolution of 0.1 nm against methanol as blank. The spectra of samples were exported into an Excel sheet (Microsoft, USA) as a matrix with each column as a different sample. The development of PCR and PLS models, as well as validation and prediction steps were operated in Matlab (MathWorks, USA). The same software was also used to plot the figures as well.

### Chemicals and Reagents

Standard materials of AT and EZ were generously provided by Neutec Pharmaceuticals (Sakarya, Türkiye). Analytical-grade methanol was obtained from Carlo Erba (Milan, Italy). The commercial sample of AT and EZ fixed-dose combination was procured from a local pharmacy, produced by Neutec Pharmaceuticals, Sakarya, Türkiye. Label claim was 10 mg AT and 10 mg EZ per tablet.

### Standard Solutions

Stock solutions of AT and EZ were individually prepared by dissolving 10 mg of each compound in 100 ml of methanol. The working concentration ranges were 4–36 µg/ml for AT and EZ. A calibration set comprising 25 solutions was generated by factorial design at five concentration levels. Solutions in calibration set were prepared by mixing the required amount of stock solutions and diluting them with methanol. The specific concentrations of each standard material in calibration sample set are listed in Table 1.

**Table 1.** Calibration set used to construct PCR and PLS models

| | AT | EZ | | AT | EZ | | AT | EZ | | AT | EZ | | AT | EZ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C1** | 4 | 4 | **C6** | 12 | 4 | **C11** | 20 | 4 | **C16** | 28 | 4 | **C21** | 36 | 4 |
| **C2** | 4 | 12 | **C7** | 12 | 12 | **C12** | 20 | 12 | **C17** | 28 | 12 | **C22** | 36 | 12 |
| **C3** | 4 | 20 | **C8** | 12 | 20 | **C13** | 20 | 20 | **C18** | 28 | 20 | **C23** | 36 | 20 |
| **C4** | 4 | 28 | **C9** | 12 | 28 | **C14** | 20 | 28 | **C19** | 28 | 28 | **C24** | 36 | 28 |
| **C5** | 4 | 36 | **C10** | 12 | 36 | **C15** | 20 | 36 | **C20** | 28 | 36 | **C25** | 36 | 36 |

Concentration values are given in µg/ml

To assess the performance of the chemometric models, an independent test set of 11 samples with varying concentrations of both analytes was prepared following the same procedure. The concentration values of these test samples, listed in Table 2, fell within the defined working ranges. As can be seen in Table 2, the concentration of the analytes in the test set was different than those in the calibration set. Another set of synthetic binary mixtures at three distinct concentration levels (Table 3) were prepared in triplicate to evaluate both intra-day and inter-day precision. Additionally, a set of standard addition samples were prepared in triplicate to evaluate the selectivity. For this purpose, the required volumes of standard stock solutions of AT and EZ were added onto a fixed volume of commercial sample solutions and were diluted with methanol up to the final volume. The final concentration of added standards were 10 µg/ml, 20 µg/ml, and 30 µg/ml for both drugs. One extra solution was prepared by diluting the fixed volume of commercial sample to the final to be used for subtraction in standard addition studies.

### Commercial Sample Solutions

Ten film-coated tablets were grounded into a powder in a mortar. A portion of the powder, equivalent to 0.5 tablet was placed in a 25 ml volumetric flask, then filled to volume with methanol. The solution was magnetically stirred for 20 minutes and, filtered from a syringe filter with a pore size of 0.45 µm. Final sample solution to be analyzed was prepared by diluting 2 ml filtrate to 10 ml, with a theoretical concentration of 20 µg/ml of each analyte. This procedure was repeated 10 times.

## RESULT AND DISCUSSION

Overlapping spectra of analytes is a common challenge for spectroscopic analyses. Multivariate calibration methods such as PCR and PLS have the ability to extract latent variables that can be used for simultaneous analysis in spite of overlapping. However, these methods still rely on the assumption that absorbance-concentration relationship follows Lambert-Beer's law because the deviations from linearity can cause the models to loose their predictive power [37-38]. Hence, the first step was to define the linear concentration range of the analytes. For this purpose, individual standard solutions were prepared by diluting the stock solution. A working range of 4-36 µg/ml, with five calibration points with equal steps was studied for both drugs. The absorbance spectra of these solutions were recorded between 200-360 nm. The absorbance values of analytes at their λmax (246 nm for AT and 233 nm for EZ) were plotted against the concentration values. Linearity was evaluated by inspecting the mentioned calibration graph, as well as studentized residual plots [39]. Linearity was confirmed as calibration plots showed a linear trend and residuals were randomly distributed (See supplemental data). The spectra of the individual analytes at calibration levels 4, 12, 20, 28 and 36 µg/ml are illustrated in Figure 1. As can be seen in this figure, conventional univariate calibration would not be useful in to determine AT and EZ in their mixtures because of the heavily overlapping spectra. In order to address this challenge the most popular multivariate models, PCR and PLS, were used to develop new analytical methods to quantify AT and EZ in their commercial samples.

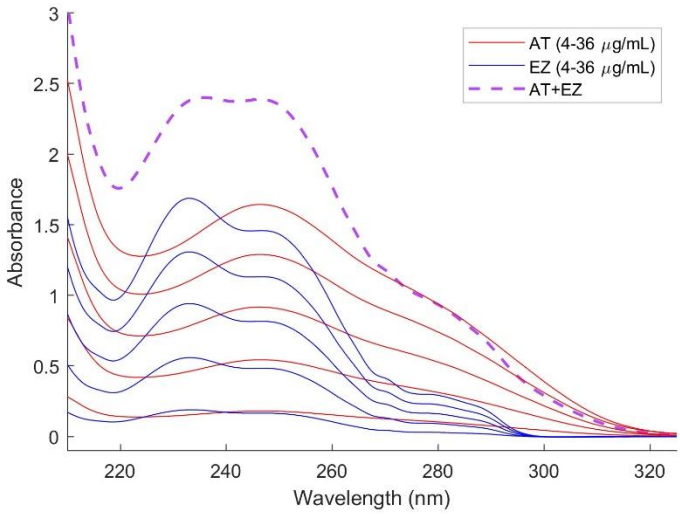


**Figure 1.** Absorption spectra of 4-36 µg/ml AT, 4-36 µg/ml EZ, and their binary mixture in methanol

### Multivariate Calibration Models

PCR and PLS regression models were applied for the quantification of AT and EZ in their mixtures. In both models, the concentration matrix and the absorbance matrix between 210-340 nm with $\Delta\lambda$=0.1 were used after mean-centering. Both methods are multivariate calibration techniques based on latent variables but differ in how these variables are computed. PCR combines principal component analysis (PCA) with inverse least squares regression, using only spectral data to extract latent variables. The latent variables describe the variance of the spectral dataset, independent of the analyte concentrations. These latent variables, also referred to as scores, are used as predictors to build a regression model that relates them to the concentration data [40].

The implementation of the PCR model was started by decomposing absorbance matrix of the calibration set into score matrix T and loading matrix. After this step, cross-validation was performed to find the number of components to be used in the predictive phase. As can be seen in Figure 2, three principal components were adequate for both drugs. Then, the truncated score matrix T with three

columns was regressed on the concentration matrix to calculate the regression coefficient matrix. Finally, the predicted concentration matrix was computed by matrix multiplication of regression coefficient matrix with the absorbance matrix of samples [40-41].

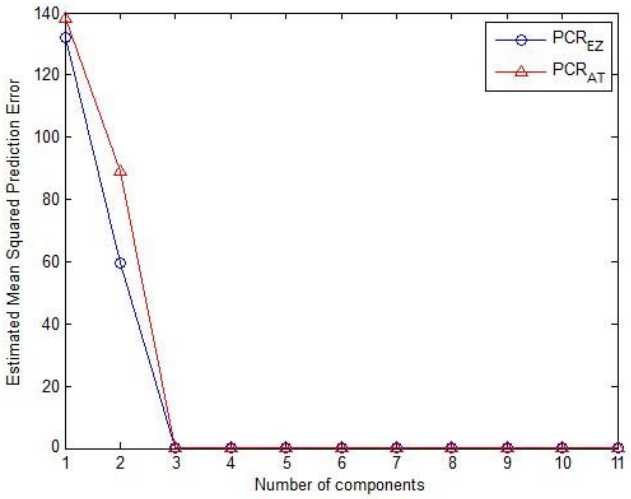


**Figure 2.** Cross validation plot of the PCR model for AT and EZ

Conversely, PLS includes both spectral and concentration data during decomposition to describe the covariance between the two datasets, resulting in analyte-dependent latent variables. In PLS implementation, absorbance and concentration matrices were simultaneously decomposed by PCA, with an internal equation relating the two score matrices P, and Q. Then, weight matrix, W, that maximizes the covariance between scores and the concentration matrix was computed. Similar to the PCR, cross validation was performed to decide on the number of latent variables as three for AT and EZ drugs (See Figure 3). Later, the regression coefficient matrix B was computed using the truncated matrices by the equation $B = W(P^TW)^{-1}Q^T$ to be used in the prediction step [38,41,42].

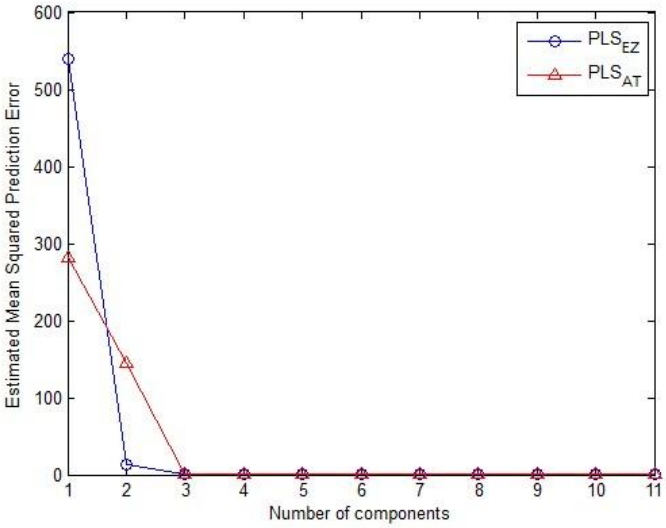


**Figure 3.** Cross validation plot of the PLS model for AT and EZ

Actual and predicted concentration values by the PCR and PLS models were plotted to evaluate the outliers and to visualize the predictive power as given in Figure 4.

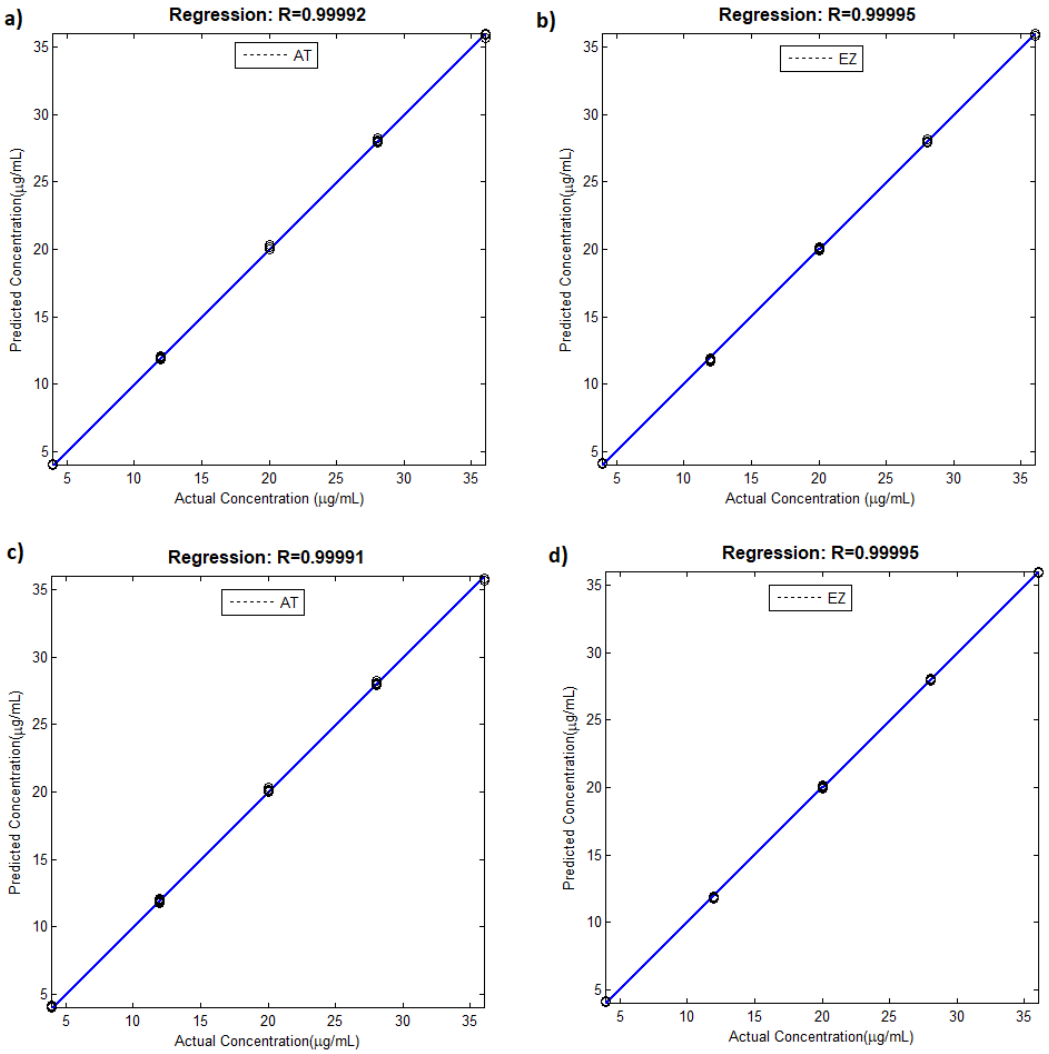


**Figure 4.** Actual and predicted concentration plots of AT and EZ obtained by the developed PCR (a and b) and PLS (c and d) models

## Analytical Validation

Analytical validation of the developed PCR and PLS methods were carried out by analyzing different sets of laboratory-made solutions and assessing their results. A test set of 11 binary mixtures were prepared by diluting stock solutions to reach various concentration levels. Actual and predicted concentration values by the PCR and PLS methods are given in Table 2. The recovery value of each sample, as well as their mean, standard deviation and relative standard deviation were also listed in the same table. Mean recovery values were reported to be between 102.3% and 98 %, with relative standard deviation values smaller below 2.0, indicating sufficient accuracy and precision of the models in spite of overlapping spectral features. Additionally, to compare how well PCR and PLS models performs on independent data, standard error of prediction (SEP) was used. As an estimation of the average deviation of predicted values from the actual values, standard deviation of residuals obtained by PCR and PLS models for both drugs were calculated and reported as SEP values in Table 2.

**Table 2.** Recovery results of independent test samples by PCR and PLS methods

| | | | PCR | | PLS | | PCR | | PLS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Added (µg/µl) | | Found (µg/µl) | | Found (µg/µl) | | Recovery (%) | | Recovery (%) | |
| **Code** | **EZ** | **AT** | **EZ** | **AT** | **EZ** | **AT** | **EZ** | **AT** | **EZ** | **AT** |
| **M1** | 4 | 30 | 4.10 | 30.08 | 4.14 | 30.08 | 102.4 | 100.3 | 103.6 | 100.3 |
| **M2** | 12 | 30 | 12.09 | 29.47 | 12.37 | 29.50 | 100.7 | 98.2 | 103.1 | 98.3 |
| **M3** | 20 | 30 | 20.12 | 29.62 | 20.38 | 29.66 | 100.6 | 98.7 | 101.9 | 98.9 |
| **M4** | 28 | 30 | 27.96 | 29.81 | 28.26 | 29.86 | 99.9 | 99.4 | 100.9 | 99.5 |
| **M5** | 36 | 30 | 36.41 | 29.59 | 36.63 | 29.61 | 101.1 | 98.6 | 101.8 | 98.7 |
| **M6** | 30 | 4 | 31.51 | 3.98 | 31.17 | 3.96 | 105.0 | 99.5 | 103.9 | 99.1 |
| **M7** | 30 | 12 | 30.73 | 11.25 | 30.49 | 11.30 | 102.4 | 93.7 | 101.6 | 94.2 |
| **M8** | 30 | 20 | 30.72 | 19.55 | 30.80 | 19.61 | 102.4 | 97.7 | 102.7 | 98.0 |
| **M9** | 30 | 28 | 30.44 | 27.31 | 30.54 | 27.38 | 101.5 | 97.5 | 101.8 | 97.8 |
| **M10** | 30 | 36 | 30.44 | 35.00 | 30.59 | 35.07 | 101.5 | 97.2 | 102.0 | 97.4 |
| **M11** | 30 | 30 | 30.41 | 29.15 | 30.51 | 29.22 | 101.4 | 97.2 | 101.7 | 97.4 |
| | | | | **Mean:** | | | 101.7 | 98.0 | 102.3 | 98.1 |
| | | | **Standard deviation:** | | | | 1.37 | 1.74 | 0.92 | 1.59 |
| | | | **Relative standard deviation:** | | | | 1.35 | 1.77 | 0.90 | 1.62 |
| | | | **Standard error of prediction:** | | | | 0.64 | 0.60 | 0.63 | 0.55 |

**Table 3.** Analysis results of intra-day and inter-day samples (n=3)

| | Added (µg/ml) | | Found (µg/ml) | | | |
|---|---|---|---|---|---|---|
| | | | PCR | | PLS | |
| | **AT** | **EZ** | **AT** | **EZ** | **AT** | **EZ** |
| **Inter-day** | 10 | 10 | 10.22 | 9.70 | 10.35 | 9.69 |
| | 20 | 20 | 19.79 | 19.45 | 19.67 | 19.44 |
| | 30 | 30 | 30.41 | 29.45 | 30.56 | 29.51 |
| **Intra-day** | 10 | 10 | 10.18 | 9.77 | 10.08 | 9.63 |
| | 20 | 20 | 20.43 | 19.61 | 20.31 | 19.54 |
| | 30 | 30 | 30.86 | 29.34 | 30.90 | 29.41 |
| | | | **Mean recovery (%)** | | | |
| | | | **PCR** | | **PLS** | |
| | | | AT | EZ | AT | EZ |
| **Inter-day** | | | 102.23 | 97.03 | 103.54 | 96.86 |
| | | | 98.96 | 97.24 | 98.35 | 97.21 |
| | | | 101.38 | 98.16 | 101.87 | 98.36 |
| **Intra-day** | | | 101.81 | 97.67 | 100.83 | 96.31 |
| | | | 102.17 | 98.04 | 101.56 | 97.72 |
| | | | 102.86 | 97.81 | 102.98 | 98.05 |
| | | | **Relative Standard Deviation** | | | |
| | | | **PCR** | | **PLS** | |
| | | | AT | EZ | AT | EZ |
| **Inter-day** | | | 3.69 | 2.34 | 2.41 | 3.22 |
| | | | 2.15 | 2.17 | 1.98 | 1.83 |
| | | | 1.38 | 0.85 | 0.68 | 0.76 |
| **Intra-day** | | | 3.01 | 0.37 | 2.21 | 2.03 |
| | | | 2.51 | 2.99 | 1.83 | 2.63 |
| | | | 1.51 | 0.61 | 1.08 | 0.57 |
| | | | **Relative Error** | | | |
| | | | **PCR** | | **PLS** | |
| | | | AT | EZ | AT | EZ |
| **Inter-day** | | | 2.23 | -2.97 | 3.54 | -3.14 |
| | | | -1.04 | -2.76 | -1.66 | -2.79 |
| | | | 1.38 | -1.84 | 1.87 | -1.64 |
| **Intra-day** | | | 1.81 | -2.33 | 0.83 | -3.69 |
| | | | 2.17 | -1.97 | 1.56 | -2.29 |
| | | | 2.86 | -2.19 | 2.98 | -1.95 |

Precision and accuracy of the methods were also evaluated by inter-day and intra-day studies. Validation samples at three concentration levels (10 µg/ml, 20 µg/ml, 30 µg/ml) of both drugs was analyzed three times in one day (intra-day) and on three consecutive days (inter-day). The analysis results were summarized in Table 3, presented as mean percent recovery, relative standard deviation, and percent relative error.

The selectivity of the proposed methods was investigated by standard addition studies. The predicted concentration of AT and EZ in the sample prepared without the addition of standard was subtracted from the predicted concentration of AT and EZ in the remaining samples. The experiments were performed in triplicates. The average of added amounts, mean recoveries and relative standard deviations were listed in Table 4. The standard addition studies demonstrated that tablet excipients had no interferent effect on the predicted concentrations.

**Table 4.** Analysis results of standard addition studies

| | **Added** | | **Found (µg/µl)** | | | |
|---|---|---|---|---|---|---|
| | **(µg/µl)** | | **PCR** | | **PLS** | |
| | **EZ** | **AT** | **EZ** | **AT** | **EZ** | **AT** |
| Sample solution + | 10 | 10 | 10.07 | 9.65 | 9.85 | 9.71 |
| Sample solution + | 20 | 20 | 20.41 | 20.14 | 20.16 | 20.21 |
| Sample solution + | 30 | 30 | 30.27 | 29.74 | 30.05 | 29.81 |
| | | | **Recovery (%)** | | | |
| | | | **PCR** | | **PLS** | |
| | | | EZ | AT | EZ | AT |
| | | | 100.7 | 96.5 | 98.5 | 97.1 |
| | | | 102.1 | 100.7 | 100.8 | 101.0 |
| | | | 100.9 | 99.1 | 100.2 | 99.4 |
| | | | **Relative standard deviation** | | | |
| | | | **PCR** | | **PLS** | |
| | | | EZ | AT | EZ | AT |
| | | | 0.04 | 0.04 | 0.04 | 0.04 |
| | | | 0.11 | 0.07 | 0.12 | 0.07 |
| | | | 0.03 | 0.10 | 0.08 | 0.09 |

## Assay Results of Commercial Samples

The developed PCR and PLS methods were applied to the quantitative analysis of commercial sample solutions. The assay results of commercial tablets are presented in Table 5 as milligrams per tablet. The average assay results confirmed well with the label claim. The assay results from using PCR and PLS were compared using the F-test and t-test. The computed F- and t-statistic values were smaller than the critical values, indicating comparable results in terms of variance and mean. The analysis results obtained by PCR and PLS models did not significantly differ for either AT or EZ.

In this study, two new spectrophotometric methods based on multivariate regression were developed for the co-estimation AT and EZ in fixed-dose tablet formulations. The regression methods, namely PCR and PLS, were used to overcome the spectral overlapping problem by using the latent variables. The analytical performance of the proposed methods was confirmed by analytical validation studies. The developed PCR and PLS models were applied to the simultaneous estimation of EZ and AT in film-coated tablet formulations. Both methods showed good agreement with the label claim, with no significant difference between them. The proposed methods were directly applicable for the pharmaceutical analysis and did not require laborious sample preparation, extraction, nor separation steps. This study shows PCR and PLS models can be combined with spectrophotometry to ensure accurate, precise, reliable, and efficient analysis of pharmaceuticals containing two active pharmaceutical ingredients.

**Table 5.** Assay results of commercial tablets containing AT and EZ

| | mg/tablet* | | | |
|---|---|---|---|---|
| | PCR | | PLS | |
| | EZ | AT | EZ | AT |
| **T1** | 9.71 | 10.19 | 10.03 | 10.10 |
| **T2** | 10.25 | 10.07 | 10.17 | 9.81 |
| **T3** | 10.25 | 10.13 | 10.22 | 9.87 |
| **T4** | 10.17 | 9.85 | 10.11 | 9.90 |
| **T5** | 10.18 | 9.82 | 10.26 | 9.97 |
| **T6** | 10.26 | 9.80 | 10.13 | 9.95 |
| **T7** | 10.44 | 10.04 | 10.18 | 9.79 |
| **T8** | 10.30 | 9.99 | 10.72 | 10.14 |
| **T9** | 10.45 | 9.95 | 10.08 | 9.71 |
| **T10** | 10.50 | 9.76 | 10.09 | 9.82 |
| **Mean** | 10.25 | 9.96 | 10.20 | 9.91 |
| **Standard deviation** | 0.22 | 0.15 | 0.19 | 0.14 |
| **Relative standard deviation** | 2.16 | 1.48 | 1.90 | 1.38 |
| **F-stat** | 1.29 | 1.18 | **F-crit** =3.18 (p=0.05) | |
| **t-stat** | 0.56 | 0.85 | **t-crit** =2.10 (p=0.05) | |

*Label claim: 10 mg AT, 10 mg EZ per tablet

## AUTHOR CONTRIBUTIONS

Concept: Z.C.E., E.D.; Design: Z.C.E., E.D.; Control: E.D.; Sources: E.D.; Materials: E.D.; Data Collection and/or Processing: Z.C.E., E.D.; Analysis and/or Interpretation: Z.C.E., E.D.; Literature Review: Z.C.E.; Manuscript Writing: Z.C.E., E.D.; Critical Review: Z.C.E., E.D.; Other: -

## CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

## ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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