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Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 313–318
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Simultaneous kinetic determination of levodopa and carbidopa by H-point standard addition method

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Received 25 September 2006; received in revised form 14 February 2007; accepted 16 February 2007
Available online 23 February 2007

Abstract

The kinetic H-point standard addition method (HPSAM) was applied to the simultaneous determination of levodopa and carbidopa. The method was based on the difference in the rate of oxidation of these compounds with Cu(II)–neocuproine system and formation of Cu(I)–neocuproine complex at pH 5.5. The absorbance of the Cu(I)–neocuproine complex was monitored at 453 nm. Experimental conditions such as pH, reagent concentrations, ionic strength and temperature were optimized. Simultaneous determination of levodopa and carbidopa was performed in the range of 0.8–4 and 0.2–1.5 $\mu g\,ml^{-1}$, respectively. The proposed method was applied to the simultaneous determination of levodopa and carbidopa in pharmaceutical samples, and satisfactory results were obtained.

Keywords: Levodopa; Carbidopa; Simultaneous determination; HPSAM

1. Introduction

Levodopa (L-dopa), [(−)-3-(3,4-dihydroxyphenyl)-L-alanine] and carbidopa [(−)-L-2-(3,4-dihydroxybenzyl)-2-hydrazinopropionic acid] are catecholamines with an alkylamine chain attached to a benzene ring bearing two hydroxyl groups. Levodopa, the medication of choice for the treatment of Parkinson's disease, is principally metabolized by an enzymatic reaction (dopa-decarboxylase) to dopamine compensating for the deficiency of dopamine in the brain [1]. Parkinson's disease is a progressive neurological disorder that occurs when the brain fails to produce enough dopamine. This condition causes tremor, muscle stiffness or rigidity, slowness of movement (bradykinesia) and loss of balance. Dopamine cannot be administered directly because it cannot penetrate the blood–brain barrier. Therefore, levodopa, which can be orally administered, is used to provide a source of dopamine, and is used in the treatment of Parkinson's disease to provide symptomatic relief to most patients at the initial stages of the disease.

However, elevated levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias [2]. Carbidopa has been used as an inhibitor of the decarboxylase activity. By administering levodopa combined with carbidopa, the concentration of dopamine is controlled at an appropriate level effectively and with generally reduced side effects.

In order to achieve better curative effect and lower toxicity, it is very important to control the content of levodopa and carbidopa in pharmaceutical tablets. Various methods like spectrophotometry [3–5], gas chromatography (GC) [6], high performance liquid chromatography (HPLC) [7], chemiluminescence [8,9], amperometric and voltammetric determination [10–12], potentiometry [13], radio-immunoassay [14] and flow injection analysis (FIA) [15–17] have been described in literature for the determination of levodopa or carbidopa in various biological samples and pharmaceutical preparations.

Their simultaneous determination in pharmaceutical preparations and biological fluids has been traditionally achieved using high performance liquid chromatography (HPLC) [18–20]. In recent years, capillary electrophoresis (CE) has been alternatively used, and several separation methodologies have been developed in order to resolve both compounds and their enantiomers [21–23]. A method utilizing NMR spectroscopy has also been developed [24]. However, simultaneous spectrophotometric determination of levodopa and carbidopa has rarely been reported. The only few methods reported recently [25,26] lacks high sensitivity and thus there is a need for a simple, accurate and

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0731-7085/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.jpba.2007.02.020

sensitive method for simultaneous determination of levodopa and carbidopa.

The modification of the standard addition method is called "H-point standard addition method (HPSAM)" [27,28] and is used where the error resulting from the presence of a direct interference in the presence of an analyte is transformed into a systematic error. This error can then be evaluated and eliminated. This method also permits both proportional and constant errors produced by the matrix of the sample to be corrected. In 1991, Campíns-Falco and coworkers [29] established the application of HPSAM to kinetic data for simultaneous determination of binary mixtures or the calculation of analyte concentration completely free from bias error. The greatest advantage of HPSAM is that, it can remove errors resulting from the presence of an interfering and blank reagent. So, HPSAM can determine the two components simultaneously [30–41]. If another version of HPSAM involving the use of the absorbance increment as analytical signal is employed using ΔA_{t1-t2}, the resulting graph will allow the analyte concentration, C_X, to be calculated with no systematic, constant, or proportional error thanks to the intrinsic features of the HPSAM and the nature of standard addition method.

This work describes a simple, inexpensive and accurate method for the simultaneous kinetic determination of levodopa and carbidopa. The formation of a charge-transfer complex between Cu(I) and neocuproine (Nc) (2,9-dimethyl-1,10-phenantheroline) [42] is the basis of the existing spectrophotometric method for the indirect determination of levodopa and carbidopa.

The proposed method is based on the difference between the rate of the reactions of levodopa and carbidopa with Cu(II) in the presence of neocuproine in a buffered medium. The absorbance of the complex formed, i.e. $[Cu(Nc)_2]^+$, was monitored at the maximum wavelength of 453 nm as a function of time. Difference in kinetic behavior of levodopa and carbidopa accompanied with mathematical treatment of data, using HPSAM permits simultaneous analysis of the two compounds. Also, for the determination of levodopa in the presence of carbidopa, the absorbance increment as an analytical signal can be employed in another version of HPSAM to allow the analyte concentration, C_{levodopa} to be calculated with no systematic, constant or proportional error due to the intrinsic features of the HPSAM.

2. Experimental

2.1. Chemicals

An ethanolic stock solution of neocuproine (Nc) (3.0×10^{-2} M) was prepared by dissolving 0.1562 g of Nc (Merck) in ethanol and diluting to 25 ml with the same solvent. Copper stock solution, 0.1 M, was prepared by dissolving 0.6040 g of $Cu(NO_3)_2{\cdot}3H_2O$ in water and diluting to 25 ml. Hydrochloric and acetic acid solutions (1.0 M) were prepared by dilution of their concentrated solutions (Fluka). NaOH stock solution (1.0 M) was prepared by dissolving 4.0 g of NaOH (Merck) in water and diluting to 100 ml with distilled water. Acetic-acetate buffer (pH 5.5, 1.0 M) was prepared by using acetic acid (1.0 M) and sodium hydroxide (1.0 M) solution and adjusting the pH with a pH-meter to 5.5. A stock solution of levodopa (1500 μg ml^{-1}, Fluka) was prepared daily by dissolving 15.0 mg of levodopa in a minimum amount of a weak acidic solution and diluting to 10 ml with water. Carbidopa stock solution (300 μg ml^{-1}, Fluka) was prepared daily by dissolving 3.0 mg of carbidopa in water and diluting to 10 ml. All chemicals used were of analytical reagent grade. Triply distilled water was used throughout.

2.2. Instruments

The absorbance measurements as a function of time, were made using a Shimadzu 1601 PC UV–Vis spectrophotometer attached to a Pentium 200 MHz computer. Measurements of pH were made with a Metrohm 780 pH-meter using a combined glass electrode.

2.3. Procedure

The solution containing Cu(II) and neocuproine reagents was prepared in 100 ml flask by the addition of 20 ml of stock Nc solution, 6 ml of stock Cu(II) solution, 20 ml of buffer solution (pH 5.5, 1.0 M) and diluting with water to the mark. For each measurement, 3 ml of the reagent solution was transferred to the spectrophotometric cell and the absorbance of this solution was zeroed at 453 nm before injecting the analyte(s). Then, an appropriate amount of levodopa and/or carbidopa in the concentration ranges of 0.80–4.00 and 0.20–1.50 μg ml^{-1}, respectively, was injected into the cell using a 100-μl syringe and stirred manually for few seconds. The variation of the absorbance versus time was recorded immediately. The absorbance was measured at 453 nm with 1 s time intervals for each sample. Simultaneous determination of levodopa and carbidopa with HPSAM was performed by measuring the absorbances at 12 and 100 s after initiation of the reaction for each sample.

For the analysis of real samples, eight commercially available tablets (SINEMET® PLUS RETARD) containing levodopa and carbidopa were weighted and ground and the solution was prepared by dissolving a certain amount of grounded tablets in the weak acidic solutions and filtering the solution. Three milliliters of the prepared solution containing levodopa and carbidopa was analyzed ($n = 3$) by the proposed method.

3. Results and discussion

3.1. Principle of the method

The Cu(II)–Nc system allows the spectrophotometric determination of a reducing agent, A_{red}, provided that the redox reaction:

$$n\text{Cu}^{2+} + 2n\text{Nc} + A_{red} \rightarrow n[\text{Cu(Nc)}_2]^+ + A_{OX}$$

is complete with the formation of an equivalent amount of $[Cu(Nc)_2]^+$ with respect to the n-electron reductant, A_{red}.

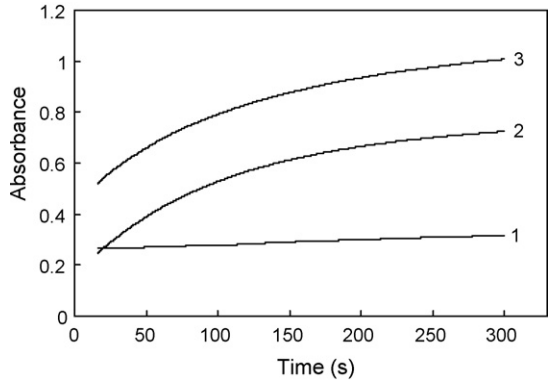

Fig. 1. Absorbance changes of Cu(II)–Nc system vs. time in the reaction with (1) carbidopa (1.5 μg ml^{-1}), (2) levodopa (2.0 μg ml^{-1}) and (3) a mixture of carbidopa (1.5 μg ml^{-1}) and levodopa (2.0 μg ml^{-1}) recorded at 453 nm.

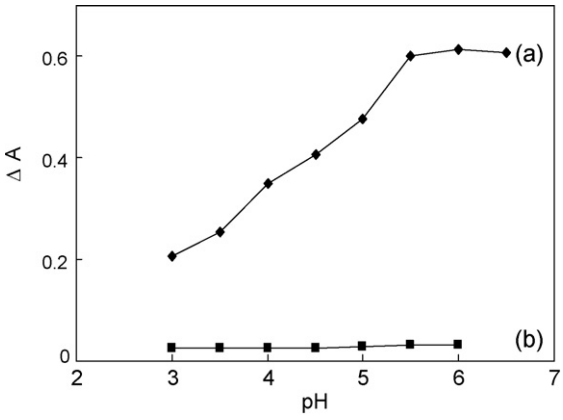

Fig. 2. Effect of pH of Cu(II)–Nc solution on the reaction rates of (a) levodopa (2.0 μg ml^{-1}) and (b) carbidopa (2.0 μg ml^{-1}) systems. Experimental conditions: temperature 25.0 °C, 6.0×10^{-3} M Cu(II) and 6.0×10^{-3} M Nc. ΔA was calculated as the difference in the absorbances at 40 and 250 s.

Copper(II) is a strong oxidizing agent only when its reduction product, Cu(I), is stabilized by a strong complex-forming ligand, e.g. Nc. The standard potential of the Cu(II)–Cu(I) couple (0.17 V) is shifted to more positive values by preferential complexation of Cu(I). The stronger the reductant, the more quantitative will be the reduction of Cu(II) with the subsequent formation of $[Cu(Nc)_2]^+$ [42].

The reduction of Cu(II) to Cu(I) by levodopa and carbidopa is taken place in the presence of Nc. Subsequent complex formation between the resulted Cu(I) and Nc produces $[Cu(Nc)_2]^+$ that has a maximum absorbance at 453 nm. Difference in kinetic behavior of the two analytes accompanied with mathematical treatment of data using HPSAM permits simultaneous analysis of the two compounds. As it is illustrated in Fig. 1, the reaction rate of carbidopa with Cu(II)–Nc system was very low and the difference in the absorbance was very small. However, the reaction rate of levodopa is faster.

3.2. Effect of operational parameters

Selectivity is one of the most important requirements of any analytical method. Employing suitable experimental conditions such as pH, concentration of Nc and Cu(II) and using desirable mathematical algorithms can improve selectivity. The kinetic behavior of most chemical species in chemical reactions can be controlled by changing the microenvironment of reaction.

3.2.1. Effect of pH

The effect of pH on the reaction rates of levodopa and carbidopa over the pH range of 3.0–6.5 was examined. Increase in pH up to 5.5, caused an increase in the reaction rates for levodopa (Fig. 2(a)). However, over the examined range of pH, no significant change in the reaction rate of carbidopa with Cu(II)–Nc system was observed (Fig. 2(b)). So, pH 5.5 was chosen as the optimum pH value for simultaneous determination of levodopa and carbidopa and was used in further studies.

3.2.2. Effects of Cu(II) and neocuproine concentrations

The effect of Cu(II) concentration, at constant concentration of Nc (6.0×10^{-3} M), was studied. The increase of Cu(II) concentration up to 6.0×10^{-3} M caused an increase in the reaction rate of levodopa with Cu(II)–Nc system. However, Cu(II) concentrations above 6.0×10^{-3} M decreased the reaction rate of levodopa. The increase of Cu(II) concentration caused no significant change in the reaction rate of carbidopa with Cu(II)–Nc system. The oxidizing power of Cu(II) in a solution containing Nc is dependent on the ease of formation of $[Cu(Nc)_2]^+$. At high concentrations of Nc with respect to Cu(II), the reaction rate of levodopa with Cu(II)–Nc system is decreased. This might be due to the fact that high concentrations of Nc would result in a positive interference from Cu(II) which could have arisen from incomplete conversion of Cu(I) into the Cu(I)–Nc complex via mixed ligand complex formation. On the other hand, a large excess of Cu(II) with respect to Nc can also exhibit an affinity for Nc, thereby preventing the preferential quantitative formation of $[Cu(Nc)_2]^+$. Thus, large excess of Cu(II) competes with Cu(I) for complex formation with Nc [42]. The experimental results showed that 1:1 ratio of Nc and Cu(II) was the best ratio for further work. Among the 1:1 ratios of Cu(II) and Nc with different equal concentrations, the concentration ratio of 6.0×10^{-3} M:6.0×10^{-3} M was the best because this concentration provides higher sensitivity and reasonable time interval for analysis of levodopa.

3.2.3. Effect of surfactants

Micelles change the effective microenvironment around dissolved solutes and as a consequence their physicochemical properties, such as rate constant, spectral profile, etc., can be affected. The fact that micelles can accelerate reactions has been increasingly exploited in the last few years to improve the features of both catalytic [43–45] and non-catalytic [46] kinetics methods [47]. Micelles can alter reaction kinetics through their rate, control pathways, change reaction mechanisms and/or the observed rate constant ratio of two or more species interacting with a common reagent. Because micelles can affect the kinetics of reactions; the effects of addition of different surfactants on the system was studied under optimum conditions. Cetyltrimethyl ammonium bromide (CTAB) as a cationic surfactant, sodium

dodecyl sulfate (SDS) as an anionic surfactant and Triton X-100 as a nonionic surfactant were selected at concentrations above their critical micelle concentrations (cmc). However, none of these had a significant effect on the reaction rates of levodopa and carbidopa with Cu(II)–Nc system.

3.2.4. Effect of ionic strength

The influence of ionic strength on the reaction rates of levodopa and carbidopa was studied by the addition of KNO_3 up to 0.5 M. Increase in the ionic strength (i.e. increase in KNO_3 concentration) caused no significant change in the reaction rates of levodopa and carbidopa with Cu(II)–Nc system.

3.2.5. Effect of temperature

The effect of temperature on the reaction rates of levodopa and carbidopa was studied in the range of 10–60 °C. Increase in the temperature caused an increase in the reaction rates of levodopa and carbidopa with Cu(II)–Nc system. Increasing temperature higher than 25.0 °C, causes the reaction rate of levodopa to become very fast and this limits the time range of analysis (Fig. 3(a)). The reaction rate of carbidopa also increased above 25.0 °C, which is not suitable for application in HPSAM (Fig. 3(b)). Moreover, for simplicity and better control of temperature, 25.0 °C was chosen as the optimum temperature. This temperature was used in further studies.

3.3. Selection of appropriate times for applying H-point standard addition method (HPSAM)

The reaction of carbidopa with Cu(II)–Nc system under the optimized conditions was very slow. Thus, the changes in the absorbance between 1 and 200 s were very small. However, the reaction of levodopa with Cu(II)–Nc system under similar conditions was nearly complete within 200 s.

For selection of suitable time pairs, some time pairs such as 12–100, 12–95, 70–150, 80–120, and 12–80 s were examined and the corresponding H-point plots were plotted. The time pair that gave the greatest slope increment, lower error, and shortest analysis time, was selected. For this reason, the time pair of 12–100 s was employed. The absorbances corresponding to levodopa at the two selected times of 12 and 100 s were selected to calculate levodopa concentrations in the range of 0.8–4.0 μg ml^{-1} in the presence of carbidopa. The concentrations of carbidopa in the range of 0.2–1.5 μg ml^{-1} were calculated in each sample by obtaining the ordinate values of the H-point (A_H). The calibration graph for carbidopa was plotted using ordinate values of H-points (A_H) versus corresponding carbidopa concentrations.

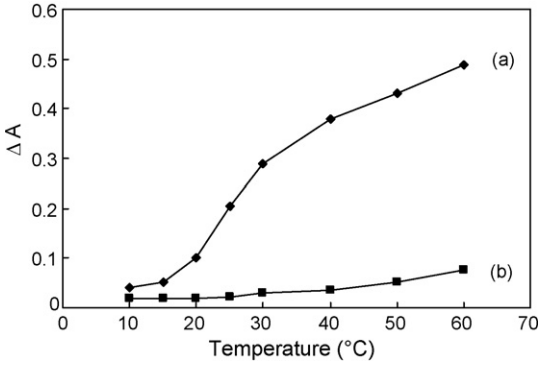

Fig. 3. Effect of temperature (10–60 °C) on the reaction rates of (a) levodopa (2.0 μg ml^{-1}) and (b) carbidopa (2.0 μg ml^{-1}) systems. Experimental conditions: 6.0×10^{-3} M Cu(II), 6.0×10^{-3} M Nc, pH 5.5. ΔA was calculated as the difference in the absorbances at 12 and 100 s.

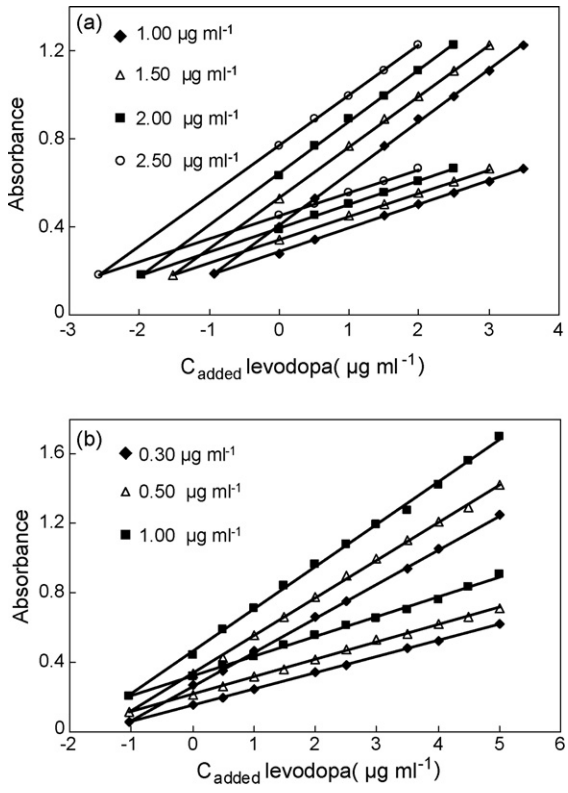

Fig. 4. Plots of H-point standard addition method. (a) Fixed carbidopa concentration (0.8 μg ml^{-1}) and different concentrations of levodopa; and (b) fixed levodopa concentration (1.0 μg ml^{-1}) and different concentrations of carbidopa.

3.4. Data analysis using H-point standard addition method

At H-point, C_H is independent of the concentration of interferent and A_H is also independent of the analyte concentration. As shown in Fig. 4, the value of C_H is independent of the concentration of carbidopa, and the value of A_H is independent of the concentration of levodopa.

Under optimum conditions, several synthetic mixed samples with different concentrations of levodopa and carbidopa were analyzed using the proposed method. The accuracy and precision of the results were all satisfactory.

As shown in Table 1, for four replicate experiments, the obtained levodopa and carbidopa concentrations are in good agreement with the added concentrations. The accuracy and precision of the results were all satisfactory.

Application of the absorbance increment version of HPSAM, i.e. the ΔA_{t1-t2} versus C_{added} variant, yields the concentration of levodopa directly from the intercept on *Y*-axis. Fig. 5 shows the results.

Table 1
Results of four replicate experiments for the analysis of levodopa and carbidopa mixtures

A–C equation	R^2	Sample ($\mu g\,ml^{-1}$)		Found ($\mu g\,ml^{-1}$)	
		Levodopa	Carbidopa	Levodopa	Carbidopa
$A_{100} = 0.2152C_i + 0.5681$	0.9968	2.00	0.60	2.04	0.59
$A_{12} = 0.0997C_i + 0.3327$	0.9991				
$A_{100} = 0.2234C_i + 0.5962$	0.9998	2.00	0.60	2.06	0.58
$A_{12} = 0.1001C_i + 0.3427$	0.9999				
$A_{100} = 0.2089C_i + 0.5645$	0.9996	2.00	0.60	2.06	0.61
$A_{12} = 0.0993C_i + 0.3383$	0.9993				
$A_{100} = 0.2164C_i + 0.5745$	0.9994	2.00	0.60	2.05	0.59
$A_{12} = 0.0992C_i + 0.3337$	0.9993				
Mean				2.05	0.59
Standard deviation				0.01	0.013

C_i = concentration of analyte (levodopa).

Table 2
Results for levodopa and carbidopa quantification in pharmaceutical samples (three replicates)

Sample	Nominal ($\mu g\,ml^{-1}$)		Found[a] ($\mu g\,ml^{-1}$)		Recovery (%)	
	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa
1	2.00	0.50	1.96	0.51	98.0	102.0
2	2.80	0.70	2.79	0.72	99.6	102.9
3	3.20	0.80	3.18	0.82	99.4	102.5

[a] Mean value ($n = 3$).

Detection limits were obtained as 0.15 and 0.03 $\mu g\,ml^{-1}$, for levodopa and carbidopa, respectively.

3.5. Interference studies

Some substances that were supposed to be present in pharmaceuticals were tested for their possible interferences. Absorbance changes of a solution containing levodopa (2.0 $\mu g\,ml^{-1}$) and carbidopa (1.0 $\mu g\,ml^{-1}$) were analyzed for seven times and then, the effect of interfering species at different concentrations on the absorbance of this solution were studied. A species was considered as interference when its presence produced a variation in the absorbance change of the sample (in the period of 200 s) greater than two times the standard deviation of the sample. The following excipients did not interfere in the maximum tested concentrations ($\mu g\,ml^{-1}$) shown in parentheses: sucrose (1000), glucose (500), starch (1000), saccharin (1000), and riboflavin (65). Caffeine did not show any interfering effect up to 50 $\mu g\,ml^{-1}$ but interfered above this concentration. Some other compounds with significant redox properties (e.g. ascorbic acid) may interfere in the proposed procedure. But our goal was the determination of levodopa and carbidopa in tablets. In these tablets the other redox compounds are absent.

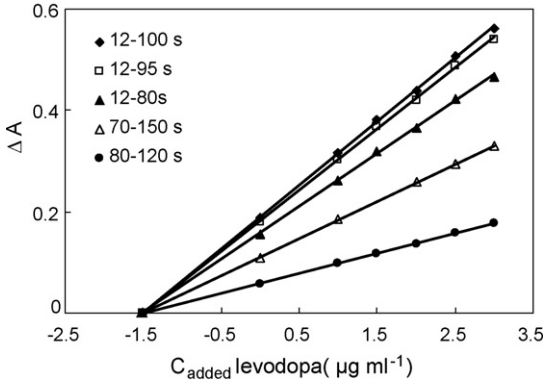

Fig. 5. ΔA vs. added levodopa concentrations at different time intervals for a synthetic mixture with 1.5 $\mu g\,ml^{-1}$ levodopa and 0.8 $\mu g\,ml^{-1}$ carbidopa.

3.6. Prediction of levodopa and carbidopa concentrations in real samples

The proposed method was applied to the determination of levodopa and carbidopa in a commercially available tablet (SINEMET® PLUS RETARD) containing 25 mg carbidopa and 100 mg levodopa. The quantitative results of this analysis are summarized in Table 2. As it is shown, levodopa and carbidopa can be determined with satisfactory accuracy and precision in pharmaceutical preparations.

4. Conclusion

An accurate and simple method was proposed for the simultaneous determination of levodopa and carbidopa in pharmaceuticals. The results showed the successful applicability of the proposed method for their simultaneous quantification because of good selectivity, accuracy, and precision. The method is more sensitive than previously reported methods based on simultaneous spectrophotometric methods [25,26]. It can also determine lower concentrations of carbidopa in the presence of higher amounts of levodopa, and thus can be successfully applied

to the analysis of tablets which usually contain higher amounts of levodopa with respect to carbidopa. The obtained detection limits for levodopa and carbidopa were 0.15 and 0.03 $\mu g\,ml^{-1}$, respectively.

Acknowledgements

The authors wish to express their gratitude to Shiraz University Research Council and Third World Academy of Science, Iran Chapter (TWASIC) for the support of this work. They are also thankful to Dr. T. Madrakian for the kind donation of samples of levodopa and carbidopa.

References

[1] H.V. Barnes, Clinical Medicine, Year Book Medical Publisher, New York, 1988, pp. 745–750.
[2] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopaeia, The Pharmaceutical Press, London, 1993.
[3] P. Nagaraja, K.C. Srinivasa Murthy, K.S. Rangappa, N.M. Made Gowda, Talanta 46 (1998) 39–44.
[4] J. Coello, S. Maspoch, N. Villegas, Talanta 53 (2000) 627–637.
[5] P. Nagaraja, R.A. Vasantha, K.R. Sunitha, J. Pharm. Biomed. Anal. 25 (2001) 417–424.
[6] P.S. Doshi, D.J. Edwards, J. Chromatogr. 210 (1981) 505–511.
[7] G. Cannazza, A.D. Stefano, B. Mosciatti, D. Braghiroli, M. Baraldi, F. Pinnen, P. Sozio, C. Benatti, C. Parenti, J. Pharm. Biomed. Anal. 36 (2005) 1079–1084.
[8] N.T. Deftereos, A.C. Calokerinos, C.E. Efstathiou, Analyst 118 (1993) 627–632.
[9] M.L. Yang, L.Q. Li, M.L. Feng, J.R. Lu, Yaowu. Fenxi. Zazhi. 18 (1998) 41–45.
[10] E.M. Garrido, J.L.F.C. Lima, C. DelerueMatos, J. Pharm. Biomed. Anal. 15 (1997) 845–849.
[11] M.F.S. Teixeira, M.F. Bergamini, C.M.P. Marques, N. Bocchi, Talanta 63 (2004) 1083–1088.
[12] M.F. Bergamini, A. Santos, N.R. Stradiotto, M.V.B. Zanoni, J. Pharm. Biomed. Anal. 39 (2005) 54–59.
[13] S.S. Badawy, Y.M. Issa, A.S. Tag-Eldin, Electroanalysis 8 (1996) 1060–1064.
[14] C.J. Waugh, I.F. Gow, C.R.W. Edwards, B.C. Williams, Biochem. Soc. Trans. 17 (1989) 163–164.
[15] O. Fatibello-Filho, I.C. Vieira, Analyst 122 (1997) 345–350.
[16] L.H. Marcolino-Júnior, M.F.S. Teixeira, A.V. Pereira, O. Fatibello-Filho, J. Pharm. Biomed. Anal. 25 (2001) 393–398.
[17] M. Pistonesi, M.E. Centurión, B.S. Fernández Band, P.C. Damiani, A.C. Olivieri, J. Pharm. Biomed. Anal. 36 (2004) 541–547.
[18] J.B. Kafil, B.S. Dhingra, J. Chromatogr. A 667 (1994) 175–181.
[19] A. Tolokán, I. Klebovich, K. Balogh-Nemes, G. Horvai, J. Chromatogr. B 698 (1997) 201–207.
[20] K.A. Sagar, M.R. Smyth, J. Pharm. Biomed. Anal. 22 (2000) 613–624.
[21] S. Fanali, V. Pucci, C. Sabbioni, M.A. Raggi, Electrophoresis 21 (2000) 2432–2437.
[22] L. Zhang, G. Chen, Q. Hu, Y. Fung, Anal. Chim. Acta 431 (2001) 287–292.
[23] P.T.T. Ha, A. Van-Schepdael, T. Hauta-Aho, E. Roets, J. Hoogmartens, Electrophoresis 23 (2002) 3404–3409.
[24] Z. Talebpour, S. Haghgoo, M. Shamsipur, Anal. Chim. Acta 506 (2004) 97–104.
[25] T. Madrakian, A. Afkhami, M. Borazjani, M. Bahram, Bull. Korean Chem. Soc. 25 (2004) 1764–1768.
[26] P.C. Damiani, A.C. Moschetti, A.J. Rovetto, F. Benavente, A.C. Olivieri, Anal. Chim. Acta 543 (2005) 192–198.
[27] F. Bosch-Reig, P. Campíns-Falcó, Analyst 113 (1988) 1011–1016.
[28] F. Bosch-Reig, P. Campíns-Falcó, J. Verdú-Andrés, Anal. Chim. Acta 283 (1993) 831–844.
[29] F. Bosch-Reig, P. Campíns-Falco, A. Sevillano-Cabeza, R. Herraez-Hernandez, C. Molins-Legua, Anal. Chem. 63 (1991) 2424–2429.
[30] J. Yang, G. Zhou, N. Jie, R. Han., C. Lin, J. Hu, Anal. Chim. Acta 325 (1996) 195–200.
[31] P. Campins-Falco, J. Verdu-Andres, F. Bosch-Reig, Anal. Chim. Acta 348 (1997) 39–49.
[32] P. Campins-Falco, F. Bosch-Reig, F. Blasco-Gomez, Talanta 47 (1998) 193–202.
[33] J. Verdu-Andres, F. Bosch-Reig, P. Campins-Falco, J. Chemom. 12 (1998) 27–40.
[34] A. Safavi, H. Abdollahi, F. Sedaghatpour, S. Zeinali, Anal. Chim. Acta 409 (2000) 275–282.
[35] A. Safavi, H. Abdollahi, Talanta 54 (2001) 727–734.
[36] A. Safavi, H. Abdollahi, M.R. Hormozi Nezhad, Talanta 56 (2002) 699–704.
[37] A. Safavi, H. Abdollahi, M. Bagheri, Anal. Chim. Acta 459 (2002) 119–131.
[38] A. Safavi, H. Abdollahi, F. Sedaghatpour, M.R. Hormozi Nezhad, Talanta 59 (2003) 147–153.
[39] H. Abdollahi, S. Zeinali, Talanta 62 (2004) 151–163.
[40] A. Safavi, O. Moradlou, Anal. Lett. 37 (2004) 2337–2349.
[41] A. Safavi, F. Towhidi, H. Abdollahi, Can. J. Anal. Sci. Spect. 49 (2004) 309–313.
[42] E. Tutem, R. Apak, F. Baykut, Analyst 116 (1991) 89–94.
[43] M.L. Lunar, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 237 (1990) 207–214.
[44] D. Sicilia, S. Rubio, D. Pérez-Bendito, Talanta 38 (1991) 1147–1153.
[45] D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem. 64 (1992) 1490–1495.
[46] E. Athanasiou-Malaki, M.A. Koupparis, Anal. Chim. Acta 219 (1989) 295–307.
[47] D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta 284 (1993) 149–157.