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Chemometric Determination of Parkinson's Drugs Containing Multiple Active Substances

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ABSTRACT

In this work, the active ingredients of Entacapone, Levodopa and Carbidopa in drug samples used in the treatment of Parkinson's disease were quantitatively determined by Ultraviolet Visible (UV-VIS) Spectroscopy and chemometrics. Firstly, the spectra of each drug active ingredient were taken individually and then synthetic mixtures identical to the drug sample were analyzed. In our method, validation parameters were calculated for each method. Percent (%) recoveries were found on average for both the synthetic mixture and the commercial sample. The recoveries were quantitative for each method. The accuracy of the methods was tested by applying ANOVA test to the results obtained from the PLS and PCR calibration methods. The developed methods are reproducible, sensitive, and accurate, and can be recommended for the analysis of drug samples containing Entacapone, Levodopa, and Carbidopa

Keywords: Entacapone, Levodopa, Carbidopa, Chemometry.

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Introduction

Parkinson's disease was defined by James Parkinson in 1817 [1]. Parkinson's disease is a type of disease known as trembling paralysis, which occurs with serious losses in brain cells and is more common in men than women. It progresses more slowly than other disease types. With the decrease in dopamine cells in the brain, the communication between brain cells decreases, which negatively affects movement control [2]. Parkinson's disease is treated with medication to compensate for the loss of dopamine-producing cells and improve quality of life. In the treatment of this disease, levodopa, a dopamine precursor, is given orally to the patient to replenish the diminished dopamine in the body. When levodopa is taken alone, a large amount of levodopa will be destroyed before reaching the brain, causing factors such as dizziness and nausea in the patient, and treatment will not be provided. For this reason, auxiliary active ingredients are used to ensure that levodopa reaches the brain more easily without being broken down in the blood. These are mainly active ingredients such as carbidopa and entacapone. Today, drugs used in the treatment of this disease contain one or more of these excipients [3,4].

It would be more specific to state that the active ingredients (levodopa, carbidopa, and entacapone) were quantitatively determined in drug samples used in the treatment of Parkinson's disease. Advances in chemometrics have made it possible to work with a wide variety of calibration methods to analyze complex chemical mixtures. Levodopa (L-DOPA) is an amino acid in the structure of 3,4-dihydroxy-L-phenylalanine. It has a molecular weight of 197.19 g/mol and the chemical formula $C_9H_{11}N_1O_4$ [5] (Figure 1.).

Carbidopa is a $C_{10}H_{14}N_2O_4$ white crystalline substance with a molecular weight of 244.3 g/mol that allows levodopa to cross the blood-brain barrier faster [6].

Entacapone is a nitrocatechol compound with a chemical structure of $C_{14}H_{15}N_3O_5$ and molecular weight of 305.29 g/mol. It is used in the treatment of Parkinson's disease in addition to levodopa and carbidopa to increase the effectiveness of treatment [7].

Developments in the fields of computers, software, statistics, and applied mathematics have led to a new discipline in chemistry called chemometrics for the solution of complex systems. This new discipline provides ease of operation not only in chemistry but also in many other disciplines such as biology, medicine, engineering, and economics [8].

Advances in chemometrics have made it possible to work with a wide variety of calibration methods for the analysis of complex chemical mixtures. Some of the most widely used multivariate calibration methods are called Partial Least Squares (PLS) and Principal Component Regression (PCR). The advantages of chemometrics are as follows:

i) To apply chemometrics, which is the application of software, mathematics, and statistics to chemistry, to the simultaneous determination of the active substances to be analyzed.

ii) With the chemometrics program, to develop a new method that is alternative, faster, and more cost-effective than previous classical methods.
iii) To facilitate spectrophotometric analysis of intricate systems without prior separationthus.
iv) With the chemometrics method, it is aimed to prevent the loss of time and work caused by the trial and error method by going through a correct experimental design [9].

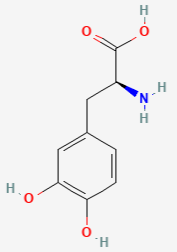
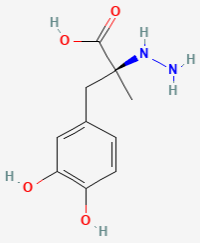
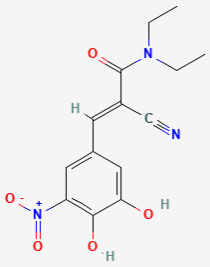
Active Substance	Formula	Molecular Weights	Structures
Levodopa	$C_9H_{11}N_1O_4$	197.19 g/mol	
Carbidopa	$C_{10}H_{14}N_2O_4$	244.3 g/mol	
Entacapone	$C_{14}H_{15}N_3O_5$	305.29 g/mol	

Figure 1. Chemical Structure of the Active Substance Levodopa, Carbidopa and Entacapone

Studies in the literature for the analysis of levodopa, carbidopa and entacapone are [10,11], [12,13] and [14,15], respectively. After the spectrophotometric method was applied to the drug tablet sample containing the active ingredients levodopa, carbidopa, and entacapone, the data obtained were evaluated by two different chemometric methods. Chemometric methods enabled the simultaneous evaluation of all data without any prior sorting of the drug samples. Furthermore, it was noted that the approach employed was precise, accurate, valid, consistent, and dependable when it came to the validation process—that is, it included the steps necessary to demonstrate that the approach is accurate, precise, and consistently delivers the desired results.

Materials and Methods

Levodopa, carbidopa, and entacapone, the active components of medications used to treat Parkinson's disease, were quantified in this work using UV/VIS spectrophotometry and chemometrics approaches. Analytical-grade chemicals were obtained from (Sigma-Aldrich) and used in the experiment. Levodopa, carbidopa, and entacapone, the active components of medications used to treat Parkinson's disease, were determined using UV-VIS spectra using a UV 1700 PHARMASPEC SHIMADZU spectrophotometer equipped with a 1 cm long cell and controlled by a computer. The Minitab 17 [16] application was used to calculate the data. Chemometric techniques were used to evaluate the results. The spectra of artificial mixtures were recorded with the ratios of levodopa, carbidopa, and entacapone ranging from table 1. Finally, measurements were made on a sample of medicinal tablets that are sold commercially.

For the spectrophotometric measurements in the study, stock solutions of levodopa, carbidopa and entacapone were prepared as 25 mg / 250 mL in 0.1 M HCl. In this study, the spectra of levodopa, carbidopa and entacapone, first individually and then of synthetic mixtures prepared in different ratios, were obtained by spectrophotometric measurements. Finally, measurements were performed on a commercially available tablet sample. The data obtained were

evaluated by different chemometric methods. In the first step, calibration (zeroing) of the UV spectrophotometer device was performed. The calibration was first performed against air, leaving both cells empty. Then, the same procedure was performed by placing a blank sample prepared with our solvent in both light paths. The blank was always prepared in this way for all readings. When choosing the blank, solvent was preferred as the blank to eliminate interference effects. In the last step, the commercial tablet (Stalevo) is analyzed. In the preparation of the drug sample, all tablets in the package were crushed in an agate mortar, diluted and mixed. One tablet is weighed, dissolved in solvent, homogenized by stirring in a magnetic stirrer and absorbance readings are taken.

Results

Solutions of 100 ppm of the active substances levodopa, carbidopa and entacapone were prepared by using 0.1 M HCl as solvent at 25 mg/250 mL. In the next step, solutions were prepared to analyze the spectroscopic properties of each individual substance in the range of 1-40 µg/mL for each substance. The wavelengths at which levodopa, carbidopa and entacapone gave maximum absorbance (levodopa and carbidopa: 280 nm; entacapone: 306 nm) were determined.

Twenty synthetic mixture solutions of levodopa, carbidopa and entacapone in the range of 1-40 µg/mL were prepared (Table1.).

Table 1. Calibration set containing Levodopa, Carbidopa and Entacapone (µg/mL).

	Levodopa	Carbidopa	Entacapone
1	4	1	8
2	8	2	8
3	12	3	8
4	16	4	8
5	20	5	8
6	4	1	16
7	8	2	16
8	12	3	16
9	16	4	16
10	20	5	16
11	4	1	24
12	8	2	24
13	12	3	24
14	16	4	24
15	4	5	24
16	8	1	32
17	12	2	32
18	4	3	32
19	8	4	32
20	12	5	40

The absorbance values of the mixture solutions were obtained using UV spectroscopy (see Figure 2). Subsequently, chemometric methods were applied to these results. Chemometric calculations are currently the most popular, fast, and reliable methods used to quantify each component in multicomponent mixtures.

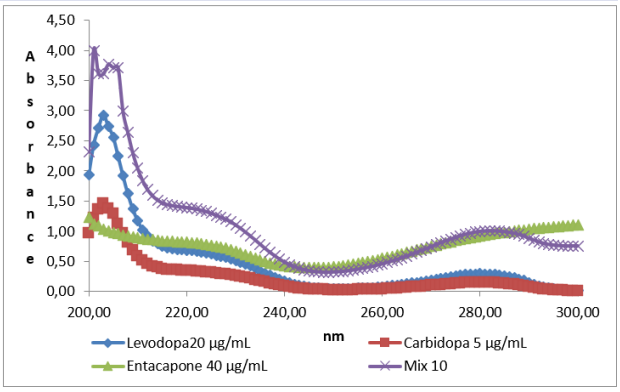


Figure 2. Absorption-Wavelength of Calibration Set containing Levodopa, Carbidopa and Entacapone

The relationship between absorbance and concentration is based on the principle of obtaining orthogonal lines by decomposing the absorbance values measured for the concentration set when examined using the principal component regression method. These lines are the coordinate order of the calibration to be established and calculations are based on this accuracy [17]. Principal component analysis (PCA) was performed as the first step before proceeding to chemometric calculations. Therefore, principal component analysis (PCA) was performed to reduce the dimensionality of the data and make it more manageable for chemometric calculations [18].

Values Calculated by Principal Component Regression (PCR)

Table 2. The results for each substance in the mixture containing Levodopa, Carbidopa, and Entacapone were calculated using principal component regression.

No	Levodopa			Carbidopa		
	Added (µg/mL)	Found (µg/mL)	Recovery %	Added (µg/mL)	Found (µg/mL)	Recovery %
1	4	3.95	98.75	1	1.01	101
2	8	7.94	99.25	2	1.99	99.5
3	12	11.95	99.58	3	2.97	99.00
4	16	15.99	99.94	4	3.98	99.50
5	20	19.96	99.80	5	4.96	99.20
6	4	3.98	99.50	1	0.96	96.00
7	8	7.94	99.25	2	1.97	98.50
8	12	11.95	99.58	3	2.98	99.33
9	16	15.98	99.88	4	3.98	99.50
10	20	19.96	99.80	5	4.98	99.60
11	4	3.86	96.50	1	0.94	94.00
12	8	7.98	99.75	2	1.89	94.50
13	12	11.97	99.75	3	3.00	100
14	16	15.94	99.63	4	3.98	99.50
15	4	3.86	96.50	5	4.96	99.20
16	8	7.98	99.75	1	0.98	98.00
17	12	11.89	99.08	2	1.97	98.50
18	4	3.65	91.25	3	2.95	98.33
19	8	7.84	98.00	4	3.89	97.25
20	12	11.79	98.25	5	4.72	94.40
		Mean	98.689		Mean	98.240
		Relative Standard Deviation	2.030		Relative Standard Deviation	1.992

Table 3. The results for each substance in the mixture containing Levodopa, Carbidopa, and Entacapone were calculated using principal component regression. (more).

No	Entacapone		
	Added (µg/mL)	Found (µg/mL)	Recovery %
1	8	7.98	99.75
2	8	7.95	99.38
3	8	7.89	98.63
4	8	7.94	99.25
5	8	7.96	99.50
6	16	15.96	99.75
7	16	15.87	99.19
8	16	15.97	99.81
9	16	15.96	99.75
10	16	15.99	99.94
11	24	23.96	99.83
12	24	23.97	99.88
13	24	23.89	99.54
14	24	23.56	98.17
15	24	23.88	99.50
16	32	31.97	99.91
17	32	31.88	99.63
18	32	31.97	99.91
19	32	31.94	99.81
20	40	39.45	98.63
		Mean	99.486
		Relative Standard Deviation	0.494

The recovery and relative standard deviation (RSD) data are shown in Table 3. Precision and accuracy data were checked.

ANOVA test [19] was applied to the calculated results to ensure the appropriateness of principal component regression. The F-test was used to determine whether the principal component regression model was statistically significant. For levodopa, carbidopa and entacapon, respectively, the calculated F-value (0.0024; 0.01; 0.01) was less than the critical F-value (4.09), indicating that the model is statistically significant. Since the calculated F value is less than the critical value, it was decided that the method is applicable. The Pearson correlation coefficient is defined in statistics as the measurement of the strength of the relationship between two variables and their relationship to each other. In simple terms, the Pearson correlation coefficient calculates the effect of the change in one variable when the other variable changes. The calculated p values were 0.96, 0.92 and 0.97 for levodopa, carbidopa and entacapone, respectively. The Pearson correlation coefficient with a p value should be greater than 0.05. The calculated ANOVA data were evaluated according to these steps. The significant F-value and p-value greater than 0.05 for all three active ingredients indicate that the principal component regression model is suitable for the simultaneous determination of levodopa, carbidopa, and entacapone.

Validation of the Principal Component Regression Method

Minimal data of the sum of squares of predicted errors (Predicted Residual Error Some of Squares→ PRESS) were obtained in the cross-validation process for the calibration of PLS and PCR calibrations for the quantification of substances in mixtures containing the active substances levodopa, carbidopa and entacapone. PRESS (Equation 1.) value close to zero increases the degree of accuracy [20]. The obtained PRESS values are small enough. Other parameters in the validation are the standard error of the calibration: SEC (Equation 2.) and the square root of the mean square error of the estimate: RMSEC (Equation 3.) [21].

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

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

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

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

The limit of observability (LOD) and limit of detection (LOQ) parameters are related but have different definitions (Equations 4., 5.) [22].

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

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

Sa: Standard deviation, m: Mean.

LOQ values were considered to be valid only if LOQ > LOD [23].

Table 3. Validation Parameters for Principal Component Regression

Parameter	Levodopa	Carbidopa	Entacapone
SEC	0.027	0.016	0.034
PRESS	0.014	0.0059	0.029
RMSEC	0.026	0.017	0.038
LOD	0.131	0.107	0.225
LOQ	0.397	0.324	0.681

Application of Principal Component Regression and Partial Least Squares Method to Commercial Pharmaceutical Tablets

In a final step, the chemometric techniques used in this study were applied to commercial drug tablets after assessing the suitability of the methods and the analytical quality of the calculated data.

Table 4. Drug Sample Results

NO	Levodopa (gram) PCR	Carbidopa (gram) PCR	Entacapone (gram) PCR
1	0.097	0.0248	0.0198
2	0.098	0.0245	0.0195
3	0.089	0.0239	0.0197
4	0.101	0.0247	0.201
5	0.095	0.0246	0.0198
Mean	0.096	0.025	0.056
Relative Standard Deviation	0.047	0.014	1.449

It was studied with the drug Stalevo. It contains 100 mg levodopa, 25 mg carbidopa and 200 mg entacapone.

Values Calculated with Partial Least Squares Method (PLS)

The chemometric methods used in this study are partial least squares method (PLS) and principal component regression (PCR) [24,25]. The chemometric model is created with the help of the matrix formed from the relationship between absorbance and concentration and chemometric calculations are created [26]. The most well-known chemometric calibration is the partial least squares method (PLS). According to the PLS algorithms used to create the calibration in the PLS method, orthogonalized PLS algorithm and non-orthogonalized PLS algorithm are used. The data obtained is more reliable than the classical methods.

Table 5. The results for each substance in the mixture containing Levodopa, Carbidopa, and Entacapon were calculated using the partial least squares method.

No	Levodopa			Karbidopa		
	Added (µg/mL)	Found (µg/mL)	Recovery %	Added (µg/mL)	Found (µg/mL)	Recovery %
1	4	3.86	96.5	1	0.95	95.00
2	8	7.78	97.25	2	1.96	98.00
3	12	11.65	97.08	3	2.98	99.33
4	16	15.94	99.63	4	3.94	98.50
5	20	19.86	99.30	5	4.95	99.00
6	4	3.51	87.75	1	0.92	92.00
7	8	7.69	96.13	2	1.96	98.00
8	12	11.75	97.92	3	2.95	98.33
9	16	15.63	97.69	4	3.99	99.75
10	20	19.95	99.75	5	4.95	99.00
11	4	3.78	94.50	1	1.01	101.0
12	8	7.96	99.50	2	1.96	98.00
13	12	11.92	99.33	3	2.97	99.00
14	16	15.83	98.94	4	3.96	99.00
15	4	3.82	95.50	5	4.95	99.00
16	8	7.95	99.38	1	0.86	86.00
17	12	11.96	99.67	2	1.94	97.00
18	4	3.86	96.50	3	2.86	95.33
19	8	7.95	99.38	4	3.95	98.75
20	12	11.98	99.83	5	4.98	99.60
		Mean	97.575		Mean	97.48
		Relative Standard Deviation	2.826		Relative Standard Deviation	3.356

Table 5. The results for each substance in the mixture containing Levodopa, Carbidopa, and Entacapon were calculated using the partial least squares method. (more).

No	Entakapon		
	Added (µg/mL)	Found (µg/mL)	Recovery %
1	8	7.89	98.63
2	8	7.95	99.38
3	8	7.91	98.88
4	8	7.99	99.88
5	8	7.89	98.63
6	16	15.95	99.69
7	16	15.86	99.13
8	16	15.92	99.50
9	16	15.94	99.63
10	16	15.98	99.88
11	24	23.89	99.54
12	24	23.96	99.83
13	24	23.94	99.75
14	24	23.94	99.75
15	24	23.99	99.96
16	32	31.94	99.81
17	32	31.95	99.84
18	32	31.96	99.88
19	32	31.97	99.91
20	40	38.99	97.48
		Mean	99.447
		Relative Standard Deviation	0.625

When calculating the concentrations found against the added concentration, cross-validation was applied to avoid errors in the drug sample [27,28].

The method was deemed applicable for levodopa, carbidopa, and entacapone as the calculated F-values (0.01) were less than the critical value (4.09). The p-values were calculated as 0.92, 0.91, and 0.97 for levodopa, carbidopa, and entacapone, respectively. The Pearson correlation coefficient should have a p-value greater than 0.05.

Validation of Partial Least Squares Method (PLS)

Parameters were calculated for the quantification of substances in mixtures containing active pharmaceutical ingredients.

Table 6. Validation Parameters for the Partial Least Squares Method

Parameter	Levodopa	Carbidopa	Entacapone
SEC	0.056	0.017	0.037
PRESS	0.044	0.004	0.056
RMSEC	0.047	0.014	0.053
LOD	0.141	0.063	0.350
LOQ	0.427	0.191	1.061

Application of Partial Least Squares Method to Commercial Pharmaceutical Tablets

After assessing the suitability of the methods and the analytical quality of the calculated data, the chemometric techniques employed in this study were applied to commercial drug tablets in the final stage.

Table 7. Drug Sample Results.

NO	Levodopa (gram) PCR	Carbidopa (gram) PCR	Entacapone (gram) PCR
1	0.095	0.25	0.0193
2	0.089	0.0249	0.0198
3	0.094	0.0242	0.0196
4	0.094	0.0248	0.0194
5	0.097	0.0251	0.0195
Mean	0.094	0.07	0.02
Relative Standard Deviation	0.031	1.44	0.0099

It was studied with the drug Stalevo. It contains 100 mg levodopa, 25 mg carbidopa and 200 mg entacapone.

Discussion

The active substances levodopa, carbidopa, and entacapone were quantified in mixtures using UV Spectroscopy data supported by chemometric programs. For the active substances levodopa, carbidopa and entacapone, the method was statistically supported by developing UV. The values found were first quantified by principal component analysis (PCA). Then partial least squares (PLS) and principal component regression (PCR) chemometric methods were applied. In order to test the accuracy of the PLS and PCR methods, the ANOVA test was applied to both methods.

Both principal component regression (PCR) and partial least squares (PLS) methods yielded very high recovery values and sufficiently small relative standard deviation values. The correlation coefficients between the actual and estimated values were close to one, indicating a good fit of the chemometric models. Upon examination of the validation results for the method, it is evident that the LOD values are smaller than the LOQ values. Additionally, all other calculated parameters should be close to zero. Our own calculated data also showed values close to zero.

The suitability of the methods used was verified before proceeding to the commercial drug tablets. An ANOVA test was conducted to perform this procedure. The calculated F-values should be lower than the F-measure or F-theoretical values. Pearson's correlation coefficient with a P-value greater than 0.05 should be used. Based on the calculated values, it was determined that the methods used were appropriate. The chemometric methods applied in this study can be used to quantify multiple active ingredients in complex drug mixtures.

Conclusion

Statistical analysis of UV spectroscopy data using chemometric programmes was used to quantify the active substances levodopa, carbidopa, and entacapone in mixtures. Mixtures containing both substances were prepared. Before proceeding to commercial drug tablets containing these mixtures, both methods were tested, and the reliability of the data was examined. It was concluded that the applied methods could be recommended for the quantitative analysis of complex two-component drug mixtures.

Conflicts of interest

There are no conflicts of interest in this work.

References

[1] Yalıman A., Şen E.İ., Parkinson Hastalığı ve Rehabilitasyonu, *Türk Fiz Tıp Rehab. Derg.*, 57 (2011) 38-44.

[2] Şahin A.R., Arık A.C., Depresyon ve Parkinson hastalığı, *O.M.Ü. Tıp Dergisi,* 11(3) (1994) 239-246.

[3] Yiğit G., Arıcıoğlu F., Günümüz ve Gelecekte Parkinson Hastalığı için Farmakolojik Tedavi Yaklaşımları, *MÜSBED*, 5(4) (2015) 265-273.

[4] Pekcan Ertokuş G., Özer T., Parkinson Hastalığının Tedavisinde Kullanılan Levodopa ve Benserazid Etken Maddelerinin Kemometrik Tayini, *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 13(2), (2020) 669-676.

[5] Tashkhourian J., Hormozi-Nezhad M.R., Khodaveisi J., Application of silver nanoparticles and principal component-artificial neural network models for simultaneous determination of levodopa and benserazide hydrochloride by a kinetic spectrophotometric method, *Spectrochimica acta. Part A, Molecular and Biomolecular Spectroscopy,* 82(1) (2011) 25-30.

[6] Damiani P.C., Moschetti A.C., Rovetto A.J., Benavente F., Olivieri A.C., Design and optimization of a chemometrics-assisted spectrophotometric method for the simultaneous determination of levodopa and carbidopa in pharmaceutical products, *Analytica Chimica Acta*, 543 (2005) 192–198.

[7] Bugamelli F., Marcheselli C., Barba E., Raggi M.A., Determination of L-dopa, carbidopa, 3-*O*-methyldopa and entacapone in human plasma by HPLC–ED, *Journal of Pharmaceutical and Biomedical Analysis*, 54 (2011) 562-567.

[8] Dinç E., Kemometri Çok Değişkenli Kalibrasyon Yöntemleri, *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi,* 27(1) (2007) 61-92.

[9] Arslan A.M., Çoklu Etken Madde İçeren Parkinson İlaçlarının Kemometrik Tayini, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, 2023.

[10] Hosseini M.G., Faraji M.,Momeni M.M., Ershad S., An innovative electrochemical approach for voltammetric determination of levodopa using gold nanoparticles doped on titanium dioxide nanotubes. *Microchim Acta*, 172 (2011) 103-108.

[11] Benvidi A., Dehghani-Firouzabadi A., Mazloum-Ardakani M., Mirjalili B.F., Zare R., Electrochemical deposition of gold nanoparticles on reduced graphene oxide modified glassy carbon electrode for simultaneous determination of levodopa, uric acid and folic acid: Application of silver nanoparticles and principal component-artificial neural network models for simultaneous determination of

levodopa and benserazide hydrochloride by a kinetic spectrophotometric. *Journal of Electroanalytical Chemistry*, 736 (2015) 22-29.

[12] Gholivanda M.B., Jalalvanda A.R., Goicoechea H.C., Multivariate analysis for resolving interactions of carbidopa withdsDNA at a fullerene-C60, *International Journal of Biological Macromolecules*, 69 (2014) 369-381.

[13] Tajika, S., Taher, M.A., Beitollahi, H., 2013, Simultaneous determination of droxidopa and carbidopa using a carbon nanotubes paste electrode, *Sensors and Actuators B: Chemical*, 188 (2013) 923-930.

[14] Abdel-Ghany, M.F., Hussein, L.A., Ayad, M.F., Youssef, M.M., Investigation of different spectrophotometric and chemometric methods for determination of entacapone, levodopa and carbidopa in ternary mixture, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 171 (2017) 236-245.

[15] Mantena, B.P.V., Rao, S.V., Rao, K.M.C.A., Ramakrishna, K., Reddy, R.S., Rapid Separation Technique for the Determination of Potential Impurities Present in Levodopa, Carbidopa, and Entacapone in Fixed Dose Combination Drug Product Using Trifunctionally Bonded Phase Ethylene Bridged Sorbent Column with Smaller Ion-Pair Reagent, *Journal of Liquid Chromatography & Related Technologies*, 38 (2015) 1073–1087.

[16] Minitab, Available at: https://www.inovadanismanlik.com.tr/ , Retrieved: 21.08.2023.

[17] Dinç E., Kemometrik İşlem ve Yöntemlerin Analitik Kimyadaki Tipik Uygulamaları, *Uygulamalı Kemometri Yaz Okulu Notları*. (2009) 13-17.

[18] Saganowska P., Wesolowski M., Principal Component and Cluster Analyses as Supporting Tools for Co-CrystalsDetection, *J.Therm.Anal.Calorim*., (2017) 45-55.

[19] Bajpai V., Kumar S., Singh A., Singh J., Negi M.P.S., Bag S.K., Kumar N., Konwar R., Kumar B., Chemometric based identification and validation of specific chemical markers for goegraphical, seasonal and gender variations in tinospora cordifolia stem using HPLC-ESI-QTOF-MS Analysis, *Photochemical Analysis,* 28(4), (2017) 277-288.

[20] Uyanık A., Analitik Kimyacılar İçin İstatistik ve Kemometri", Pegem Akademi Yayıncılık, 6. Baskı (2012) 254-259.

[21] Bilgili A.V., Çullu M.A, Aydemir S., 2014, Tuzdan etkilenmiş toprakların yakın kızılötesi yansıma spektroradyometre ve elektromanyetik indüksiyon tekniği yardımıyla karakterize edilebilme potansiyelinin araştırılması , *Harran Tarım ve Gıda Bilimleri Dergisi*, 18(1) (2014) 32-45.

[22] A. Shrivastava, V.B. Gupta, Methods for the determination of limit of detection and limit of quantitation of tha analytical methods, *Chronicles of Young Scientist,* 2 (1), (2011) 21-25.

[23] Armbruster D.A., Pty T., Limit of blank, limit of detection and limit of quantitation, *Clin Biochem Rev*, 29 (2008) 49-52.

[24] Dinç E., Baleanu D., Spectrophotometric quantitative determination of cilazapril and hydrochlorothiazide in tablets by chemometric methods, *Journal of Pharmaceutical and Biomedical Analysis*, 30 (2002) 715-723.

[25] Dinç E., Özdemir A., Baleanu D., Comparative study of the continuous wavelent transform, derivative and partial least squares methods applied to the overlapping spectra for the simultaneous quantitative resolution of ascorbic acid and asetilsalicylic acid in effervescent tablets, , *Journal of Pharmaceutical and Biomedical Analysis*, 37 (2005) 715-723.

[26] Aktaş A.H., Sarıdağ A.M., Liquid chromatografic-chemometric tecnhniques for the simultaneous HPLC determination of lansoprazole, amoksisilin and clarithromisin in commercial preparation, *Journal of Chromatografic Science*, (2017) 1-7.

[27] Porfire A., Muntean D., Achim M., Vlase L., Tomuta I., Simultaneous Quantification of Simvastatin and Excipients in Liposomes Using Near Spectroscopy and Chemometry, *Journal of Pharmaceutical and Biomedical Analysis*, 107 (2015) 40-49.

[28] Tarhan I., Kara A.A.I.H, Quantitative Determination of Free Fatty Acids in Extra Virgin Olive Oils by Multivariate Methods and Fourier Transform Infrared Spectroscopy Considering Different Absorption Modes, *International Journal of Food Properties,* (2017) 1-8.