Simultaneous Spectrophotometric Determination of Levodopa and Carbidopa in Pharmaceutical Formulations and Water Samples by Using Mean Centering of Ratio Spectra and H-Point Standard Addition Methods

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Two spectrophotometric methods are described for the simultaneous determination of binary mixtures of carbidopa and levodopa in pharmaceutical formulations, without prior separation steps, using the mean centering of ratio spectra and H-point standard addition methods (HPSAM). The methods are based on the difference in the absorption spectra for the products of the reaction of carbidopa and levodopa with 4-aminobenzoic acid in the presence of periodate ion at pH 4.0. The methods allow rapid and accurate determination of carbidopa and levodopa. The results showed that the methods were capable to simultaneous determination of 0.30—10.00 μg ml^{-1} and 0.50—10.00 μg ml^{-1} each of carbidopa and levodopa. The proposed methods were successfully applied to the simultaneous determination of carbidopa and levodopa in pharmaceutical samples.

Key words carbidopa; levodopa; simultaneous determination; spectrophotometry

Catecholamine drugs are aromatic vic-diols in which either their 3- or 4-position is unsubstituted or these positions are not sterically blocked. They can be oxidized chemically or electrochemically to *o*-quinones that are quite reactive and can be attacked by a variety of nucleophiles.[1)]

Several methods have been reported for simultaneous determination of derivatives of catecholamines. Michotte *et al.*[2)] determined levodopa, carbidopa and 3-*o*-methyldopa simultaneously in plasma by HPLC with electrochemical detection. Sagar and Smyth[3)] reported simultaneous determination of levodopa, carbidopa and their metabolites by electrochemical detectors combined with HPLC. Coello *et al.*[4)] studied simultaneous kinetic-spectrophotometric determination of levodopa and benserazide by bi- and three-way partial least squares calibration that permit the quantification of both analytes with a precision of 0.7% for levodopa and 1.5% for benserazide. Uslu and Özkhan[5)] determined the binary mixtures of levodopa and benserazide by derivative spectrophotometry. Recently we reported a spectrophotometric method for simultaneous determination of levodopa and carbidopa in pharmaceutical preparations by derivative spectrophotometry in basic media.[6)]

The simultaneous determination of two or more compounds in the same sample without previous chemical separation has been developed from several mathematical procedures. In 1988, Bosch-Reig and Campins-Falco[7)] presented H-point standard addition method. The H-point standard addition method (HPSAM) was based on the principle of dual wavelength spectrophotometry and the standard addition method. They studied its principle and applications.[8—12)] The greatest advantage of HPSAM is that it can remove errors resulting from the presence of an interfering and blank reagent. So HPSAM can be used for simultaneous determination of two components in the solution.[13)]

Recently, Afkhami and Bahram[14)] presented a new spectrophotometric method for the analysis of binary and ternary mixtures, without prior separation steps which called "mean centering of ratio spectra" method.

In this paper, we applied two methods, H-point standard addition method and mean centering of ratio spectra for simultaneous determination of binary mixtures of levodopa and carbidopa without any preliminary separation steps. The methods are based on the difference in the reactions of carbidopa and levodopa with 4-aminobenzoic acid in the presence of periodate ion.

Theoretical Background

Requirements for Applying HPSAM Consider an unknown sample containing an analyte X and an interferent Y. The determination of the concentration of X by HPSAM under these conditions requires the selection of two wavelengths λ_1 and λ_2, at which the interferent species, Y, has the same absorbance.[7)] Then known amounts of X are successively added to the mixture, and the resulting absorbances are measured at the two wavelengths and expressed by the following equations:

$$A_{(\lambda 1)}=b_0+b+M_{\lambda 1}C_i \quad (1)$$

$$A_{(\lambda 2)}=A_0+A'+M_{\lambda 2}C_i \quad (2)$$

Where $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$ are the analytical signals measured at λ_1 and λ_2, respectively. b_0 and A_0 ($b_0 \neq A_0$) are the original analytical signal of X at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$, respectively. b and A' are the analytical signals of Y at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$, respectively. $M_{\lambda 1}$ and $M_{\lambda 2}$ are the slopes of the standard addition calibration lines at λ_1 and λ_2, respectively, and C_i is the added X concentration. The two straight lines obtained intersect at the so-called H-point ($-C_H$, A_H). If the component Y is the known interferent, and the analytical signal corresponding to Y, b (at λ_1 or λ_2), does not change with the additions of analyte, X, hence, A_H is only related to the signal of the interferent Y at the two selected wavelengths. To evaluate the interferent concentration from the ordinate value of the H-point (A_H), a calibration graph or the absorbance value of an interferent standard is needed. If the component Y is the unknown interferent, the Y analytical signals remain equal with the addition of analyte X. According to the above discussion, at the H-point, C_H is independent on the concentration of interferent and so A_H is also independent of the analyte concentration.[13)]

Requirements for Applying Mean Centering of Ratio Spectra Consider a mixture of two compounds X and Y. If there is no interaction among the compounds and Beer's low is obeyed for each compound, it can be written:

$$A_m=\alpha_X C_X+\alpha_Y C_Y \quad (3)$$

here, A_m is the vector of the absorbance of the mixture, α_X and α_Y are the molar absorptivity vectors of X and Y and C_X and C_Y are the concentrations of X and Y, respectively.

If Eq. 3 is divided by α_Y corresponding to the spectrum of a standard so-

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lution of Y in binary mixture, the first ratio spectrum is obtained in the form of Eq. 4 (for possibility of dividing operation, the zero values of α_Y should not be used in the divisor):

$$B = \frac{A_m}{\alpha_Y} = \frac{\alpha_X C_X}{\alpha_Y} + C_Y \quad (4)$$

If the Eq. 4 is mean centered (MC), since the mean centering of a constant (C_Y) is zero, Eq. 5 would be obtained:

$$\mathrm{MC}(B) = \mathrm{MC}\left[\frac{\alpha_X C_X}{\alpha_Y}\right] \quad (5)$$

Equation 5 is the mathematical foundation of multi-component analysis that permits the determination of concentration of each of the active compounds in the solution (X in this equation) without interfering from the other compounds of the binary system (Y in these equations). As Eq. 5 shows, there is a linear relation between the amount of MC(B) and the concentration of X in the solution. A calibration curve could be constructed by plotting MC(B) against concentration of X in the standard solutions of X or in the standard binary mixtures. For more sensitivity the amount of MC(B) corresponding to maximum or minimum wavelength should be measured. Calibration graphs for Y could also be constructed as described for X.[14,15]

Experimental

Apparatus A Perkin-Elmer Lambda 45 UV–Vis spectrometer was used for recording and storage of UV–Visible absorbance spectra using quartz cells and slit width of 0.5 nm. A Metrohm model 713 pH-meter with a combined glass electrode was used for pH measurements. All calculations in the computing process were done in Matlab 6.5 and Microsoft Excel for Windows.

Reagents Distilled water and analytical-reagent grade chemicals were used. Standard solutions of carbidopa and levodopa were prepared by dissolving appropriate amounts of carbidopa and levodopa (both from Aldrich) in pH 4.0 acetate buffer solution. A 1000 mg l^{-1} carbidopa and levodopa solutions were prepared by dissolving 0.1000 g carbidopa or levodopa in pH 4.0 acetate buffer solution and diluting to the mark in a 100-ml volumetric flask. A 0.01 mol l^{-1} 4-aminobenzoic acid solution and 0.01 mol l^{-1} periodate solution were prepared (Merck). Acetic acid-acetate (1 mol l^{-1}) buffer solution of pH 4.0 was prepared from acetic acid-sodium acetate (both from Merck).

H-Point Standard Addition Method An aliquot of the solution containing 5.00—100.00 μg of levodopa and 3.00—100.00 μg of carbidopa, 0.4 ml of 1.0×10^{-2} mol l^{-1} of 4-aminobenzoic acid solution, 1 ml of pH 4.0 buffer solution and 0.3 ml of 1.0×10^{-2} mol l^{-1} periodate solution were added into a 10 ml volumetric flask and made up to the mark with water. The solution was then allowed to stand for 15 min at room temperature. After that, a portion of the solution was transferred into a quartz cell to measure it absorbance at 394 and 498 nm. Synthetic samples containing different concentration ratios of carbidopa and levodopa were prepared and standard addition of carbidopa were performed. Simultaneous determination of carbidopa and levodopa with HPSAM was performed by measuring the absorbance of the solutions at 394 and 498 nm. The concentration ranges of carbidopa and levodopa for construction of HPSAM calibration graphs were 0.30—10.00 and 0.50—10.00 μg ml^{-1}, respectively.

Mean Centering of Ratio Spectra For preparation of all standard and prediction mixtures, an aliquot of the solution containing 3.00—100.00 μg carbidopa and 5.00—100.00 μg levodopa, 0.4 ml 4-aminobenzoic acid solution, 1 ml pH 4.0 buffer solution and 0.3 ml periodate solution were added into a 10 ml volumetric flask and made up to the mark with water. The solution was then allowed to stand for 15 min. After that, a portion of the solution was transferred into a quartz cell to record its absorbance from 350—700 nm against water blank.

A calibration graph for carbidopa is obtained by recording and storing the spectra of standard solutions containing different concentrations of carbidopa. The stored spectra of the solution of carbidopa are divided by standard spectrum of levodopa according to Eq. 3. Then mean centering of these vectors with respect to wavelength are obtained according to Eq. 4. The minimum or maximum of these vectors with respect to wavelength is used for the construction of calibration graph for carbidopa. For the prediction of concentration of carbidopa in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of carbidopa. For samples with unknown matrices standard addition can be used for removing matrix effect. The construction of calibration curves for other active compound and also its prediction step was performed as described for carbidopa.

Results and Discussion

The electrochemical oxidation behavior of some catecholamines in the presence of 4-aminobenzoic acid showed 1,4 [Michael] addition of 4-aminobenzoic acid on corresponding *o*-benzoquinone of catecholamines at pH 4.0.[16]

Figure 1 shows the absorption spectra for levodopa, carbidopa and their mixture in the presence of priodate and 4-aminobenzoic acid at pH 4.0. As it is seen, levodopa in the presence of 4-aminobenzoic acid and periodate shows an absorption band at 450 nm, and carbidopa shows an absorption band at 370 nm.

Periodate oxidizes levodopa and carbidopa to their corresponding quinones. In the presence of 4-aminobenzoic acid the produced *o*-benzoquinone is converted to the final product by a 1,4 [Michael] addition reaction[17] (Chart 1).

To take full advantages of the procedures, the reagent concentrations must be optimized. The parameters were optimized by setting all parameters constant and optimizing one each time. The effect of periodate concentration was studied in the range $(0.1—4.0)\times10^{-4}$ mol l^{-1}. The absorbance of the solution at 450 nm increased by increasing periodate concentration up to 2.0×10^{-4} mol l^{-1} and remained constant at higher concentrations. Therefore, 3.0×10^{-4} mol l^{-1} of periodate was selected as optimum concentration. The effect of the 4-aminobenzoic acid concentration was studied in the range of $(0.5—5.0)\times10^{-4}$ mol l^{-1}. The results show that the absorbance increased at 450 nm by increasing 4-aminobenzoic acid concentration up to 3.0×10^{-4} mol l^{-1} solution and remained constant at higher concentrations. Therefore

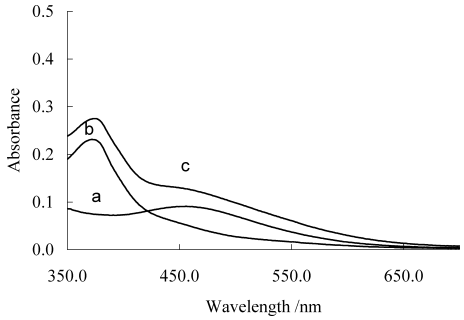

Fig. 1. Absorption Spectra for the Reaction of 5.00 μg ml^{-1} of Levodopa (a), 5.00 μg ml^{-1} Carbidopa (b) and Their Mixture (5.00 μg ml^{-1} Each of Them) (c) with 4×10^{-4} mol l^{-1} 4-Aminobenzoic Acid and 3×10^{-4} mol l^{-1} Periodate Solution at pH 4.0

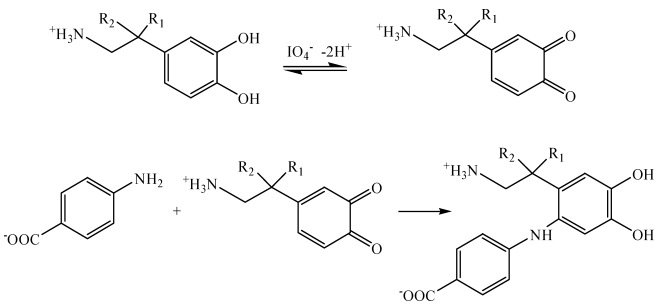

Chart 1

4.0×10^{-4} mol l^{-1} of 4-aminobenzoic acid was selected.

HPSAM In this special system, carbidopa and levodopa were considered as the analyte and interferent, respectively. Figure 2 shows the two straight lines obtained from Eqs. 1 and 2, which indicates $-C_H$ and A_H.

Wavelength Selection To select the appropriate wavelength pair for HPSAM, the following principles were applied. At the selected wavelengths the analyte signal must be linear to its concentration, and the interferent signal must remain unchanged when changing the analyte concentration. The analytical signal obtained from a mixture containing the analyte and the interferent should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at the two selected wavelengths (λ_1 and λ_2) must be as large as possible in order to obtain good accuracy and sensitivity.[13] In this case there were several pairs of wavelengths. The best wavelength pair was 394 and 498 nm (λ_1 and λ_2) and therefore these wavelengths were chosen. Standard solutions of carbidopa and levodopa were initially tested to validate the applicability of the chosen wavelengths. Figure 3 shows the H-point standard addition plots for several synthetic test solutions.

Analytical Characteristics Under the optimum conditions described above, simultaneous determination of levodopa and carbidopa, was performed applying HPSAM. To check the reproducibility of the method, four replicate measurements of levodopa and carbidopa were performed. The concentration of the interfering component (levodopa) was calculated in each test solution by means of the calibration method using standard solutions and the ordinate value of H-point (A_H). The concentration of carbidopa was directly obtained ($-C_H$).

In order to ensure the accuracy of the method, several synthetic mixtures with different concentration ratios of levodopa and carbidopa were analyzed with the proposed method. The results are given in Table 1. As the amounts of obtained recoveries show, the accuracy of the method is satisfactory. The relative standard deviation for four replicate measurements of a mixture of 5.00 μg ml^{-1} of levodopa and 5.00 μg ml^{-1} carbidopa was 4.45%, 2.89%, respectively.

Limit of detection was calculated as LOD=3S_{CH}, where S_{CH} is the standard deviation (S.D.) of several (n=5) repli-

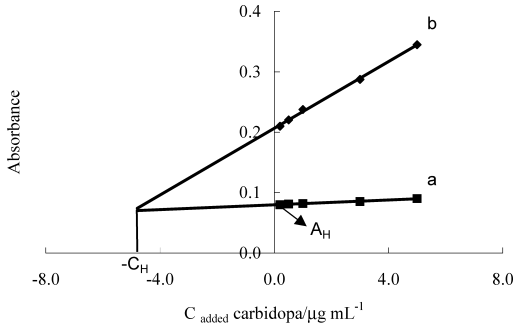

Fig. 2. Plot Absorbance of HPSAM for Simultaneous Determination of 5.00 μg ml^{-1} Carbidopa and 5.00 μg ml^{-1} of Levodopa in (a) 498 nm and (b) 394 nm

Conditions: 4-aminobenzoic acid 4×10^{-4} mol l^{-1}; periodate 3×10^{-4} mol l^{-1}. pH 4.0.

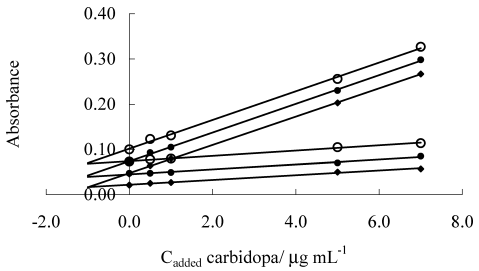

Fig. 3. Plots of HPSAM for Simultaneous Determination of Carbidopa and Levodopa in Different Concentrations, Levodopa=1.00 μg ml^{-1}, Carbidopa =1.00 μg ml^{-1} (◆), Levodopa=3.00 μg ml^{-1}, Carbidopa=1.00 μg ml^{-1} (●) and Levodopa=5.00 μg ml^{-1}, Carbidopa=1.00 μg ml^{-1} (○)

Conditions: 4-aminobenzoic acid 4×10^{-4} mol l^{-1}; periodate, 3×10^{-4} mol l^{-1}. pH 4.0.

Table 1. Results Obtained for Resolving of the Mixture of Carbidopa and Levodopa by HPSAM

A–C equation	r^2	Taken (μg ml^{-1})		Found[a] (μg ml^{-1})	
		Carbidopa	Levodopa	Carbidopa	Levodopa
A_{394}=0.0329C+0.0466	0.998	1.00	1.00	0.94 (94.0)	0.96 (96.0)
A_{498}=0.0048C+0.0202	0.996				
A_{394}=0.0252C+0.2089	0.996	5.00	5.00	4.98 (99.6)	5.21 (104.2)
A_{498}=0.0037C+0.1018	0.996				
A_{394}=0.0202C+0.1557	0.996	1.00	10.00	0.98 (98.0)	10.08 (100.8)
A_{498}=0.0057C+0.1413	0.998				
A_{394}=0.0306C+0.173	0.999	5.00	1.00	5.13 (102.6)	0.97 (97.0)
A_{498}=0.0055C+0.044	0.992				
A_{394}=0.0321C+0.081	0.999	0.50	5.00	0.49 (98.0)	4.81 (96.0)
A_{498}=0.0048C+0.0676	0.998				
A_{394}=0.0249C+0.1128	0.996	2.00	4.00	2.09 (104.5)	4.24 (106.0)
A_{498}=0.0041C+0.0693	0.961				
A_{394}=0.0303C+0.2044	0.992	6.00	1.00	6.12 (102.0)	1.09 (109.0)
A_{498}=0.0052C+0.0506	0.988				
A_{394}=0.0241C+0.1272	0.994	5.00	0.50	4.81 (96.2)	0.54 (108.0)
A_{498}=0.0016C+0.0188	0.964				
A_{394}=0.0264C+0.0514	0.997	0.30	3.00	0.33 (110.0)	3.04 (101.3)
A_{498}=0.005C+0.0442	0.986				
A_{394}=0.027C+0.2972	0.997	8.00	6.00	8.19 (102.3)	5.62 (93.6)
A_{498}=0.0048C+0.1153	0.905				

a) Values of recovery are given in parentheses.

cate measurements of zero concentration of analyte with HPSAM.[13] The corresponding value obtained for levodopa was 0.21 μg ml^{-1} and for carbidopa was 0.10 μg ml^{-1}. Relative error for the determination of each 5.00 μg ml^{-1} of levodopa and carbidopa was 3.78% and 2.54% for levodopa and carbidopa, respectively. Levodopa and carbidopa can be determined in the range 0.50—10.00 and 0.30—10.00 μg ml^{-1} with satisfactory accuracy and precision.

Mean Centering of Ratio Spectra The absorption spectra of the standard solutions of carbidopa with different concentrations were recorded in the wavelength range 350—550 nm with 1 nm intervals (Fig. 4a) and divided by the normalized spectrum of the levodopa and the ratio profiles were obtained (Fig. 4b). Mean centering (MC) of the ratio profiles was obtained in the wavelength range 390—420 nm (Fig. 4c). The concentration of carbidopa was determined by measuring the amplitude at 390 nm corresponding to a maximum wavelength shown in Fig. 4c. For the prediction of concentration of carbidopa in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of carbidopa.

The absorption spectra of the standard solutions of levodopa with different concentrations were recorded in the wavelength range 350—550 nm with 1 nm intervals (Fig. 5a) and divided by the normalized spectrum of the carbidopa and the ratio profiles were obtained (Fig. 5b). Mean centering (MC) of the ratio profiles was obtained in the wavelength range of 410—440 nm (Fig. 5c). The concentration of levodopa was determined by measuring the amplitude at 410 nm corresponding to a maximum wavelength shown in Fig. 5c. For the prediction of concentration of levodopa in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixtures were used instead of the spectra of standard solution of levodopa.

Analytical Characteristics In the proposed method mean centering of ratio spectra, Beer's law was obeyed in the concentration range 0.30—10.00 μg ml^{-1} for carbidopa, and 0.50—10.00 μg ml^{-1} for levodopa. Table 2 shows the linear regression parameters for calibration data for simultaneous determination of carbidopa and levodopa in their binary mixtures. Limit of detection of the method for determination of

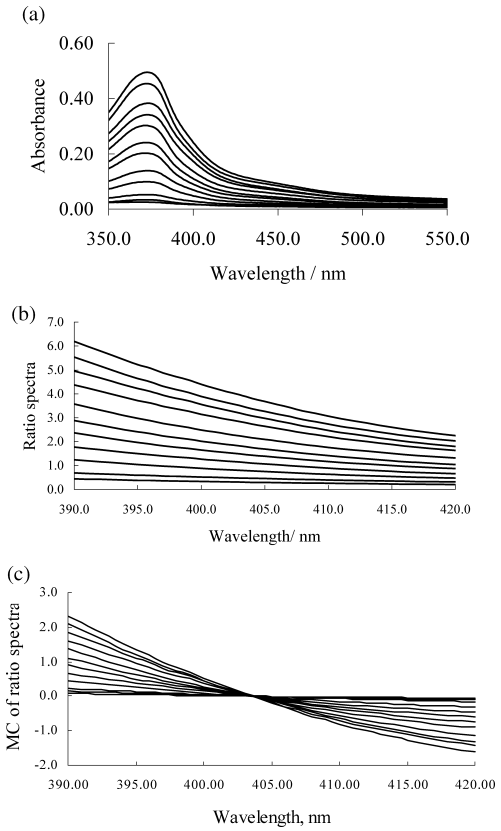

Fig. 4. The Absorption Spectra for the Standard Solutions of the Carbidopa with Different Concentrations (0.30—10.00 μg ml^{-1}) (a), the Ratio Spectra Obtained by Dividing the Normalized Spectra of the Levodopa (b) and the Mean Centering of Ratio Spectra (c)

Conditions: 4-aminobenzoic acid 4×10^{-4} mol l^{-1}; periodate, 3×10^{-4} mol l^{-1}. pH 4.0.

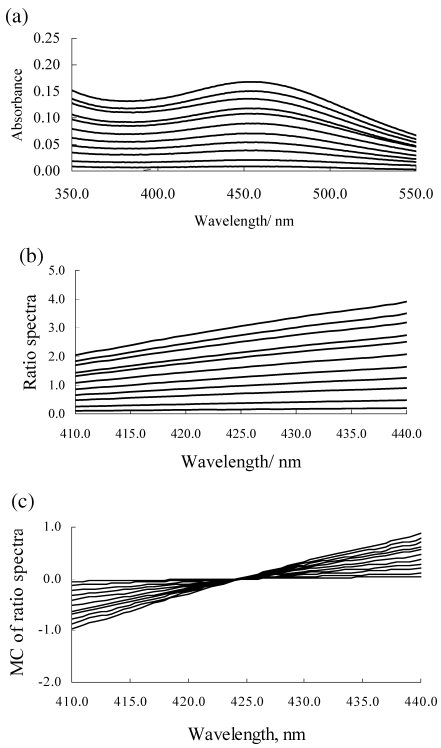

Fig. 5. The Absorption Spectra of the Standard Solutions of the Levodopa with Different Concentrations (0.50—10.00 μg ml^{-1}) (a), the Ratio Spectra Obtained by Dividing the Normalized Spectra of the Carbidopa (b) and the Mean Centering of Ratio Spectra (c)

Conditions: 4-aminobenzoic acid 4×10^{-4} mol l^{-1}; periodate, 3×10^{-4} mol l^{-1}. pH 4.0.

Table 2. Analytical Characteristics for Analysis of Levodopa and Carbidopa in Binary Mixtures by Mean Centering of Ratio Spectra Method

Analyte	Wavelength (nm)	Calibration equation[a)]	(R^2)[b)]	Linear range (μg ml^{-1})	LOD[c)] (μg ml^{-1})
Carbidopa	390	$y=0.2312x-0.0098$	0.9992	0.30—10.00	0.08
Levodopa	410	$y=0.0934x+0.0092$	0.9991	0.50—10.00	0.17

a) x is the concentration of drug in μg ml^{-1}, *b*) squared correlation coefficient, *c*) limit of detection.

Table 3. The Effect of Wavelength Range on the Analytical Parameter

Analyte	Wavelength range (nm)	Correlation coefficient ($n=10$)	Slope (ml μg^{-1})	Intercept
Carbidopa	390—490	0.9984	0.4053	−0.0161
	390—450	0.9986	0.3327	−0.0147
	410—450	0.9929	0.1118	−0.0025
	450—490	0.9966	0.0259	−0.0025
	390—420	0.9992	0.2312	−0.0098
Levodopa	390—420	0.9986	0.0930	0.0090
	380—410	0.9982	0.0821	0.0089
	410—470	0.9987	0.1827	0.0198
	410—440	0.9991	0.0934	0.0092
	420—450	0.9985	0.0896	0.0117

carbidopa and levodopa are also shown in Table 2.

To check the reproducibility of the method five replicate resolving of carbidopa and levodopa mixtures were performed. The relative standard deviation (RSD) for five replicate determinations of 5.00 μg ml^{-1} each of carbidopa and levodopa was 2.26 and 2.92%, respectively. The mean recoveries for simultaneous determination of these species in binary mixtures were 101.6 and 99.4% for carbidopa and levodopa, respectively.

The effect of wavelength range on the analytical parameters such as slope, intercept and correlation coefficient of the calibration graph was also tested (Table 3). It was observed that changing the wavelength range had significant effect on the analytical parameters. Therefore, selection of the wavelength range was performed for carbidopa and levodopa separately. The best wavelength range for levodopa and carbidopa by the proposed method was obtained 410—440 nm and 390—420 nm, respectively.

The effect of divisor concentration on the analytical parameters such as detection limit, slope, intercept and correlation coefficient of the calibration graphs was also tested. It was observed that changing the concentration of divisors in their linear calibration range had no significant effect on the analytical parameters. Therefore, a normalized spectrum of each of the carbidopa and levodopa was used as divisor profile in the proposed method.

In order to obtain the accuracy and precision of the method, several synthetic mixtures with different concentration ratios of carbidopa and levodopa were analyzed using the proposed method. The results are given in Table 4. The prediction error of a single component in the mixtures was calculated as the relative standard error (R.S.E) of the prediction concentration[18]:

$$\text{R.S.E. (\%)} = \left(\frac{\sum_{j=1}^{N} (\hat{C}_j - C_j)^2}{\sum_{J=1}^{N} (C_j)^2} \right)^{1/2} \times 100 \quad (6)$$

where N is the number of samples, C_j the concentration of the component in the jth mixture and $\hat{C}_j$ the estimated concentration. The total prediction error of N samples is calculated as follows:

$$\text{R.S.E.}_t \text{ (\%)} = \left(\frac{\sum_{i=1}^{M} \sum_{J=1}^{N} (\hat{C}_{ij} - C_{ij})^2}{\sum_{i=1}^{M} \sum_{J=1}^{N} (C_{ij})^2} \right)^{1/2} \times 100 \quad (7)$$

Table 4. Results for Analysis of Levodopa and Carbidopa in Binary Mixtures in Different Concentration Ratios by Mean Centering of Ratio Spectra Method

Taken (μg ml^{-1})		Found (μg ml^{-1})		Recovery (%)	
Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa
1.00	1.00	1.07	0.96	107	96
5.00	5.00	5.02	4.73	100.4	94.6
1.00	10.00	1.07	9.45	94.5	95.3
5.00	1.00	4.96	0.97	98	97.0
0.50	5.00	0.51	5.01	102	100.2
2.00	4.00	2.00	3.84	96	96.0
6.00	1.00	6.01	1.05	100.1	105
5.00	0.50	4.98	0.49	99.6	98.0
0.30	3.00	0.31	2.88	103.3	96.0
8.00	6.00	8.12	5.93	101.5	98.8
Meanrecovery				102	97.7
R.S.E (%)[a] single				1.22	4.00
R.S.E$_t$ (%)[b] total					3.06

a) Calculated from Eq. 6. *b*) Calculated from Eq. 7.

where C_{ij} is the concentration of the component in the jth samples and $\hat{C}_{ij}$ is its estimation. Table 4 also shows the reasonable single and total relative errors for such system.

Interference Study To study the selectivity of the proposed methods, the effect of various species on the determination of a mixture of 5.00 μg ml^{-1} each of levodopa and carbidopa by both the methods was tested under the optimum conditions. The tolerance limit was defined as the concentration of added ion causing less than a ±5% relative error. Ions Na^+, K^+, Al^{3+}, Cl^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}, ascorbic acid, glycine, lactose, glucose, fructose did not interfere up to 5000 μg ml^{-1}. Zn^{2+}, Mg^{2+} and PO_4^{3-} interfered at 2500, 250 and 100 μg ml^{-1}, respectively. Pb^{2+} and Cu^{2+} interfered at 50 μg ml^{-1}.

Application The proposed methods were successfully applied to the simultaneous determination of mixtures of carbidopa-levodopa in the LevodopaC tablets and after addition in tap water samples. The results are shown in Table 5. The good agreement between these results and known values indicate the successful applicability of the proposed methods for simultaneous determination of carbidopa and levodopa in mixture sample.

Conclusion

The proposed methods, HPSAM and mean centering of ratio spectra, are very suitable for simultaneous determination of levodopa and carbidopa and can be applied to the analysis of pharmaceutical samples. The results show that accuracy, precision, reproducibility, sensitivity and linear range are nearly the same for both the methods. A comparison between the results obtained by the methods indicate that the detection limit and RSD% obtained by mean centering of ratio spectra method are lower than those obtained by HPSAM. These methods are very simple, rapid, accurate and cheap. Standard addition can be used in the proposed methods and matrix effects can be removed easily. Therefore, these methods can be used for resolving binary mixtures in the complex samples with unknown matrices.

Table 5. Determination of Levodopa and Carbidopa Mixtures in Tap Water Samples and Tablet by the Mean Centering of Ratio Spectra Method and HPSAM

Sample	Spiked or present in sample ($\mu g\,ml^{-1}$)		Found			
			Mean centering of ratio spectra ($\mu g\,ml^{-1}$)		HPSAM ($\mu g\,ml^{-1}$)	
	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa
Tap water	5.00	1.00	4.82±0.02	1.06±0.10	4.84±0.24	1.09±0.01
	1.00	10.00	1.04±0.07	10.35±0.04	0.84±0.01	10.75±0.06
Tablet	1.00	10.00	0.97±0.05	10.13±0.11	1.10±0.04	9.78±1.01
	1.50	5.00	1.42±0.03	5.06±0.05	1.41±0.03	4.91±0.54

a) Mean±S.D. ($n=3$).

References

1) Dryhurst G., Kadish K. M., Scheller F., Renneberg R., "Biological Electrochemistry," Vol. 1, Academic Press, New York, 1982.
2) Michotte Y., Moors M., Deleu D., Herregodts P., Ebinger G., *J. Pharm. Biomed. Anal.*, **5**, 659—664 (1987).
3) Sagar A. K., Smyth M. R., *J. Pharm. Biomed. Anal.*, **22**, 613—624 (2000).
4) Coello J., Maspoch S., Villegas N., *Talanta*, **53**, 627—637 (2000).
5) Uslu B., Özkan S. A., *Anal. Lett.*, **35**, 303—314 (2002).
6) Madrakian T., Afkhami A., Borazjani M., Bahram M., *Bull. Korean Chem. Soc.*, **25**, 1764—1768 (2004).
7) Bosch-Reig F., Campins-Falco P., *Analyst*, **113**, 1011—1016 (1988).
8) Bosch-Reig F., Campins-Falco P., Sevillano-Cabeza A., Herraez-Hernandez B., Molins-Legua C., *Anal. Chem.*, **63**, 2424—2429 (1991).
9) Bosch-Reig F., Campins-Falco P., Verdu-Andres J., *Talanta*, **39**, 1—7 (1992).
10) Bosch-Reig F., Campins-Falco P., Verdu-Andres J., *Anal. Chim. Acta*, **270**, 253—265 (1992).
11) Bosch-Reig F., Campins-Falco P., Verdu-Andres J., Molins-Legua C., *Talanta*, **41**, 39—52 (1994).
12) Bosch-Reig F., Campins-Falco P., Verdu-Andres J., *Analyst*, **119**, 2123—2127 (1994).
13) Safavi A., Abdollahi H., *Talanta*, **54**, 727—734 (2001).
14) Afkhami A., Bahram M., *Talanta*, **66**, 712—720 (2005).
15) Massart D. L., Vandeginste B. G. M., Buydens L. M. C., Jong S. D. E., Lewi P. J., Smeyers-Verbeke J., "Handbook of Chemometrics and Qualimetrics: Part A," Elsevier Science Ltd., 1997.
16) Afkhami A., Nematollahi D., Madrakian T., Khalafi L., *Electrochim. Acta*, **50**, 5633—5640 (2005).
17) Madrakian T., Afkhami A., Khalafi L., Mohammadnejad M., *J. Braz. Chem. Soc.*, **17**, 1259—1265 (2006).
18) Abdollahi A., *Anal. Chim. Acta*, **442**, 327—336 (2001).