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SIMULTANEOUS DETERMINATION OF LEVODOPA AND CARBIDOPA IN PHARMACEUTICALS BY PRINCIPAL COMPONENT REGRESSION

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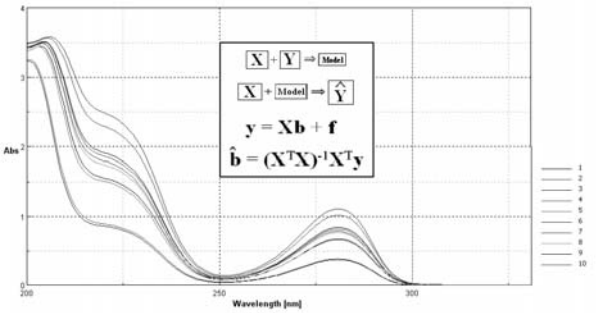
Simultaneous determination of levodopa and carbidopa with minimum sample pre-treatment and without analyte separation has been successfully achieved using principal component analysis (PCA). The developed method is based on an experimental design approach combined with PCR methodologies and the training set was carefully chosen using a partial factorial calibration design at five concentration levels. The good results obtained for the statistical parameters (R^2, Q^2, s, F, SDEC, SDEP, SDEC, PRESS and RSS) sustain the fact that the experimental model has a great power of prediction. The method was validated and its applicability was assessed using commercial pharmaceutical tablets containing carbidopa and levodopa as active compounds in mixture.



INTRODUCTION

Parkinsonism is a degenerative disorder of the vegetative neural system. Its primary symptoms which include movement disorders such as shaking, rigidity, slowness of movement and difficulty with walking, result from the reduced activity of dopamine-secreting cells and they have been efficiently treated by medication increasing the dopamine level in the brain.[1]

Levodopa [(-)-3-(3,4-dihydroxylphenyl)-l-alanine] is a catechol related compound which acts as an important neurotransmiter and also a dopamine precursor which can be orally administrated and reaches the brain where it is enzimatically decarboxylated to dopamine.[2] However, elevated levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias.[3] Consequently an inhibitor of the decarboxylase activity must be used and usually this is carbidopa [(-)-l-2-(3,4-dihydroxybenzyl)-2-hydrazinopropionic acid] which is also a catechol related compound. By administering levodopa combined with carbidopa, the side effects are generally reduced and the concentration of dopamine is effectively controlled at an appropriate level.

Several methods such as spectrophotometry,[4-6] gas chromatography (GC),[7] high performance liquid chromatography (HPLC),[8] chemiluminescence (CL),[9] amperometric and voltammetric determination,[10-12] potentiometry,[13] radio-immunoassay[14] and flow injection analysis (FIA)[15-17] have been reported in literature for the determination of levodopa or carbidopa in biological samples and pharmaceutical formulations. The major drawback of these methods is that they usually require a separation step prior to

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the analysis and given the usage of such compounds in pharmaceutical mixtures and biological fluids, simultaneous analysis is desirable, preferably by simple, rapid and selective techniques.

In this context, the solution is given by the development of chemometric methods, which include the experimental design and exploratory data analysis, the most important aspects of the experimental design development being widely presented in the literature.[18] Such methods have been limitedly applied for simultaneously determination of carbidopa and levodopa.[19-21] Multivariate methods allow extracting analytical information from the full-spectra, providing simultaneous determination of several components in the sample. Among the different regression method existent for multivariate calibration, the factor analysis based methods including partial least squares (PLS) regression and principal component regression (PCR) have received considerable attention in the chemometrics literature.[22] These techniques are powerful multivariate statistical tools that have been successfully and widely applied to the quantitative analysis of spectroscopic data due to the simplicity and sensitivity of spectroscopic methods and speed of the determination, since preliminary separation steps and sample pre-treatment are avoided.[23] The multivariate regression methods consider that a series of variables describes a system. Thus, in the PCR procedure the concentration is described by an undetermined number of scores corresponding to the first principal components (PCs), obtained by applying the algorithms of principal components analysis (PCA) over the digitized chemical information.[24-29]

In view of the above considerations and because of the coexistence of levodopa and carbidopa in pharmaceuticals, the development of a simultaneous quantification method by multivariate calibration has appeared to be of great importance in the pharmaceutical field, for routine analysis of the drugs in their formulations.

MATERIALS AND METHODS

1. Materials and reagents

The analytical purity carbidopa and levodopa, used in this study, were obtained from Sigma-Aldrich (Steinheim, Germany). The involved solvents, also of analytical grade, were methanol (Chemical Company, Roumania) and distilled water. The pharmaceutical tablets tested in the study were Isicom tablets from ISIS Pharma France.

2. Equipment and software

The UV spectra were obtained using a UV-VIS Jasco double beam spectrophotometer (V-50 model) equipped with a deuterium lamp for the UV and a halogen lamp for visible domain. The spectra were registered in the UV domain, from 200 nm to 350 nm and using a computer loaded with the Spectra Manager software, the spectral data were processed. The multidimensional analysis that involved various statistical approaches was performed by using the Statistica 7 and MobiDigs software.

3. Experimental design and sample preparation

To develop the experimental design, firstly the individual stock solutions of levodopa and carbidopa were prepared in a mixture of methanol and distilled water (1:10 v/v) with a concentration of 0.1 mg/mL for each. Then the calibration solutions were prepared in 25 ml volumetric flasks by mixing appropriate volumes of stock solutions of each compound. Because the study was carried out for two compounds, the matrix upon which the calibration solutions were made was constructed by encoding 5 concentrations of the investigated compounds (Table 1). This design is known as the partial factorial design and the concentrations were encoded as follows: code 1 – 0.01 mg/mL, code 2 – 0.02 mg/mL, code 3 – 0.03 mg/mL, code 4 – 0.04 mg/mL and code 5 – 0.05 mg/mL.

The applicability of the method was assessed by analysis of the Isicom pharmaceutical tablets containing both of the investigated compounds (250 mg/tablet levodopa and 25 mg/tablet carbidopa, respectively). The sample preparation involved few simple steps. Firstly a tablet was dissolved with methanol in a 50 mL volumetric flask. Then using an ultrasonic bath for 30 minutes, the powder tablet was completely disintegrated and the resulted solution was clarified by passing it through 0.45 μm syringe filter (Nylon, 25 mmθ, Teknokroma). The resulted solution was properly diluted with distilled water and used for further analysis.

RESULTS AND DISCUSSION

For the spectrometric analysis the selected UV domain was between 200 and 350 nm. As shown in Fig. 1, the UV spectrum of each component is relatively simple and overlapped with each other. Thus, these compounds cannot be measured in the presence of each other by a simple calibration procedure without prior separation. Also by investigating the spectrum corresponding to their mixture it can be observed that the direct evaluation is not possible, thus it is necessary to resort to a more advanced chemometric method. Therefore, multivariate calibration was used for the determination of each compound in the mixtures using partial factorial design. Multivariate calibration methods demand a suitable experimental design of the standards belonging to the calibration set in order to have good predictions. The composition data of the calibration and validation solutions are listed in Table 1.

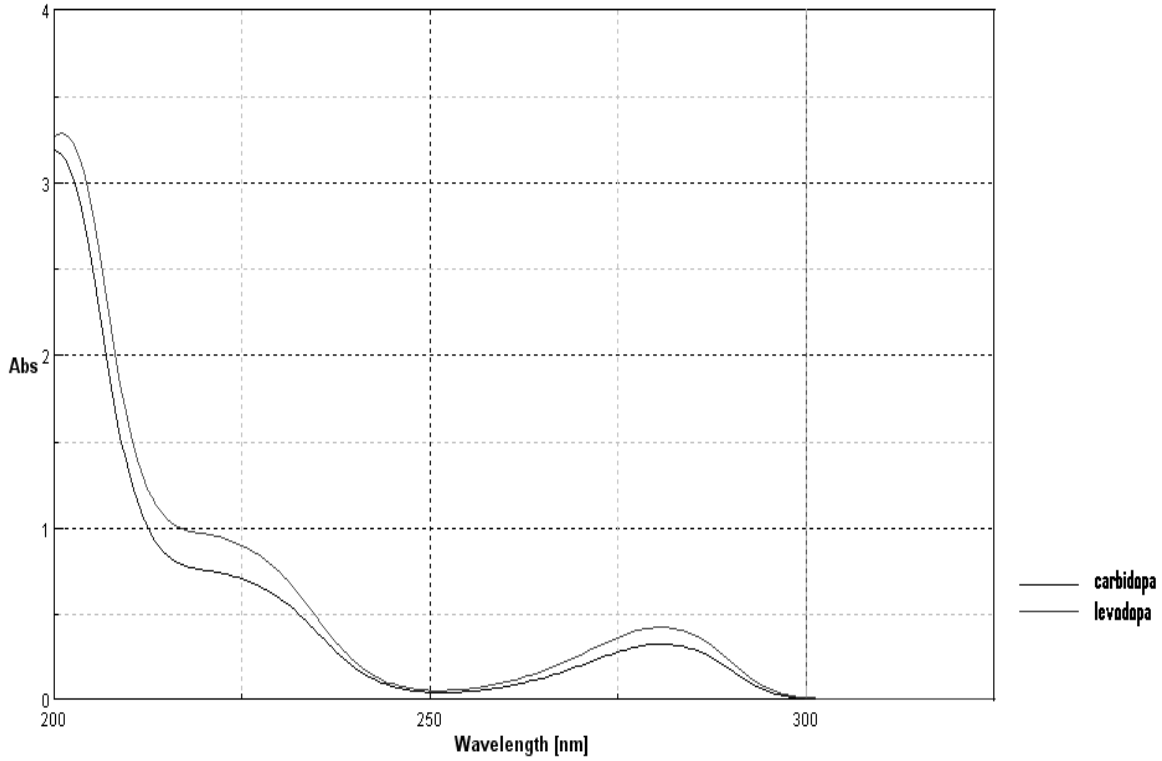

Fig. 1 – The UV absorption spectra of levodopa and carbidopa.

Table 1

The composition data of the calibration and synthetic solutions

No.	Sample	Levidopa (mg/mL)	Carbidopa (mg/mL)
1	Multivariate calibration	0.03	0.03
2		0.01	0.02
3		0.02	0.01
4		0.01	0.04
5		0.01	0.05
6		0.05	0.02
7		0.02	0.04
8		0.04	0.03
9		0.03	0.05
10		0.05	0.01
11	Synthetic samples	0.02	0.02
12		0.02	0.02
13		0.02	0.02
14		0.02	0.02

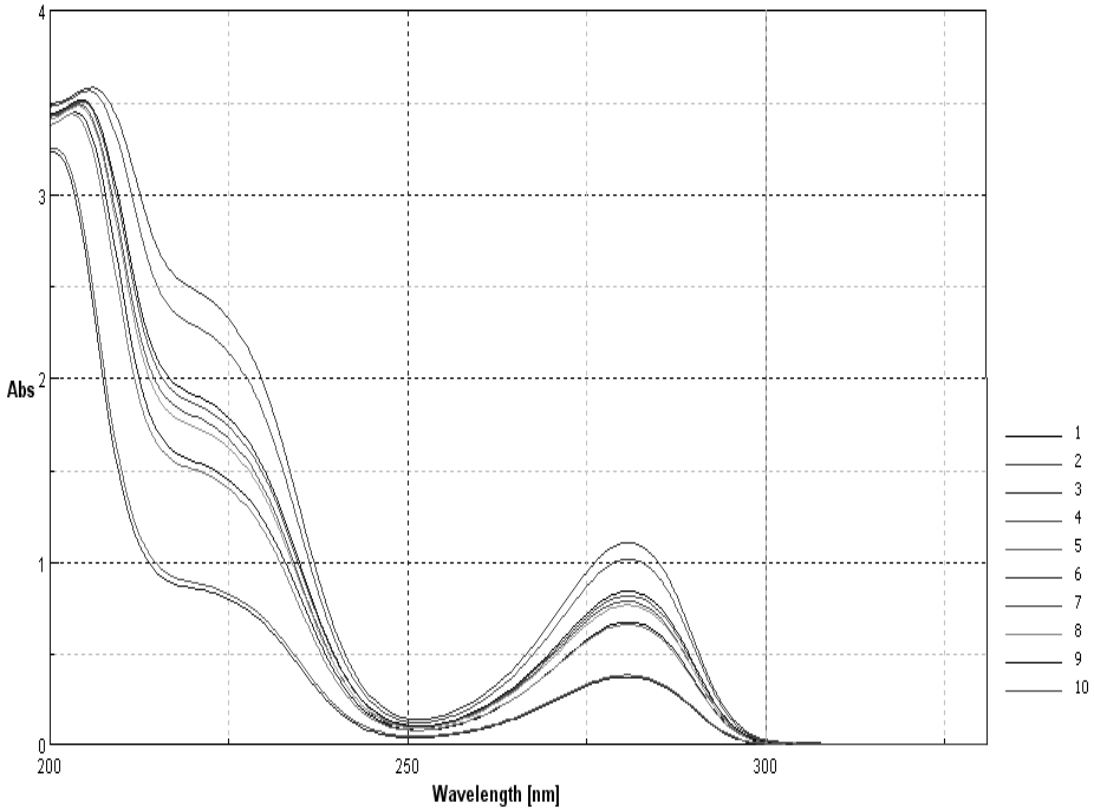

Fig. 2 – The partial factorial calibration profile (1-10 numbers corresponding to the calibration solution).

Table 2

Statistical parameters of the developed models

Statistical Parameter	Model	
	Levodopa	Carbidopa
Accepted factors	11	11
Q^2	93.62	92.60
R^2	97.44	96.66
s	0.004	0.004
F	69	53
SDEP	0.0046	0.0050
SDEC	0.0029	0.0033
PRESS	0.001	0.001
RSS	0.000	0.000

By measuring the full wavelengths in between 200-350 nm the absorbance data matrix corresponding to the concentration set was obtained. The absorption spectra of the calibration solutions set corresponding to Table 1 are shown in Fig. 2.

The quality of the prediction model obtained by applying PCR on the obtained principal components (PCs) can be judged by the performance criteria, such as cross validated determination coefficient (Q^2), standard deviation error in calculation (SDEC), standard deviation error of prediction (SDEP), the prediction sum of squares (PRESS) or root sum square (RSS), which are presented in Table 2. As it can be observed the obtained values of these parameters are very good and they indicate that the obtained models have high quality, thus they may be used for the evaluation of real samples.

The obtained model was validated in terms of precision and accuracy, by analysing artificial samples and also pharmaceutical samples. The precision, characterized as repeatability, was expressed as relative standard deviation and determined at three concentration levels. The repeatability was assessed by analysing four replicate samples for each concentration and both investigated compounds. The values obtained for

these parameters RSD < 2.86% for levodopa and RSD < 2.99% for carbidopa, indicate that the model has good precision (Table 2).

The accuracy, expressed as recovery degree, was investigated for standard solutions by analysing four replicate samples for each compound, at three concentration levels. In this case the results (Table 3) were included in range of 99.3%–101.3%, 101.7%–103.3%, and 99.6%–100.7% at low, intermediate and higher concentrations respectively, indicating that the model gives very accurate predictions. If comparing the obtained results, in terms of accuracy and precision, to the other studies[19-21] involving simultaneous determination of carbidopa and levodopa, it's easy to observe that the proposed method presents better characteristics, except of that involving last-square support vector,[19] which led to similar results.

The applicability of the model was tested on pharmaceutical tablets containing carbidopa and levodopa as active principles (250 mg/tablet levodopa and 25 mg/tablet carbidopa, respectively). The analysis was carried out using the standard addition method at two concentration levels. As shown in Table 4 the values obtained for the concentration of both investigated compounds were very similar to the real concentration values (labelled by the manufacturer), with recoveries between 98.03% and 101.16% for levodopa and 99.63% and 100.33% for carbidopa, respectively.

CONCLUSIONS

A rapid, precise and accurate chemometric technique based on PCR analysis of UV spectra was developed for the simultaneous determination of levodopa and carbidopa in synthetic samples and pharmaceutical tablets. The excellent recovery values obtained for artificial and commercial samples indicate good accuracy of the method for both investigated compounds. Also, the proposed technique does not require a separation step or any complicated sample treatment. The assay results obtained in this study strongly encourage the application of this technique for routine analysis and quality control of the commercial pharmaceutical products containing levodopa and carbidopa in mixture.

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Table 3

Precision and accuracy of the developed method

Compound	Concentration (mg/mL)	Found concentration* (mg/mL)	Recovery (%)	Standard deviation	RSD (%)
Levodopa	0.0150	0.0152	101.3	0.0004	2.86
	0.0300	0.0310	103.3	0.0004	1.42
	0.0450	0.0448	99.6	0.0007	1.47
Carbidopa	0.0150	0.0149	99.3	0.0004	2.39
	0.0300	0.0305	101.7	0.0009	2.99
	0.0450	0.0453	100.7	0.0005	1.16

*Data are mean of four replicates.

Table 4

Estimation of levodopa and carbidopa from pharmaceutical tablets

Compound	Concentration of the pharmaceutical sample (mg/mL)	Added concentration to the sample (mg/mL)	Observed concentration (mg/mL)	Obtained concentration (mg/mL)	Recovery (%)
Levodopa	0.030	0.000	0.0300	0.0300	100.06
	0.030	0.010	0.0394	0.0294	98.03
	0.030	0.020	0.0503	0.0303	101.16
Carbidopa	0.030	0.000	0.0299	0.0299	99.96
	0.030	0.010	0.0401	0.0301	100.33
	0.030	0.020	0.0499	0.0299	99.63

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