Quim. Nova, Vol. 34, No. 3, 373-376, 2011

Artigo

SPECTROPHOTOMETRIC DETERMINATION OF CATECHOLAMINE USING VANADIUM AND ERIOCHROME CYANINE R

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Recebido em 31/1/10; aceito em 29/9/10; publicado na web em 26/1/11

A highly sensitive spectrophotometric method for the analysis of catecholamine drugs; L-dopa and methyldopa, is described. The analysis is based on the reaction of drug molecules with vanadium (V) which is reduced to vanadium (IV) and form complex with eriochrome cyanine R to give products having maximum absorbance (λ_{max}) at 565 nm. Beer's law is obeyed in the range 0.028-0.84 and 0.099-0.996 μg mL^{-1} for L-dopa and methyldopa, respectively. The statistical analysis as well as comparison with reported methods demonstrated high precision and accuracy of the proposed method. The method was successfully applied in the analysis of pharmaceutical preparations.

Keywords: L-dopa; methyldopa; vanadium.

INTRODUCTION

Catecholamine drugs are aromatic vic-diols in which either the 3- or 4- position is unsubstituted and these positions are not sterically blocked. These drugs are now widely used to treat several disorders. L-Dopa, also known as levodopa is used as a first-line treatment for Parkinson's disease, usually along with carbidopa or benserazide.[1] The drug readily crosses the blood brain barrier and is decarboxylated to dopamine in the brain. It has also found to ameliorate the conditions in acromegaly.[2] Methyldopa is an effective antihypertensive agent when given in conjunction with a diuretic. It is a prodrug, which is metabolised to an active metabolite, α-methylnorepinephrine, which acts as a α_2-adrenergic receptor agonist in the brainstem to attenuate the output of vasoconstrictor adrenergic signals to the peripheral sympathetic nervous system.[3]

A vast number of methods have been developed for the analysis of these compounds. L-Dopa is estimated by LC-MS-MS,[4] chemiluminescence,[5] HPLC-DAD,[6] voltametry[7] and HPTLC.[8] Similarly, methyldopa has been reported to be estimated by NMR[9] and HPLC-MS[10] methods. Also, several spectrophotometric methods are reported for the determination of these drugs. L-Dopa is estimated by tris(1,10-phenanthroline),[11] p-nitro aniline,[12] sulphanilamide,[13] sulfanilic acid,[14] 4-aminobenzoic acid,[15] isoniazid,[16] sodium metaperiodate,[17] Cu(II)-neocuproine,[18] chloranil,[19] potassium ferricyanide,[20] and 4-aminoantipyrine.[21] Methyldopa is estimated by tris(1,10-phenanthroline),[11] p-nitro aniline,[12] sulphanilamide,[13] sulfanilic acid,[14] 4-aminobenzoic acid,[15] isoniazid,[16] polyphenol oxidase[22] and barbituric acid.[23]

Herein, we have developed a highly sensitive method for the determination of catecholamine drugs based on the complex formation between reduced vanadium and eriochrome cyanine R. The method is simple, accurate and free from interference by excipients.

EXPERIMENTAL

Apparatus

A Jasco (Model UVIDEC-610 UV-VIS spectrophotometry with 1-cm matched quartz cuvettes was used for all absorbance measurements.

Materials

Analytical reagent grade chemicals and bidistilled water were used throughout the experiment. L-Dopa (LD) and methyldopa (MD) were all purchased from Sigma (USA) and used as working solution without further treatment. Sodium metavandate was purchased from E. Merk (Germany) and eriochrome cyanine R (ECR) was obtained from E. Merk (Germany). Stock solutions of each drug containing 100 μg mL^{-1} were prepared by dissolving 10 mg of the respective drugs in 100 mL of water. The resulting solutions were standardised by the reported method.[24] The solutions were further diluted quantitatively according to their linearity range. A 50 μg mL^{-1} solution of vanadium was prepared by dissolving 12 mg of sodium metavandate in few drops of dilute HCl and diluting the solution to 100 mL with water. A 500 μg mL^{-1} solution of eriochrome cyanine R was prepared by dissolving 50 mg in 100 mL of water. An acetic acid/sodium acetate buffer (0.02M) of pH 5.0 was prepared by dissolving suitable quantity in water.

Preparation of tablet/capsule sample solution

Twenty tablets of each drug was weighed, powdered and mixed thoroughly. A quantity equivalent to 10 mg of each drug was transferred to 100 mL volumetric flask. The drugs were dissolved in water, shaken well, and made up to the volume with water. The resultant solutions were filtered, further diluted according to their linearity range and analysed as described under the analytical procedure.

Recommended analytical procedure

Accurately measured suitable volume of LD and MD were transferred from stock solution to 10 mL volumetric flasks, which could be diluted quantitatively to obtain 0.028-0.84 and 0.099-0.993 μg mL^{-1}, respectively. To each flask containing drugs in the order mentioned above, 1.5 mL of vanadium, 1 mL of acetic acid/sodium acetate buffer (0.02M) of pH 5.0 and 3 mL of ECR were added. The

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solutions were swirled and allowed to stand for 45 min. The absorbances were measured at 565 nm against the blank containing all the reagents mentioned above except drugs.

RESULTS AND DISCUSSION

Spectral characteristic

The absorption spectra of the reaction product were measured at 565 nm against the blank solutions. The method is based on reduction vanadium (V) by the drugs to vanadium (IV), which formed complex with ECR. An attempt to form a ternary complex by adding cationic surfactant such as CTAB was not successful because the violet color so formed was not stable. The reaction was carried out at room temperature. The absorption spectra of the colored products are shown in Figure 1.

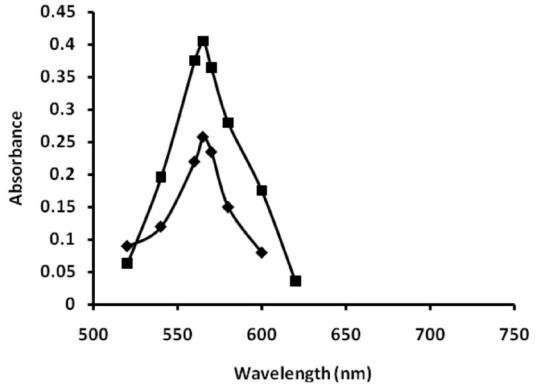

Figure 1. *Absorbance spectra of LD (0.45 µg mL^{-1}) and MD (0.35 µg mL^{-1})*

Optimum reaction condition

By varying one and keeping other experimental parameters and the amount of drug constant, the effect of vanadium (V), ECR and buffer were studied. Maximum colour intensity was obtained when 1.5 mL of vanadium, 3.0 mL of ECR were added. The effect of buffer was studied using acetic acid/sodium acetate buffer of different pH and maximum absorbance was observed at pH 5.0. The effect of pH for LD in the reaction is shown in Figure 2. The sensitivity was found to be enhanced when the reaction was allowed to be complete in 45 min at room temperature. Attempt to use other metal ions such as Al^{+3}, Ce^{+4}, Cu^{+2}, Fe^{+3} and Zn^{+2} was not successful as the blank itself produced colored product.

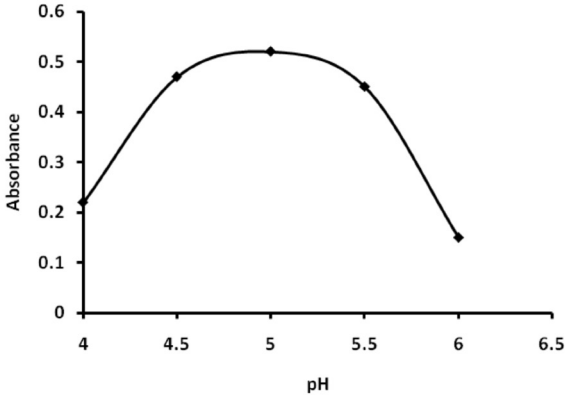

Figure 2. *Effect of pH in the absorbance of LD at 0.56 µg mL^{-1}*

Validation of the proposed method

Linearity, detection and quantification limit

Calibration graphs were constructed using standard solutions under optimum experimental condition. A linear relationship was observed between the absorbance and concentration of drugs from 0.028-0.84 and 0.099-0.993 µg mL^{-1}, respectively LD and MD, respectively. The molar absorptivity and sandell's sensitivity for each drug were calculated from beer's law. The ringbom plots demonstrated the optimum range of concentration of 0.03-0.84 and 0.11-0.97 µg mL^{-1} for LD and MD, respectively by the proposed method. The graph showed a negligible intercept, which was calculated by the least-square method's regression equation:

$$Y = a + bc$$

where, Y is the absorbance of solution in a 1 cm cell, a is the intercept, b is the slope, and c is the concentration of the measured solution in µg mL^{-1}. The confidence limits for the slope of line of regression and the intercept were computed using the relation, b ± tSb and a ± tSa at 95% confidence level. The limit of detection (LOD) and the limit of quantification (LOQ) value were determined using the formula

$$LOD \text{ or } LOQ = K\ SD/b$$

where K = 3 for LOD and 10 for LOQ, SD and b stand for standard deviation of the intercept and slope, respectively. The LOD was 6.6 x 10^{-3} and 26 x 10^{-3} µg mL^{-1}. Similarly, LOQ was found to be 0.022 and 0.087 µg mL^{-1}. The high correlation coefficients indicate the excellent linearity. Repeatability and level of precision was tested by analyzing 6 replicate samples and was found to be adequate for quantification of drugs as shown by their relative standard deviation. The optical characteristic is given in Table 1.

Table 1. Optical characteristics and statistical data of the regression analysis

Parameter	LD	MD
λ_{max} (nm)	565	565
Beer's Law range (µg mL^{-1})	0.028-0.840	0.099-0.996
Ringbom plots (µg mL^{-1})	0.03-0.84	0.11-0.97
Molar absorptivity (L mol^{-1} cm^{-1} x 10^5)	2.67	1.68
Sandell's sensitivity (µg/cm^2) x 10^{-3}	0.73	1.41
Limit of detection (µg mL^{-1}) x 10^{-3}	6.6	26
Limit of quantification (µg mL^{-1})	0.022	0.087
Regression Equation(Y= a + bc)[a]		
Slope (b) ± tS_b[b]	0.7807±0.0473	0.824±0.028
Intercept (a) ± tS_a[c]	0.0590±0.0205	0.032±0.015
Correlation coefficient (R)	0.9991	0.9993
Relative standard deviation[d]	0.44	0.78

[a]Y = a + bc, where c is the concentration of the measured solution in µg mL-1 and Y is the absorbance unit. [b]Confidence interval for slope at 95% confidence limit for 6 degree of freedom. [c]Confidence interval for intercept at 95% confidence limit 6 degree of freedom. [d]Average of 6 determinations (concentrations of 0.28 and 0.5 µg mL-1 of pure drugs of LD and MD respectively)

The error (Sc) in the determination of a given concentration of drugs was defined by the expression where,

$$Sc = \frac{Sy/x}{b}\left[1 + \frac{1}{n} + \frac{(yo - \bar{y})^2}{b^2 \sum (x - \bar{x})^2}\right]^{1/2}$$

$\bar{y}$ and $\bar{x}$ are the average values of the absorbance and concentration, respectively, for n standard samples. The graph shows that the error is reached minimum when absorbance corresponding to about 0.4 and 0.52 μg mL^{-1} of LD and MD, respectively, when the actual absorbance is equal to the average absorbance. The plots of error, Sc vs. concentration of drugs are shown in Figure 3.

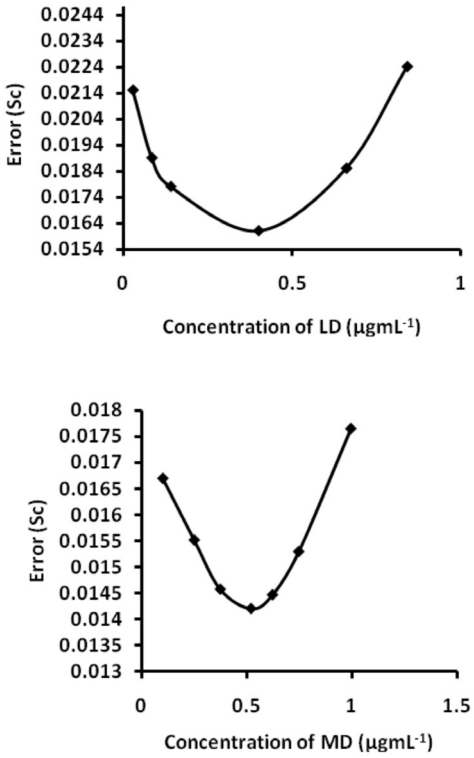

Figure 3*. Plot of error in the determination of LD and MD*

Interference studies

The effect of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drugs as mentioned in Table 2 in presence of 100 fold more concentration of each excipients. The tolerance limit was defined as the concentration which gave an error of ± 3.0% in the determination of drugs. The common excipients such as sodium chloride, starch, dextrose, lactose, talc, carboxymethyl cellulose, magnesium stearate, sucrose, had no effect in the analysis.

Table 2. Recovery of drugs from solution with a 100-fold excess of various additives* used as excipients

	Recovery (%)[a]	
Excipients	LD[b]	MD[c]
Dextrose	99.4 ± 0.2	99.7 ± 0.3
Lactose	99.7 ± 0.6	99.9 ± 0.3
Starch	99.4 ± 0.4	99.8 ± 0.7
Sucrose	99.4 ± 0.6	99.3 ± 0.4
Carboxymethyl cellulose	99.4 ± 0.1	99.7 ± 0.8
Talc	99.5 ± 0.3	99.5 ± 0.3
Magnesium Sterate	99.8 ± 0.2	99.5 ± 0.2
Sodium Chloride	100.0 ± 0.5	100.0 ± 0.3

[a] Mean ± R.S.D n = 3. [b] Concentration of LD used- 0.28 μg mL^{-1}. [c] Concentration of MD used- 0.5 μg mL^{-1}

Precision studies

The short term precision (intraday precision) of the drugs were evaluated by measuring 5 independent samples at 3 different concentration levels (0.14, 0.28, 0.56 μg mL^{-1} for LD and 0.50, 0.62 and 0.74 μg mL^{-1} for MD. Similarly, the assay for daily precision (interday precision) at the same concentration level was repeated for 5 consecutive days (Table 3). The relative standard deviation ranged between 0.3-0.6 and 0.5-0.9 for LD and MD, respectively. The values of relative standard deviation can be considered to be satisfactory for the routine determination of the drugs.

Table 3. Intra-day and Inter-day precision data

	Concentration taken (μg mL^{-1})	Intra-day Recovery[a]	Inter-day Recovery[b]
LD	0.14	0.14 ± 0.6	0.14 ± 0.9
	0.28	0.28 ± 0.3	0.28 ± 0.5
	0.56	0.56 ± 0.4	0.56 ± 0.7
MD	0.50	0.5 ± 0.7	0.5 ± 0.8
	0.62	0.62 ± 0.5	0.62 ± 0.6
	0.74	0.74 ± 0.5	0.74 ± 0.8

[a] Mean ± R.S.D n= 5. [b] Mean ± R. S.D n= 5, performed over a period of 5 days

The available pharmaceutical dosage forms of the investigated drugs were analysed by the proposed method. The precision of the method was checked by taking 6 replicate measurements. The results obtained by the proposed and the reference methods for the dosage forms were compared statistically.

Student's t- value ≤ 2.44 for pharmaceutical samples at the 95% confidence limit indicate insignificant difference between the found and true contents of the test. Analytical results measured for the same pharmaceuticals by the proposed method and reference method were compared using F-test. The F-values ≤ 5.05 thus obtained indicate insignificant difference in precision between both methods at 95% confidence limit. The standard deviation ranged between 0.15-0.20. The reliability and accuracy of the proposed method were further ascertained through recovery studies using the standard addition method by adding different amount of standard drugs to the pre-analyzed dosage forms such that the cumulative amount after adding the drugs did not exceed their linearity range. The mean percentage recoveries, relative to the labeled amounts ranged from 99.9±0.14 to 100.0±0.11 (Table 4).

CONCLUSION

We have proposed a new spectrophotometric method for the determination of catecholamine drugs, which is fairly sensitive, simple, and economical with reasonable precision and accuracy. The optical parameters and statistical comparison justify this method for application in routine drug estimation in pure and dosage forms. Also, the procedures do not involve any critical reaction conditions or tedious sample preparation steps. So, the recommended method is well suited for the assay and evaluation of drugs in pharmaceutical preparation and can also be considered as a general method for the quantification of listed catecholamine drugs.

ACKNOWLEDGMENTS

One of the authors A. K. Shrestha thanks the Jawaharlal Nehru Memorial Fund, India (Ref: SU/2/552/2008-09/760) for providing scholarship and University of Mysore for providing facilities for the investigation.

Table 4. Analysis of drugs in pharmaceutical formulations

Formulation	Mass per dosage form (mg)	Proposed method (mg)[a]	Reported methods (mg)[a, c]	Recovery (%)[b]	Error (%)
Bidopal[d]	500	500.0 ± 0.20 F=1.08 t=1.03	499.9 ± 0.20 (6)	100.0 ± 0.15	0.07
Alphadopa[e]	250	250.1 ± 0.15 F=1.19 t=0.76	250.0 ± 0.20 (6)	100.0 ± 0.11	0.06
Sembrina[f]	250	249.9 ± 0.13 F=1.26 t=2.12	250.1 ± 0.14 (6)	99.9 ± 0.14	0.05

[a] Mean ± S.D n = 6; the t- and F- values obtained after comparison to the reference methods have following theoretical values at 95% confidence limit; t = 2.44 F= 5.05. [b] Mean ± R.S.D n = 6 after adding 4 different amounts of pure drugs to a fixed concentration of pre-analysed pharmaceutical formulations. [c] Numbers inside the bracket indicates reference number of the reported methods. [d] LD equivalent to 500 mg/tablet (GSK, India). [e] MD equivalent to 250 mg/ tablet (Merind Ltd, India). [f] MD equivalent to 10 mg/tablet (Aventis, India)

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