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Application of Chemometric Methods to Quantative Analysis of Drugs used in Parkinson's Disease

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Abstract

In this study, precise, sensitive and accurate spectrophotometric with combinated chemometric methods were developed for drugs used in Parkinson's disease in pharmaceutical tablet. The commonly used Parkinson's drugs are levodopa and carbidopa. The used chemometric methods are partial least squares regression (PLS) and principal component regression (PCR). PLS and PCR were successfully applied for chemometric analysis of levodopa and carbidopa in synthetic mixtures and pharmaceutical tablets. Absorbance and concentration values were used in Minitab and other chemometric programs to calculate estimated concentrations with PCR and PLS. In the first step, the synthetic mixtures including levodopa and carbidopa were prepared and absorbance values are obtained from spectrophotometry. The second step, in pharmaceutical tablet containing levodopa and carbidopa (Sinemet®) was calculated amounts for each drug active substance. As a result of the determination, high recoveries and low standard deviations were found. Because of recoveries and standard deviations were accomplished, this study encouraged us to apply for drug analysis. The proposed methods are highly sensitive, precise, therefore, they were successfully applied for active substances in pharmaceutical tablet.

Keywords: Levodopa; Carbidopa; PLS; PCR

Introduction

Levodopa is an antiparkinsonian and a dopamine reporter. Levodopa is integrated with an amino acid decarboxylase inhibitor such as carbidopa [1]. Levodopa is converted to dopamine in the brain and easily passes the blood brain barrier for the treatment of Parkinson's disease [2]. Another drug used in the treatment of Parkinson's disease is carbidopa, a compound related to catechol [3,4]. The chemical structures of levodopa and carbidopa are shown in Figure 1 and Figure 2.

Chemometric calibration methods have been developed from various mathematical processes to simultaneously identify two or more compounds in the same sample without chemical separation [5]. Finally, chemometric calibration methods are used for spectral data analysis involving two or more compounds with overlapping spectra [6]. The Minitab 17 program (Inova, Ankara, Turkey) was used for the analysis of all the concentration and absorbance data to do the statistical calculations. Minitab is statistical analysis software. In addition to statistical research, statistics can be used to learn [7]. The most accepted chemometric methods in drug analysis are principal component

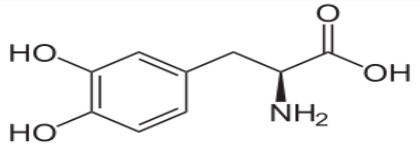

Figure 1: Chemical Structures of Levodopa.

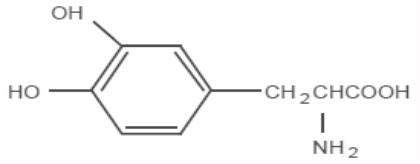

Figure 2: Chemical Structures of Carbidopa.

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Table 1: Concentration Set for Levodopa and Carbidopa.

No.	Concentration, mg/L	
	Levodopa	Carbidopa
1	4	5
2	4	10
3	4	15
4	4	20
5	4	25
6	8	5
7	8	10
8	8	15
9	8	20
10	12	5
11	12	10
12	12	15
13	16	5
14	16	10
15	20	5

regression (PCR) and partial least squares regression (PLS) [8]. There are differant analytical methods in the literatüre in which both levodopa and carbidopa are identified separately and together. Several methods have been used for the determination of Parkinson's disease, spectrophotometric methods [1-11], electrochemical methods [12-19], chromatographic methods [20-49], ATR-FTIR spectrometric method [50], NMR [51], capillary electrophoresis [52,53].

In this study, the quantative determination of levodopa and carbidopa in pharmaceutical tablet using chemometric methods. The validation of the chemometric and spectrophotometric method has acquired precision, accuracy, selectivity. PLS and PCR methods were successfully performed to the simultaneous determination of levodopa and carbidopa in pharmaceutical tablet without any separation method. Mean recoveries (%) and standard deviation of principal component regression (PCR) and partial least squares regression (PLS) methods were calculated for the validation of the methods. The acquired results were statistically compared each other.

Experimental

Materials and preparation of standard solution for absorbance measurements

All materials used were of analytical grade. Stock solutions of 25mg/250ml of levodopa (Sigma) and carbidopa (Sigma) were prepared in 0.1M HCl. A training set and validation set contained the drugs in various proportions, 15 synthetic mixtures (for validation and calibration) were made. Shimadzu UV-1700 PharmaSpec Spectrophotometer connected to an IBM PC with UV Probe Software was used for all measurements and data processing.

Absorbance spectra of levodopa and amoxicillin were recorded between 210 and 310 nm with the 0.1 range. The calibration matrix, training, and validation sets contain two components in mixtures at different rates and to calculate concentrations. The concentration sets and analysis of pharmaceutical tablet have been calculated using PLS and PCR. Samples of 4.0 and 25.0 mg/L of drugs were placed in volumetric flasks (25ml) and dissolved containing 0.1M HCl was added. A training set and validation set contain the drugs in various

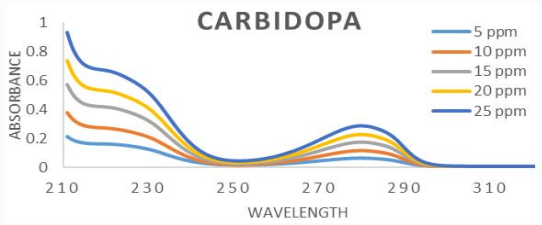

Figure 3: The Absorption Spectrum of Carbidopa.

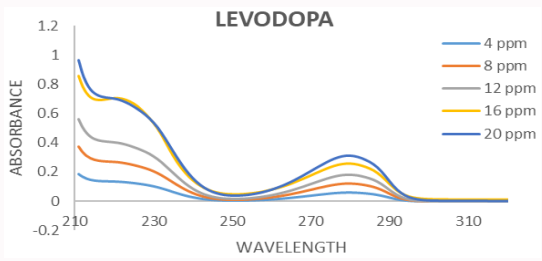

Figure 4: The Absorption Spectrum of Levodopa.

proportions, 15 synthetic mixtures (for validation and calibration) were prepared and presented in Table 1.

Preparation of pharmaceutical tablet

Sinemet produced by Merck Sharp & Dohme, containing 25mg of carbidopa and 250mg of levodopa per tablet was analyzed by the principal component regression (PCR) and partial least squares regression (PLS) chemometric methods. For this purpose, 1g of the samples were mechanically mixed with 0.1M HCl and transferred into 25ml bottles. All the techniques were applied to the final solution.

Results and Discussion

Absorption spectrum of levodopa, carbidopa and mix solution

Absorption of levodopa and carbidopa and mix solution is all in the visible region of high absorbent substances. Figure 3,4,5 show the absorbance-wavelength curves. The spectra of levodopa and carbidopa are in the range of 210-310 nm.

When the absorbance-concentration graphs for levodopa and carbidopa are examined, it is seen that the absorbance value increases as the concentration increases. The linear relationship [54], between absorbance and concentration was confirmed by the fact that the regression coefficient [55] is close to the individual (Figure 6,7).

Principal Component Regression (PCR) and Partial Least Squares Regression (PLS)

Some statistical parameters were given for validation of calibrations for synthetic mixtures of drugs.

The statistical parameters were found to produce a satisfactory validity for the PLS and PCR methods. The PLS and PCR methods have reliable accuracy and higher precision. From the results (Table 2 and 3) obtained, the standard deviation values are sufficiently small and the recovery values are sufficiently close to 100. This shows us that the results obtained are appropriated.

Validation of the method

For calibration the prediction residual error sum-of-squares (PRESS) (equation 1) [56] was calculated as:

Table 2: Composition of prediction set and recovery results obtained in synthetic mixtures for PLS method.

	Levodopa			Carbidopa		
Mix No	Added (mg/L)	Found (mg/L)	Recovery %	Added (mg/L)	Found (mg/L)	Recovery %
1	4	3.98	99.86	5	4.99	99.8
2	4	3.89	99.50	10	9.97	99.7
3	4	3.97	97.25	15	14.96	99.73
4	4	3.95	99.25	20	19.97	99.85
5	4	3.92	98.75	25	24.95	99.8
6	8	7.95	98.00	5	4.95	99
7	8	7.95	99.38	10	9.96	99.6
8	8	7.89	99.38	15	14.95	99.67
9	8	7.96	98.63	20	19.95	99.75
10	12	11.96	99.50	5	4.99	99.8
11	12	11.92	99.67	10	9.89	98.9
12	12	11.97	99.33	15	14.99	99.93
13	16	15.95	99.75	5	4.96	99.2
14	16	15.98	99.69	10	9.98	99.8
15	20	19.96	99.88	5	4.98	99.6
			Mean=99.18 **Relative Standard Deviation**=0.74			**Mean**=99.61 **Relative Standard Deviation**=0.32

Table 3: Composition of prediction set and recovery results obtained in synthetic mixtures for PCR method.

	Levodopa			Carbidopa		
Mix No	Added (mg/L)	Found (mg/L)	Mix No	Added (mg/L)	Found (mg/L)	Mix No
1	4	3.99	99.75	5	5	100
2	4	3.97	99.25	10	9.96	99.6
3	4	3.98	99.50	15	14.89	99.27
4	4	4	100.00	20	19.94	99.7
5	4	3.89	97.25	25	24.96	99.84
6	8	7.98	99.75	5	4.99	99.8
7	8	7.97	99.63	10	9.95	99.5
8	8	7.95	99.38	15	14.97	99.8
9	8	7.96	99.50	20	19.98	99.9
10	12	11.95	99.58	5	4.92	98.4
11	12	11.88	99.00	10	9.94	99.4
12	12	11.96	99.67	15	14.96	99.73
13	16	15.98	99.88	5	4.99	99.8
14	16	15.96	99.75	10	9.98	99.8
15	20	19.96	99.80	5	4.97	99.4
			Mean=99.18 **Relative Standard Deviation**=0.74			**Mean**=99.61 **Relative Standard Deviation**=0.32

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

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

According to the actual and predicted concentrations of the samples. *PRESS* values of levodopa and carbidopa were calculated and listed in Table 4.

It is important to emphasize that this is not a correct way to normalize the *PRESS* values when not all of the data sets contain the same number of samples. But the standard error of prediction (*SEC*) (equation 2) values contains the number of samples. Some statistical parameters determined the effectiveness of the calibration. The *SEP* was calculated using the following expression:

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

where n : the total number of synthetic mixtures.

Another validation parameter is RMSEC (equation 1.) [57] shown equiation 3.

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

The observable limit (LOD) and the detection limit (LOQ)

Table 4: Statistical parameter values for calibration step-simultaneous determination of levodopa and carbidopa using PLS and PCR methods.

PARAMETER	METHOT	Levodopa	Carbidopa
SEC	PLS	0,018	0,012
	PCR	0,014	0,013
PRESS	PLS	0,004	0,002
	PCR	0,0001	0,002
RMSEC	PLS	$4{,}21\times10^{-3}$	$9{,}42\times10^{-3}$
	PCR	$6{,}6\times10^{-4}$	$9{,}42\times10^{-3}$
LOD	PLS	0,052043	0,04631
	PCR	0,04631	0,140332
LOQ	PLS	0,157706	0,052817
	PCR	0,140332	0,160051

Table 5: Determination of levodopa and carbidopa in pharmaceutical tablet using PLS and PCR methods.

	Levodopa		Carbidopa	
	(gram)		(gram)	
NO	PLS	PCR	PLS	PCR
1	0.2490	0.2470	0.0249	0.0248
2	0.2460	0.2480	0.0245	0.0248
3	0.2450	0.2420	0.0246	0.0247
4	0.2480	0.2450	0.0247	0.0249
5	0.2470	0.2480	0.0249	0.0250
Mean	0.2470	0.2460	0.0250	0.0250
SD	0.0010	0.0030	0.0002	0.0001

parameters are interrelated but have different definitions (equation 4.and 5.) [58].

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

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

m: Slope

LOQ> LOD and LOQ = LOD were taken into consideration while evaluating the calculated LOD values [59].

PRESS and *SEP* values are close to zero. the degree of accuracy is increasing. The obtained *PRESS* and *SEP* values are close to zero for PLS and PCR methods.

In order to compare the performances of the investigated chemometric techniques according to UV spectrophotometric method for samples, we applied Snedecor's *F*-test [60]. The method used to compare the differences between the one-way ANOVA test was applied to the actual samples for each drug. In this study. Snedecor's *F*-values were calculated and compared with the *F* value. The same computation process was repeated for each drug. The experimental (calculated) *F*-values did not exceed the *F*-value in the variance analysis. For PLS method, the F-test value for levodopa was 0.00080 and the p-value was 0.98 for the inter-group degrees of freedom = 1, intra-group degrees of freedom = 28% For the carbidopa, the F-test value was calculated as 0.00020 and the p-value was 0.99 as the inter-group degree of freedom = 1, intra-group degree of freedom = 28% In the PCR method, the F-test value for levodopa was calculated to be 0.00048 and p-value 0.98 for the inter-group degree of freedom = 1, intra-group degree of freedom = 28% For the carbidopa, the

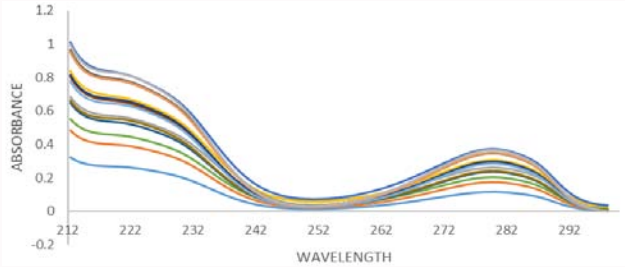

Figure 5: The Absorption Spectrum of Levodopa-Carbidopa mixtures.

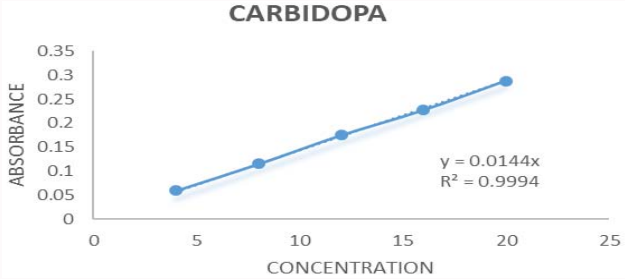

Figure 6: The Absorption-Concentration Relationship of Levodopa.

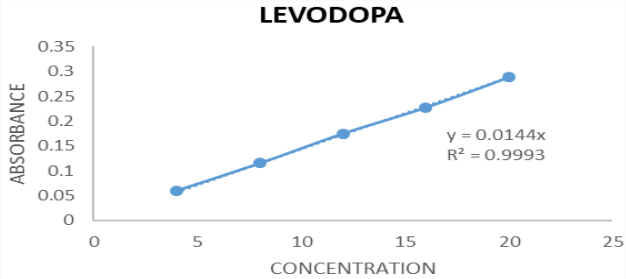

Figure 7: The Absorption-Concentration Relationship of Carbidopa.

F-test value was calculated as 0.00029 and the p-value was 0.99 as the inter-group degrees of freedom = 1, intra-group degrees of freedom = 28 and 95% confidence interval. Among all these methods, it was concluded that there was a meaningful difference.

Analysis of pharmaceutical formulation

The experimental results of two methods for pharmaceutical tablet are given in Table 5. One can see that the obtained results are very close to each other.

Conclusion

The partial least squares method and principle component regression successfully applied at the same time were able to identify drugs in synthetic solutions and pharmaceutical formulation. For all values, low prediction errors and high correlation coefficients emphasize the high linear relationship between the predicted and actual concentrations. The results obtained with this ternary mixture and some ratios of component concentrations show excellent predictive ability with these methods.

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