Use of Cerium(IV) Nitrate in the Spectrophotometric Determination of Levodopa and Methyldopa in the Pure Form and Pharmaceutical Preparations

Murad I. H. HELALEH, Nafisur RAHMAN and Eyad S. M. ABU-NAMEH

Analytical Research Division, Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India

An accurate and simple quantitative method for determining levodopa and methyldopa is proposed. Cerium(IV) has been successively used to react with drugs in 2 M H_2SO_4 at 80°C. The absorbance of colored products was measured at 510 and 550 nm for levodopa and methyldopa, respectively. The method has been extended to the determination of these drugs in dosage forms. The results have been statistically compared with those obtained by the reference method.

Keywords Levodopa, methyldopa, spectrophotometry, cerium(IV) nitrate, pharmaceutical products analysis

Levodopa and methyldopa, having a phenolic or catechol functional group, usually serve to treat Parkinson's disease. Several methods have been cited in the literature for their determination. A great deal of interest has been given to the spectrophotometric determination.¹⁻⁸ On the other hand, some sporadic methods have been recorded other than spectrophotometry, such as US Pharmacopeia⁹, B.P. official method¹⁰, amperometric oxidation¹¹ and high-performance liquid chromatography with electrochemical detection.¹² From our viewpoint, most of the above-mentioned methods lack the simplicity needed for a routine analysis.

In the present method, Ce(IV) was reacted with drug samples in a sulfuric acid medium at a temperature maintained at 80°C for 20–25 min. The final product was determined at 510 nm and 550 nm for levodopa and methyldopa, respectively.

This work was motivated by an attempt to develop a simple, accurate and sensitive assay procedure for these drugs through the formation of complexes with Ce(IV).

Experimental

Apparatus

A Bausch and Lomb spectronic 20D⁺ spectrophotometer was used for all absorbance measurements, with 1-cm cells, and a temperature-controlled water bath (NSW-133).

Reagents and samples

High-purity distilled water was used throughout. All of the chemicals and reagents were of analytical reagent or pharmaceutical grade.

Stock solutions of 2.5 mg/ml and 5 mg/ml for

methyldopa and levodopa in the pure form were prepared by dissolving exactly 250 mg and 500 mg in 100 ml of water each in a calibrated flask. The absolute purity of these materials was checked by the 1,10-phenanthroline method¹³ and found to be 99.90% and 99.98% for levodopa and methyldopa, respectively. Stock solutions of the drugs should be stored in a well-closed vessels, and direct contact with light should be avoided. They should also be freshly prepared.

Determination of levodopa and methyldopa in tablets

Five tablets were weighed and ground into a fine powder. Masses of powder containing 500 mg of levodopa and 250 mg of methyldopa were accurately mixed with water, and then filtered through Whatman No. 1 filter paper. After washing, the filtrate was made to 100 ml with water in a calibrated flask.

Cerium(IV) solution (Loba Chemie, India). A stock solution of $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (0.01 M) was prepared in 2 M sulfuric acid.

[Procedure A (Levodopa)]

After 5.0 ml of cerium(IV) nitrate solution was placed in a 10 ml calibrated flask, 0.4–1.1 ml of a levodopa solution was added. The mixture was placed on a water bath at a temperature maintained at 80°C and swirled for 20–25 min. Thereafter, it was cooled under tap and then diluted upto the mark with water. The colored product was determined at 510 nm against a reagent blank omitting the drug sample.

[Procedure B (methyldopa)]

To a known volume of methyldopa (0.5–1.0 ml), 3.5 ml of Ce(IV) solution was added. The procedure for levodopa was repeated, except that the colored product obtained in this case was determined at 550 nm.

Fig. 1 Absorption spectra of the reaction complex: A, levodopa(35 $\mu\text{g/ml}$)-Ce(IV); B, methyldopa(20 $\mu\text{g/ml}$)-Ce(IV); heated for 25 min at 80°C in 2 M sulfuric acid.

Results and Discussion

In this method, Ce(IV) in sulfuric acid is used to react with levodopa and methyldopa. It was observed that the reaction was dependent on the concentration of sulfuric acid, and that the reaction takes place only at the acid concentration range from 2 M to 12 M. The reaction rate is dependent on these factors. Thus, the optimum conditions were established by varying some parameters, including the acid concentration, temperature and heating time. The reaction is slow, but can be accelerated by increasing the acid concentration and heating time. It was found that 80°C is suitable for studying the complex products of levodopa and methyldopa. The absorbance also increases with higher acidity and time, and it reaches a maximum after 15 min. However, at high temperature (above 80°C) there is a risk of unwanted chemical changes when the reaction mixture becomes slightly turbid, and causes a hinderance in the determination.

Analytical data

Beer's law was found to be valid over the concentration range between 200–550 $\mu\text{g ml}^{-1}$ and 125–250 $\mu\text{g ml}^{-1}$, with a molar absorptivity of 1.08×10^3 and 1.15×10^3 $\text{l mol}^{-1} \text{cm}^{-1}$ for levodopa and methyldopa, respectively. The absorption spectra for the reaction of Ce(IV) nitrate with levodopa and methyldopa are shown in Fig. 1.

Molar ratio stoichiometry

In order to establish the molar ratio between the drugs and the reagent used, the continuons variation method (Figs. 2 and 3) has been applied, which indicates that 1 : 2 ratio of levodopa to Ce(IV) and a ratio of 1 : 3 for methyldopa and Ce(IV), with stability constants (K_f) of 0.48×10^9 and 1.2×10^{12} for levodopa and methyldopa

Fig. 2 Job plot for levodopa-Ce(IV) complex in 2 M H_2SO_4 at 510 nm: 0.325 mol fraction of levodopa shows the maximum absorption. Therefore, the ratio of drug to reagent is 1 : 2.

Fig. 3 Job plot for the methyldopa-Ce(IV) complex in 2 M H_2SO_4 at 550 nm: 0.25 mol fraction of methyldopa shows the maximum absorption. Therefore, the ratio of drug to reagent is 1 : 3.

respectively, which indicate the stability of complex formation. The stability constants were calculated by

$$K_f = \frac{C(1 - \alpha^-)}{(C\alpha^-)(bC\alpha^-)^b},$$

where, α^- = degree of dissociation, C = molar concentration, b = number of molecules of the reagent.

In order to determine the molar ratio for levodopa and methyldopa, reagent solutions were added in 25 ml and 50 ml volumetric flasks for levodopa and methyldopa, respectively, as shown below and the volume made

Table 1 Test on the precision and accuracy of the determination of levodopa in the pure form by the proposed method

Sample No.	Amount taken/ $\mu\text{g ml}^{-1}$	Amount found/ $\mu\text{g ml}^{-1}$	SD	RSD	%Error	%Recovery
1	30.0	30.00, 30.00, 30.13, 29.88, 30.00	0.09	0.3	0.00	100.0
2	40.0	39.93, 40.00, 39.93, 40.00, 39.93	0.04	0.1	-0.10	99.9
3	55.0	55.00, 54.98, 55.00, 55.00, 54.93	0.03	0.06	-0.04	99.9

Table 2 Test on the reproducibility of the proposed method for the determination of levodopa in tablets

Sample No.	Amount taken/ $\mu\text{g ml}^{-1}$	Amount found/ $\mu\text{g ml}^{-1}$	SD	RSD	%Error	%Recovery
1	25.0	24.88, 24.93, 25.75, 25.00, 25.00	0.36	1.44	+0.4	100.4
2	35.0	35.00, 34.98, 34.88, 35.00, 35.00	0.05	0.15	-0.1	99.9
3	50.0	50.00, 50.75, 50.00, 50.00, 49.75	0.38	0.76	+0.2	100.2

Table 3 Test on the reproducibility of the proposed method for the determination of methyldopa in tablets

Sample No.	Amount taken/ $\mu\text{g ml}^{-1}$	Amount found/ $\mu\text{g ml}^{-1}$	SD	RSD	%Error	%Recovery
1	12.5	12.50, 12.38, 12.50, 12.50, 12.50	0.56	0.45	-0.2	99.8
2	17.5	17.50, 17.44, 17.55, 17.50, 17.50	0.26	0.15	-0.06	99.9
3	22.5	22.44, 22.50, 22.50, 22.50, 22.50	0.27	0.12	-0.3	99.7

up to the mark with distilled water.

No.	Volume of drug soln./ml		Volume of Ce(IV) soln./ml	
	LD	MD	LD	MD
1	0.5	0.5	9.5	4.5
2	1.0	1.0	9.0	4.0
3	2.0	1.25	8.0	3.75
4	3.0	1.5	7.0	3.5
5	3.25	2.0	6.75	3.0
6	4.0	2.3	6.0	2.7
7	5.0	2.5	5.0	2.5
8	6.0	3.0	4.0	2.0
9	6.5	3.5	3.5	1.5
10	7.0	4.0	3.0	1.0
11	8.0	4.5	2.0	0.5

The molar concentration of levodopa is 0.025 M and of methyldopa is 0.012 M.

The molar concentration of Ce(IV) is 0.025 M.

Precision

The precision and accuracy of the proposed method and its reproducibility were checked using the pure form of drugs and tablets. The results are listed in Tables 1, 2 and 3. Therefore, the results promote sufficient accuracy to the proposed method.

Interference

The determinations of levodopa and methyldopa were successfully extended in the presence of common excipients, which are usually added to the dosage forms during their preparation, such as lactone, fructose, sucrose, glucose and starch. These excipients do not cause any interference in the determination, where a procentual change in the measured absorbance values of levodopa and methyldopa was used to determine the interferences.

Application of the proposed method

The method was applied to the determination of levodopa and methyldopa in a number of dosage forms.

Table 4 Results for the determination of levodopa and methyldopa in pharmaceutical preparations by the proposed method

Drug (Supplier)	Nominal composition/mg	Found/mg	Error, %
Levopa (tab.) (Wallace)	500 levodopa	502.25	+0.45
Emdopa (tab.) (IDPL)	250 methyldopa	249.8	-0.08

Mean of 8 determinations; error calculated to nominal content.

The obtained results are summarized in Table 4. The results were also compared with those obtained by the reference method¹³, and are given in Table 5.

The calculated t-values did not exceed the theoretical values at the 95% confidence level, showing the absence of any significant difference between the two methods. It is also mentioned that this new method is reproducible, simple, accurate, and suitable for routine analysis.

References

1. P. Sharma and K. Srinivasulu, *Indian J. Chem. Soc.*, **68**, 374 (1991).
2. P. B. Issopoulou, *Pharm. Acta Helv.*, **64**, 82 (1989).

Table 5 Determination of levodopa and methyldopa by the proposed method in contrast with the reference method¹³

Drug	Proposed method	1,10-phen. method	t
Levopa (Wallace)	100.45±0.87	99.96±0.77	1.5
Emdopa	99.92±2.7	100.17±0.38	1.7

Data presented as mean±SD for 8 determinations.
t, theoretical value=2.365.

3. M. E. El-Kommos, *Bull. Pharm. Sci. Assuit Univ.*, **10**, 34 (1987).
4. S. Sherry and E. J. Behrman, *Anal. Lett.*, **21**, 183 (1988).
5. R. T. Sane, G. J. Bhoun and S. V. Sawant, *Indian Drugs*, **24**, 207 (1987).
6. G. R. Rao and A. B. Avadhanulu, *Indian Drugs*, **23**, 699 (1986).
7. A. Berween, K. D. Khalaf and G. M. Dela, *Talanta*, **42**, 627 (1995).
8. K. Nikolic and M. Medenica, *Farmaco*, **45**, 1037 (1990).
9. U. S. Pharmacopoeia, XXII, **756**, 865 (1980).
10. British Pharmacopoeia, H. M. Stationary, 245 (1980).
11. P. T. Kissinger *Anal. Chem.*, **49**, 447A (1977).
12. M. A. Rihbany and F. D. Michael, *J. Chromatogr.*, **248**, 125 (1982).
13. P. B. Issopoulou, *Fresenius' J. Anal. Chem.*, **336**, 124 (1990).

(Received December 2, 1996)

(Accepted May 29, 1997)