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Analysis of the binary mixtures of amlodipine and atorvastatin by chemometric-spectophotometric method

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ABSTRACT

In this study, the simultaneous quantification of amlodipine and atorvastatin in pharmaceutical preparations was successfully accomplished without the need for a separation process by using chemometric calibration methods (principal component analysis method (PCA), principal component regression method (PCR), and partial least squares method (PLS). Additionally, chemometric evaluation was performed on the data obtained from UV visible field spectroscopy methods. 16 mixes containing the chemicals atorvastatin and amlodipine were created as a calibration (concentration) set to assess the validity of the designated chemometric procedures. The calibration set's absorption spectrum was captured between 200 and 500 nm. The link between the calibration set and the absorption data collected in the 215–355 nm region was used to produce three chemometric calibrations. Amlodipine and atorvastatin synthetic mixtures were analyzed to assess the validity of the PCA, PCR, and PLS approaches. The percentage (%) recovery average for atorvastatin and amlodipine was determined for both the drug sample and the synthetic mixture using the approach we used. It was discovered that the computed values were fairly high.The methodologies were controlled, and the ANOVA test was run using the results of the PLS and PCR calibration techniques. The enhanced technique might be suggested for the examination of medication samples containing atorvastatin and amlodipine since it is among the most sensitive, accurate, and repeatable techniques available.

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1. Introduction

One definition of high blood pressure is hypertension, or high blood pressure. A person with hypertension has a systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg or higher.Hypertension is described as the usage of antihypertensive medications.Hypertension is linked to cardiac problems, kidney function, and stroke.It is also possible to see disorders [1].

Amplodipine (AML) belongs to the dihydropyridine class of calcium channel blockers (Figure 1.1). It blocks the smooth muscle cell membrane's and myocardium's calcium channels from allowing calcium ions to enter the cell. AML targets smooth muscle cells specifically. AML reduces peripheral resistance and systemic blood pressure by depressing peripheral carterioles and blocking transmembrane calcium flow. After oral administration, AML is well absorbed, reaching peak plasma concentrations in 6–12 hours [2].

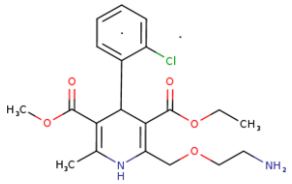

Figure 1. *Chemical Structure of Amlodipine*

The chemical formula of the active ingredient atorvastatin is [R-(R*, R*)]. -2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl) -3-phenyl-4-[(phenylamino)carbonyl] 1-Hpyrrole-1-heptanoic acid, Atorvastatin [3] is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor. This enzyme catalyzes HMG-CoA conversion through an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin has been used to reduce low-density lipoprotein (LDL) cholesterol, total cholesterol, apo-B, triglyceride levels, and C-reactive protein and increase lipoprotein levels.

Atorvastatin is used along with weight loss, diet, and exercise to reduce the risk of heart attack, heart disease, stroke, and the likelihood of developing heart disease [4].

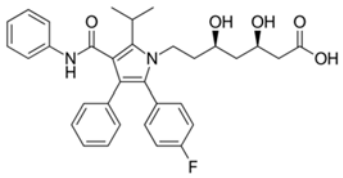

Figure 2. *Chemical Structure of Atorvasvatin*

Spectrophotometric techniques are frequently employed for the simultaneous measurement of several compounds in mixtures. It is claimed that the approach is affordable and that the outcomes are exact and accurate [5]. The two most popular chemometric techniques are principal component regression (PCR) and partial least squares regression (PLS) [6]. These techniques are widely acknowledged for the examination of pharmaceutical goods with many components [7].

In this study, chemometric determinations of cholesterol drugs were made by evaluating spectrophotometric data. The method used chemometrically is the Partial Least Squares Method (PLS). The method applied by chemometry has been given sensitivity, and both active drug substances can be analyzed next to each other without any preliminary separation. The data obtained from the method were evaluated analytically as statistical (RSD, LOD, LOQ, PRESS, SEC, RMSEC) in order to express the data more meaningfully and with higher quality.

2. Aim and method

In this study, drugs used to hypertension were evaluated in terms of UV/VIS spectrophotometric and chemometric aspects. Chemometric analysis was performed to evaluate the results. The UV 1700 Pharmaspec Shimadzu (Shimadzu, Kyoto, Japan) device was used for UV measurements in the analysis. In the first step, single spectra of substances were taken, and then the appropriate synthetic mixture was examined in the tablet sample prepared in certain proportions. Finally, measurements of commercially purchased drug tablets (Caduet) were taken. In chemometric calculations, the Minitab 17 program [8] (Inova Consulting) was used.

In this study, spectra of amlodipine, atorvastatin, and systematically prepared synthetic mixtures were taken by spectrophotometric measurements, first in single form and then in a ratio identical to the drug mixture. As a final procedure, measurements were made on the drug tablet sample obtained from the pharmacy. The spectrophotomerically collected data were calculated and evaluated by different multivariate calibration methods. In the first step, the calibration (resetting process) of the UV spectrophotometer device was performed. The calibration process was first performed against the air by leaving both cells blank. Then, the same process was done by placing a blind sample prepared with our solvent that we used in both light paths. In all readings, the blind is always prepared in this way. In order to eliminate the interference effects while choosing the blind, the blind solvent was preferred. In the last step, the commercial tablet (Caduet) is examined. While preparing the drug sample, all the tablets included in the package are crushed in an agate mortar, thinned and mixed. Weighing the weight of a tablet, it is dissolved in solvent, mixed in a magnetic stirrer to homogenize and absorbance reading is performed.

3. Findings and application

3.1. Absorption Spectra of Amlodipine and Atorvastatin Drug Active Ingredients

100 µg/mL (ppm) solutions were prepared by using 0.1 M methanol as solvent in 25 mg/250 mL of amlodipine and atorvastatin active ingredients. In the next stage, the solutions prepared to analyze the spectroscopic properties of each substance were prepared in the range of 5-50 µg/mL for each substance. The wavelength at which the active ingredients amlodipine and atorvastatin give the maximum spectrum (amplodipine: 237 nm; atorvastatin: 247 nm) was determined (Figure 3. and 4.)

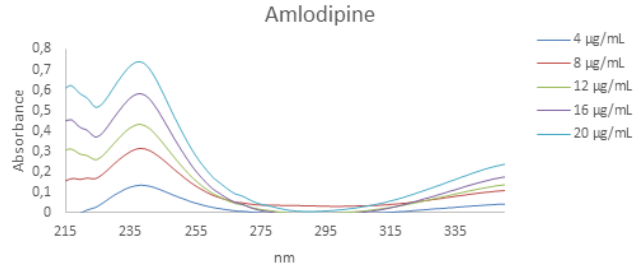

Figure 3. *Absorption spectrum of amplodipine active ingredient in the range of 4-20 µg/mL*

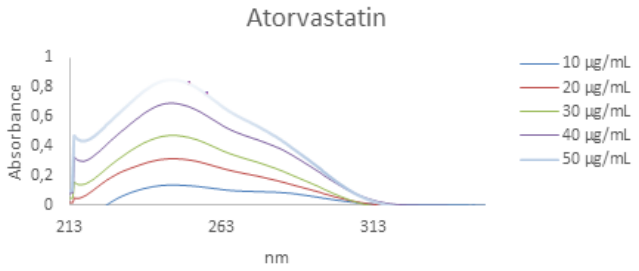

Figure 4. *Absorption spectrum of atorvastatin active ingredient in the range of 10-50 µg/mL*

The absorbance value rises with increasing concentration, as can be seen by examining the absorbance-wavelength graphs for atorvastatin and amlodipine.

Amlodipine and Atorvastatin were prepared in the range of 5-50 µg/mL (Table 1.) in 16 synthetic mixture solutions.

Table 1. *Calibration set with Amlodipine and Atorvastatin*

	Atorvastatin µg/mL	Amplodipine µg/mL
1	10	5
2	20	10
3	30	15
4	40	20
5	50	25
6	10	5
7	20	10
8	30	15
9	40	20
10	50	25
11	10	5
12	20	10
13	30	15
14	40	20
15	50	25
16	10	5

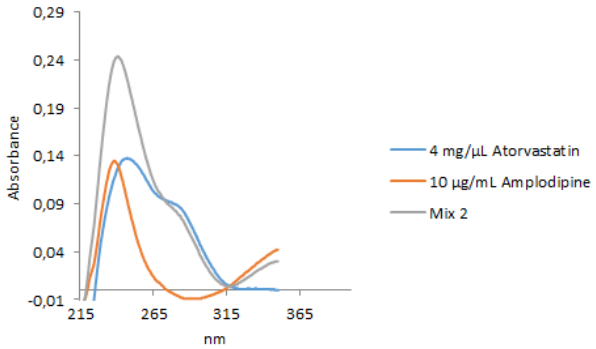

Figure 5. *Absorption-wavelength graph of the synthetic mixture containing amlodipine and atorvastatin.*

UV spectroscopy was used to determine the absorbance values of these combination solutions (Figure 5). The chemometry method was then used to analyze the obtained data. Chemometric calculations are the most widely used, dependable, and expedient techniques for figuring out how much of each component is present in multicomponent mixtures. The idea behind the relationship between absorbance and concentration is that when the principal component is analyzed using the regression approach, the absorbance values measured for the concentration set can be broken down into orthogonal lines. The computations are predicated on the correctness of these lines, which represent the coordinate order of the calibration that needs to be established [9].

In this study, partial least squares method (PLS) and principal component regression (PCR) were used as chemometric methods. The correlation matrix created with the help of the principal components obtained by principal component analysis guides other chemometric regressions. Among the chemometric methods, the methods used in this study are partial least squares method (PLS) and principal component regression (PCR) [10,11, 12,13].

Table 2. *Results calculated by principal component regression of each substance in the mixture containing Amlodipine and Atorvastatin.*

	Atorvastatin			**Amplodipine**		
No	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
1	10	9.95	99.50	5	4.97	99.4
2	20	19.92	99.60	10	9.89	98.9
3	30	29.87	99.57	15	14.79	98.6
4	40	38.97	97.43	20	19.97	99.85
5	50	49.98	99.96	25	24.69	98.76
6	10	9.05	90.50	5	4.95	99
7	20	19.88	99.40	10	9.89	98.9
8	30	29.96	99.87	15	14.97	99.8
9	40	39.95	99.88	20	19.94	99.7
10	50	49.86	99.72	25	24.45	97.8
11	10	9.97	99.70	5	5.01	100.2
12	20	18.96	94.80	10	9.97	99.7
13	30	29.95	99.83	15	14.96	99.73
14	40	39.97	99.93	20	19.98	99.9
15	50	48.99	97.98	25	24.98	99.92
16	10	9.67	96.70	5	4.97	99.4
			Mean=98.40			Mean=99.35
			% RSD=0.026			%RSD=0.063

Table 3. *Results calculated by the method of partial least squares of each substance in the mixture containing Amlodipine and Atorvastatin.*

	Atorvastatin			Amplodipine		
No	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
1	10	9.89	98.9	5	5.01	100.20
2	20	20.02	100.1	10	9.94	99.40
3	30	28.99	96.63	15	15.04	100.27
4	40	39.87	99.68	20	19.96	99.80
5	50	49.56	99.12	25	24.88	99.52
6	10	9.94	99.4	5	4.97	99.40
7	20	19.47	97.35	10	9.97	99.70
8	30	29.86	99.53	15	14.98	99.87
9	40	39.97	99.93	20	19.92	99.60
10	50	49.81	99.62	25	24.89	99.56
11	10	9.98	99.8	5	4.97	99.40
12	20	19.97	99.85	10	9.89	98.90
13	30	30.01	100.03	15	14.99	99.93
14	40	39.95	99.88	20	19.87	99.35
15	50	49.98	99.96	25	24.92	99.68
16	10	9.98	98.90	5	5.05	101.00
			Mean=99.35			Mean=99.72
			%RSD=0.01			%RSD=0.048

3.2. Chemometric Approaches

To validate calibrations for drug mixes that are synthetic, certain statistical factors were added. Tables 2, 3, and 4 display the recovery and relative standard deviation (rsd) values that were determined for each chemometric method 1. To avoid inaccuracies in the drug sample, the cross-validation process was used while the concentrations versus the additional concentrations were determined [14,15].

The ICH criteria [16,17,18] were followed in the validation process of the chemometric approach with regards to linearity, accuracy, intraday and interday precision, limit of detection, and limit of quantitation. For calibration, the following formula was used to forecast the residual error sum-of-squares (PRESS) and standard error of prediction (SEC) (Table 4) based on the actual and anticipated concentrations of the samples:

$$PRESS = \sum_{i=1}^{n} \left(C_i^{added} - C_i^{found} \right)^2 \quad (1)$$

where C_i^{added} – actual concentration, the added concentration of the drug; C_i^{found} : predicted concentration, the calculated concentration of the drug.

$$SEC = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2}{n-1}} \quad (2)$$

where n – the total number of synthetic mixtures.

Another validation parameter is RMSEC [19]. It is given in the below equation 3.

$$RMSEC = \sqrt{PRESS/n} \quad (3)$$

The equations of the observable limit (LOD) and detection limit (LOQ) parameters are shown below. These expressions are interrelated but have different equations (equation 4 and 5) [20].

$$LOD = 3Sa/m \quad (4)$$
$$LOQ = 10Sa/m \quad (5)$$

m: Slope

LOQ> LOD and LOQ = LOD were taken into consideration while evaluating the calculated LOD values [21].

Table 4. *Calculated Analytical Parameters*

Parameters	Method	Atorvastatin	Amplodipine
λ_{max} (nm)		247.0 nm	237.0 nm
Correlation Coefficient (R^2)		0.9997	0.9997
SEC	PLS	0.059	0.020
	PCR	0.106	0.036
PRESS	PLS	0.099	0.0051
	PCR	0.280	0.032
RMSEC	PLS	0.081	0.019
	PCR	0.136	0.046
LOD (µg/mL)	PLS	0.512	0.083
	PCR	0.789	0.240
LOQ(µg/mL)	PLS	1.552	0.251
	PCR	2.390	0.719

Table 5. *PLS and PCR techniques were used to extract the pharmaceutical formulation's paracetamol and amoxicillin contents.*

	Atorvastatin (gram)	
NO	PLS	PCR
1	9.89	9.98
2	9.91	9.95
3	9.97	9.89
4	9.92	10.01
5	9.85	9.94
Mean±SD*	9.908±0.004	9.954±0.005

	Amplodipine (gram)	
NO	**PLS**	**PCR**
1	4.97	5.01
2	4.89	4.96
3	4.95	4.92
4	4.82	4.97
5	4.86	4.90
Mean±SD*	4.898±0.0013	0.4952±0.009

4. Conclusion

In this study, the data obtained by UV spectrophotometry were calculated using multivariate calibration methods. Thus, theoretical and experimental calculations were evaluated together. Considering the calculations, a quantitative determination of the active ingredients of atorvastatin and amplodipine in the drug sample obtained from the pharmacy was made.

Individual UV spectra for the recognition of the active ingredients atorvastatin and amplodipine primarily Below. In the next stage, the spectrum was obtained by preparing a synthetic mixture identical to the drug sample obtained from the pharmacy. For atorvastatin and amplodipine, using the chemometric method (PCR and PLS), the method was calculated statistically.

The recovery values were acceptably large, and the standard deviations were calculated in accordance with the study. In the establishment of PLS and PCR calibrations for the quantification of these substances in mixtures containing binary drug substances, the sum of the squares of the predicted errors in the cross-validation process (Predicted Resudiual Error Some of Squares→ PRESS) and the standard error of calibration (SEC) values close to zero increase the accuracy and reliability. It has been observed that the PRESS and SEC values are small enough, or even close to zero. LOQ values are also small compared to LOD values.

The methods developed are reproducible, have high sensitivity and accuracy, produce fast results, and can be recommended for the analysis of different drug samples containing atorvastatin and amplodipine.

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