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Simultaneous Spectrophotometric Determination of Hypertension Drug's in Commercial Pharmaceutical by Chemometric Methods

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Abstract
In this study, chemometric approaches using UV spectrophotometry method developed models for simultaneous determination of valsartan (VAL) and amlodipine (AMP) in drug samples. It was used to calculate calibration mixtures between 232 and 254 nm wavelengths at 2 nm intervals for Val and AML spectra at various concentrations. For chemometric analysis of data, the least squares calibration method and basic component regression were used, and the parameters of chemometric procedures were optimized. The analytical performances of this chemometric method were compared by characterizing the sum of residual error squares (PRESS), estimated standard error (SEP), and recoveries (%). A number of synthetic mixtures containing different concentrations of VAL and AML were studied to control the predictive ability of Applied chemometric methods. This method was successfully applied to the actual samples, not affected by the auxiliaries as indicated in the recovery study results. The results obtained in this review encourage these chemometric methods to implement these strategies for standard research and quality control of the two active ingredients.

Keywords: Valsartan, Amplodipine, Partial Least Squares Calibration, Principal Component Regression

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Ticari İlaçlarda Hipertansiyon İlaçlarının Kemometrik Yöntemlerle Eşzamanlı Spektrofotometrik Tayinleri

Özet
Bu çalışmada, UV spektrofotometri yöntemi kullanılarak kemometrik yaklaşımlarla ilaç örneklerinde valsartan (VAL) ve amlodipin (AMP) eşzamanlı tayini için modeller geliştirilmiştir. Çeşitli konsantrasyonlarda VAL ve AML spektrumları için 2 nm aralıklarla 232 ile 254 nm dalga boyları arasındaki kalibrasyon karışımlarını hesaplamak için kullanıldı. Verilerin kemometrik analizi için en küçük kareler kalibrasyon yöntemi ve temel bileşen regresyonu kullanıldı ve kemometrik prosedürlerin parametreleri optimize edildi. Bu kemometrik yöntemin analitik performansları, artık hata karelerinin (PRESS), tahmini standart hatanın (SEP) ve geri kazanımların (%) toplamını karakterize ederek karşılaştırıldı. Farklı konsantrasyonlarda VAL ve AML içeren bir dizi sentetik karışım, uygulanan kemometrik yöntemlerin tahmin kabiliyetini kontrol etmek için çalışıldı. Bu yöntem, gerçek numunelere başarıyla uygulandı, geri kazanım çalışması sonuçlarında belirtildiği gibi yardımcı

maddelerden etkilenmedi. Bu incelemede elde edilen sonuçlar, bu kemometrik yöntemleri, iki aktif bileşenin standart araştırma ve kalite kontrolü için bu stratejileri uygulamaya teşvik etmektedir.

Anahtar Kelimeler: Valsartan, Amplodipin, En Küçük Kareler Kalibrasyonu, Temel Bileşen Analizi

1. Introduction

Multivariate calibration methods have been an important application area for chemometry, as they have been applied to many different branches, and especially to analytical chemistry. Many of the applied chemometric methods involve multivariate calibration. In recent studies of chemometry, most of their development, especially in the last two decades, has been based mainly on the applications of the partial least squares (PLS) algorithm and basic component regression (PCR). PLS and PCR are generally considered to be the main regression techniques for multivariate data. As a result of applying such useful chemometric methods to combined drug analyses, fast, simple, cheap and reproducible results are obtained.

Modern spectroscopic instruments used today are so fast that they can produce hundreds of spectra in minutes for a given sample containing multiple components. On the contrary, univariate calibration methods require a system without interference and are quite slow as they are not suitable for such data. Multivariate calibration is especially preferred today in drug analysis, as it is studied in many fields, as it relates to data containing device responses measured at multiple wavelengths for a sample containing multiple components.

The popular advances in chemometrics and computers in recent years have led to the development of several variable calibration methods (Haaland et al, 1988; Wentzell et al, 1997) for the analysis of complex chemical mixtures such as drug formulations.

Amlodipine/valsartan is a blood pressure lowering combination drug. It contains amlodipine, a dihydropyridine-type calcium channel blocker, and valsartan, an angiotensin receptor blocker. This combination is usually well tolerated and effective for the reduction of blood pressure (Eckert et al, 2013).

Amlodipine is used with or without other medications to treat high blood pressure. Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Amlodipine belongs to a class of drugs known as calcium channel blockers. It works by relaxing blood vessels so blood can flow more easily. Amlodipine is also used to prevent certain types of chest pain (angina). It may help to increase your ability to exercise and decrease the frequency of angina attacks. It should not be used to treat attacks of chest pain when they occur.

Valsartan, sold under the trade name Diovan among others, is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease (Corea et al, 1996). It is a reasonable initial treatment for high blood pressure. It is taken by mouth. Versions are available as the combination valsartan/hydrochlorothiazide, valsartan/amlodipine, valsartan/amlodipine/hydrochlorothi azide, or valsartan/sacubitril (Oparil et al, 1996). Common side effects include feeling tired, dizziness, high blood potassium, diarrhea, and joint pain. Other serious side effects may include kidney problems, low blood pressure, and angioedema. Use in pregnancy may harm the baby and use when breastfeeding is not recommended. It is an angiotensin II receptor antagonist and works by blocking the effects of angiotensin II.

In the presence of excipients in the examples, without any separation, the mixture, containing two or more compounds, determining the solubility of the systems at the same time is one of the important issues of the pharmaceutical industry and its analytical chemistry. The literature survey reveals that several methods were reported for the individual estimation of VAL and AMP. The simultaneous quantitative determination of both drugs at the same time in pharmaceutical tablets using various methods including HPLC and first derivative of the ratio spectrophotometric (Kul et al, 2010 ; Varghese, 2011), HPLC and Thin layer Chromatography (Varghese and Ravi, 2011), spectrophotometric (Chitlange et al, 2008; Mohammed 2011), HPLC-MS (Lena et al, 2007), TLC (Dhaneshwar , 2009) have been described for many mixtures. Working two forms of active ingredient are shown in Figure 1.

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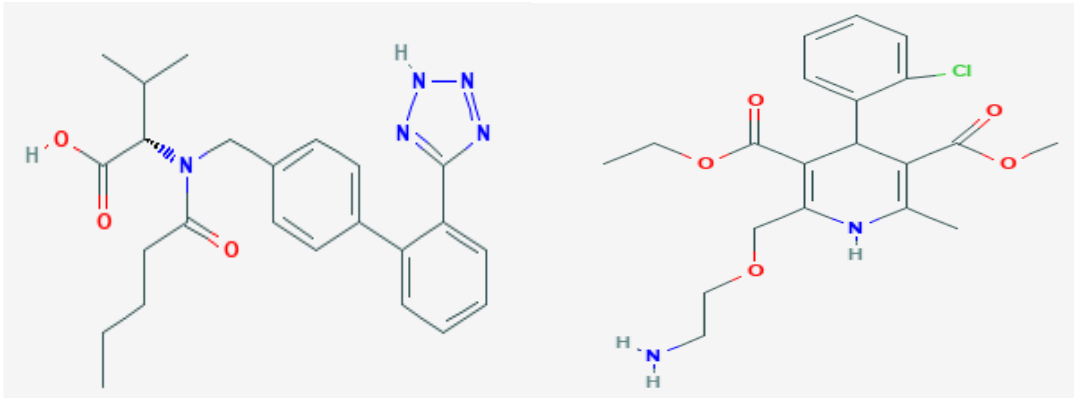

a) b)

Figure 1. Structure of the drugs a) VAL b) AML

The multivariate calibration techniques use full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection calibration model. In addition, these multivariate calibrations do not need any separation procedure, they are very cheap, very easy to apply and very sensitive. For these reasons these multivariate techniques are popular today.
In this study, two powerful chemometric methods were applied to analyze synthetic mixtures and tablets consisting of VAL and AML in the presence of interactions of absorption spectra. The application of chemometrics is vital to the success of identifying clinical drugs at the same time as it allows interpretation of multivariate data.

2. Experimental Section

2.1. Apparatus

A Shimadzu (Model UV-1700) UV-Visible spectrometer (Shimadzu, Kyoto, Japan), equipped with 1cm matched quartz cells was used for spectrometric measurements.

2.2 Standard solutions

Analytical grade materials were used in experiments. Stock solutions of 100 mg/100 mL VAL and AML were prepared in 0.1 M Methanol. The solutions were stable for the least a week if they had been stored in a cool (< 25^{o}C) and dark place.

2.3. Pharmaceutical preparations A commercial drug preparations; Exforge® tablet produced by Novartis Pharma, Turkey, containing 10 mg VAL and 160 mg AML per tablet, was analyzed by the proposed chemometric techniques.

2.4. Procedure for dosage form

A precisely weighed pummeled tablets comparable to 100 mg of the considered medications was separated with 10 mL of methanol, and sonicated for around 30 min. The concentrates were separated into 100 mL volumetric carafes at that point washed and weakened to volume with refined water. Aliquots these arrangements were moved into a progression of 10 mL volumetric jars and the examination were finished as spectrometric method. Every one of the systems were connected to the last arrangement.

2.5. Chemometric methods

The PLS and PCR chemometric methods are factor research techniques; it is a prediction attempt in which a scientific model is studied using partial foci and ghost information from a reference arrangement, followed by an adjustment attempt in which the model is used to determine. The uncertain specimen is detected in its range. These techniques are similarly called factor strategies because they convert the first factors into less symmetric factors called elements or basic components (PCs), which are flat mixtures of the first factors. At the point where multivariate tuning approaches are connected in spectrophotometric multi-segment examination, a link is created between the result and

fixing information obtained from reference tests by referring to the factors of the framework. New factors are created by PCs. The calculation of this new lattice is regulated by the calculation open to the repetition technique adopted.
The real distinction in the current capabilities of these two strategies is that PLS predict that it is superior to PCR when there are freely different large ghost segments covering random direct baselines or imaginary highlights of the study. The ideal setting technique is based on specific test conditions. Regardless, PLS seems to have made a sensible decision about a wide variety of conditions.

3. Results and Discussion

In order to build the chemometric calibration, a training set was randomly prepared by using the standard mixture solution containing 1.0 - 3.0 µg/mL VAL and 0.5 - 2.5 µg/mL AML in the variable proportions as shown in Table 1. The absorbance data matrix was obtained by measuring at the 12 wavelengths with the intervals $\Delta\lambda$ = 2 nm in the 232 – 254 nm spectral region. The prepared calibrations of two techniques using the absorbance data sets were used to predict concentration of the unknown values of VAL and AML in their mixture.

Table 1. Concentration set design for the preparation of PLS and PCR calibration

Calibration sets					
Mix	VAL(µg/mL)	AML(µg/mL)	**Mix**	VAL(µg/mL)	AML(µg/mL)
1	1.0	0.5	**9**	1.8	2.0
2	1.5	1.0	**10**	1.8	2.5
3	2.0	1.5	**11**	1.0	1.3
4	2.5	2.0	**12**	1.5	1.3
5	3.0	2.5	**13**	2.0	1.3
6	1.8	0.5	**14**	2.5	1.3
7	1.8	1.0	**15**	3.0	1.3
8	1.8	1.5			

A calibration for each technique was computed in the MINITAB 16.0 and PLS Toolbox 4.0 software by using set consisting of two drugs and their absorbance data. The multivariate calibrations of two techniques were used to predict the unknown concentrations of VAL and AML in the samples.
The application adequacy of a calibration model can be explained in several ways. Validation of the calibrations configured for the training set and synthetic binary mixtures of both drugs can be verified by statistical parameters. These results can also be examined numerically. One of the best ways to do this is by reviewing the estimated residual error frames total or PRESS. To calculate PRESS, it calculates errors between expected and predicted values for all samples, squares and is combined.

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

Strikingly speaking, this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. If we want correctly compare PRESS values for data sets that contain differing numbers of samples, we should convert to standard error of prediction (SEP), which is given by following formula.

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

Where C_i^{added} the added concentration of drug is, C_i^{found} is the found concentration of drug and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. Often, however, the performance of the calibration model

varies depending on the analyte level. In the application of two chemometric techniques to the synthetic mixtures containing two drugs in variable compositions, the mean recoveries and relative standard deviations for PLS and PCR were found to be 99.9938%, 0.4252; 100.0035%, 0.0921 and 100.0011%, 0.0005; 99.9718%, 0.0101 respectively for VAL and for AML (Table 2).

Table 2. Recovery values for the applied chemometric methods

Mixture (µg/mL)		**Recovery (%)**			
		PLS	**PCR**		
VAL	**AML**	**VAL**	**AML**	**VAL**	**AML**
20	**10**	**99.8860**	**100.0640**	**100.0005**	**99.9580**
25	**10**	**99.7856**	**100.1490**	**100.0004**	**99.9560**
30	**10**	**99.9780**	**100.0180**	**100.0003**	**99.9520**
10	**15**	**100.5740**	**99.8933**	**100.0020**	**99.9760**
15	**15**	**100.5080**	**99.8586**	**100.0013**	**99.9720**
20	**15**	**100.0810**	**99.9700**	**100.0010**	**99.9707**
10	**20**	**99.1490**	**100.1185**	**100.0020**	**99.9805**
15	**20**	**100.1907**	**99.9600**	**100.0013**	**99.9790**
20	**20**	**100.0195**	**99.9945**	**100.0010**	**99.9770**
15	**25**	**99.3406**	**100.1100**	**100.0013**	**99.9816**
20	**25**	**100.3725**	**99.9172**	**100.0010**	**99.9804**
25	**25**	**100.0408**	**99.9888**	**100.0008**	**99.9792**
	Mean	**99.9938**	**100.0035**	**100.0011**	**99.9718**
	RSD*	**0.4252**	**0.0921**	**0.0005**	**0.0101**

RSD*: Relative Standard Deviation

According to the added concentration and the concentration found in samples, the PRESS and SEP values of PLS and PCR techniques were calculated 0.0028;0.0393 and 0.0002; $4.75.10^{-7}$, 0.0550;0.0153 and 0.0044; 0.0002 respectively for VAL and AML (Table 3).

Table 3. Statistical parameters in the calibration-prediction for PLS and PCR methods

Parameter	**PLS**		**PCR**	
	VAL	AML	VAL	AML
PRESS	0.0028	0.0393	0.0002	$4.75.10^{-7}$
SEP	0.0550	0.0153	0.0044	0.0002
r	0.9999	1.0000	1.0000	1.0000
Intercept	0.9999	1.0000	1.0000	1.0000
Slope	0.0016	0.0001	0.0002	0.0039

Linear regression analysis of the concentration added for each drug and each calibration technique and the concentration contained in the synthetic mixtures was performed. In this regression analysis, the correlation coefficient (r), intercept, slope and relative standard deviation values for the chemometric

techniques suggested in Table 3 were found satisfactory. As can be seen, all statistical values showed that all techniques are suitable for determining two active ingredients in synthetic mixtures.
Accuracy and precision for the analysis of VAL and AML substances in the prepared synthetic mixtures at three different concentration levels (15.00, 20.00 and 25.00 µg/mL for VAL and 10.00, 15.00 and 20.00 µg/mL for AML) in intra-day (n=6) and inter-day (n=6), was tested for the applicability of the proposed chemometric methods. The calculated results for percent relative error, standard deviation and relative standard deviation were presented in table 4 and 5. Good accuracy and precision were observed for the results obtained by PLS and PCR calibrations.

Table4. Accuracy and precision results for PLS

Intra- day (n=6)

Added (µg/ml)		VAL					AML				
VAL	AML	Found	SD	% BSS	% RE	Recovery (%)	Found	SD	% BSS	% RE	Recovery (%)
15.00	10.00	14.9993	0.1247	0.0012	-0.0047	99.9953	9.9993	0.1841	0.0018	-0.0070	99.9930
20.00	15.00	20.0017	0.1087	0.0011	0.0085	100.0086	15.0017	0.1450	0.0014	0.0113	100.0115
25.00	20.00	24.9990	0.1400	0.0014	-0.0040	99.9960	19.9990	0.1750	0.0017	-0.0050	99.9950
					$\bar{x}$	99.9999				$\bar{x}$	99.9998
					SD	0.0075				**SD**	0.0102
					BSS	$7.48.10^{-5}$				**BSS**	0.0001
					LOD	0.0247				**LOD**	0.0335
					LOQ	0.0748				**LOQ**	0.1015

Inter-day (n=6)

Added (µg/ml)		VAL					AML				
VAL	AML	Found	SD	% BSS	% RE	Recovery (%)	Found	SD	% BSS	% RE	Recovery (%)
15.00	10.00	15.0045	0.2577	0.0025	0.0300	100.0097	10.0015	0.3865	0.0039	0.0150	100.0145
20.00	15.00	19.9978	0.2119	0.0021	-0.0110	99.9892	14.9978	0.2826	0.0028	-0.0147	99.9860
25.00	20.00	25.0006	0.1825	0.0018	0.0024	100.0027	20.0007	0.2282	0.0023	0.0035	100.0034
					$\bar{x}$	100.0005				$\bar{x}$	100.0012
					SD	0.0104				**SD**	0.0146
					% BSS	0.0001				**% BSS**	0.0002
					LOD	0.0344				**LOD**	0.0481
					LOQ	0.1042				**LOQ**	0.1458

Table5. Accuracy and precision results for PCR

Intra- day (n=6)

Added (µg/ml)		VAL					AML				
VAL	AML	Found	SD	% BSS	% RE	Recovery (%)	Found	SD	% BSS	% RE	Recovery (%)
15.00	10.00	15.0001	$3.61.10^{-5}$	$3.61.10^{-7}$	0.0007	100.0006	9.9948	0.0006	$5.62.10^{-6}$	-0.0520	99.9485
20.00	15.00	20.0001	$1.76.10^{-5}$	$1.76.10^{-7}$	0.0005	100.0004	14.9945	0.0001	$1.37.10^{-6}$	-0.0367	99.9636
25.00	20.00	25.0001	$1.71.10^{-5}$	$1.71.10^{-7}$	0.0004	100.0005	19.9924	0.0007	$7.13.10^{-6}$	-0.0380	99.9622
					x̄	100.0005				**x̄**	99.9581
					SD	0.0001				**SD**	0.0083
					BSS	1.10^{-6}				**BSS**	$8.35.10^{-5}$
					LOD	0.0003				**LOD**	0.0275
					LOQ	0.0010				**LOQ**	0.0834

Inter-day (n=6)

Added (µg/ml)		VAL					AML				
VAL	AML	Found	SD	% BSS	% RE	Recovery (%)	Found	SD	% BSS	% RE	Recovery (%)
15.00	10.00	14.9999	$1.03.10^{-5}$	$1.03.10^{-7}$	-0.0007	99.9994	9.9998	$2.16.10^{-6}$	$2.16.10^{-8}$	-0.0020	99.9986
20.00	15.00	19.9999	$1.14.10^{-5}$	$1.14.10^{-7}$	-0.0005	99.9996	14.9998	$1.30.10^{-5}$	$1.30.10^{-7}$	-0.0013	99.9991
25.00	20.00	24.9999	$9.67.10^{-5}$	$9.67.10^{-8}$	-0.0004	99.9998	19.9998	$1.53.10^{-5}$	$1.53.10^{-7}$	-0.0010	99.9993
					x̄	99.9998				**x̄**	99.9990
					SD	0.0002				**SD**	0.0004
					% BSS	2.10^{-6}				**% BSS**	$3.61.10^{-6}$
					LOD	0.0007				**LOD**	0.0012
					LOQ	0.0020				**LOQ**	0.0036

A summary of the assay results for the pharmaceutical formulation is given Table6. The results of all methods were very to each other as well as to the label value of commercial drug formulation.

Table 6. Assay results for the pharmaceutical formulation (mg/tablet)

Drug	**PLS**	**PCR**
VAL		
Mean ± SD*	159.84±0.12	160.02±0.02
AML		
Mean ± SD*	9.96±0.03	9.99±0.04

Results obtained are average of six experiments for each technique.
*SD : Standard deviation

Conclusion

PLS and PCR, powerful chemometric methods in spectrophotometric examination, have been proposed for simultaneous determination of VAL and AML in binary mixtures. Applied chemometric method strategies were successfully implemented in a commercial drug tablet. Exceptionally, the goals of covering drug mixtures have been achieved using PLS and PCR methods. As can be seen from the

results obtained, PLS and PCR chemometric methods have been shown to give accurate and reliable results in this drug mixture.

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