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A new spectrophotometric method for the determination of methyldopa

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Abstract A new, simple and low cost spectrophotometric method for the determination of methyldopa in pharmaceutical preparations was developed. The method was based on the coupling of methyldopa with 2,6-dichloroquinone-4-chlorimide (DCQ). The absorbance maximum (λ_{max}) of the resulted colored product was at 400 nm. Different buffers were used to determine the optimal pH for the reaction. About 1% w/v acetate buffer with pH 8.0 gave the optimal pH required for the reaction. Of the different solvents tried, water and ethanol were found to be the most suitable solvents. Beer's law was obeyed in concentration range of 4–20 μg/ml methyldopa. The correlation coefficient was found to be (r = 0.9975). The limit of detection and limit of quantification were 1.1 μg/ml and 3.21 μg/ml, respectively. The reaction ratio between methyldopa and DCQ was studied and found to be 1:3. The work included the study of the possible interference of hydrochlorothiazide found in combination with methyldopa tablets. The method was validated and results obtained for the assay of two different brands of methyldopa tablets were compared with the BP method (colorimetric). The repeatability and reproducibility of the developed method were evaluated and the obtained results quoted. The derivative formed as a result of the reaction of methyldopa with DCQ was isolated and its possible mechanistic pathway was suggested.

1. Introduction

Methyldopa is a catechol derivative (catecholamine) widely used as antihypertensive agent. It is a centrally acting α-2-adrenoceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure (Gilman et al., 1996). The spectrum of activity of methyldopa lies between those of the more potent agents, such as guanethidine, and the milder antihypertensive, such as reserpine. Methyldopa is a structural analogue of dihydroxyphenyl alanine (dopa); it differs only in the presence of methyl group on the α-carbon of the side chain (Graig and Stitzel, 2003).

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Methyldopa contains a chiral centre. It can therefore occur either as S or R-isomer. The activity of methyldopa as antihypertensive is due to the S-isomer of α-methyldopa.

Several types of analytical procedures have been reported for the analysis of methyldopa in bulk form, pharmaceutical form or biological fluids. These include titrimetry (Amin, 1986), spectrophotometry (El-Rabbat and Omar, 1978; Davidson, 1984; Walash et al., 1985; Issopoulos, 1989; Ribeiro et al., 2005; Krishan et al., 1990; Zivanovic et al., 1991; Babu et al., 2001), and chromatography (Hjemdahl, 1984; Das Gupta and Dhruv, 1986; Rosa et al., 1995; Benza et al., 1996). Furthermore, cyclic voltametry (Ali and Sami, 2005), electrochemical oxidation and kinetic methods (James et al., 2006; Matthieu et al., 2006) have been cited. DCQ was utilized for the spectrophotometric determination of some sympathomimetic catechol amines (Sankar et al., 1987; Rosano et al., 1991; Aly et al., 1994; Bakry et al., 1997; Mohamed et al., 2002), the phenolic drug, propofol (Gadkariem and Abounassif, 2000), thiol drugs as penicillamine and captopril (Al-Majed, 1999; El-Enany et al., 2007) and amines containing drugs as sodium floxacillin and leflunomide (Refat and El-Didamony, 2006; Abbas et al., 2006). This paper deals with the use of DCQ (Gibbs reagent) as a chromogen for the spectrophotometric assay of methyldopa.

2. Experimental

2.1. Materials and equipment

Methyldopa reference material was obtained from Shinpoong Pharm. Co. Ltd., Seoul, Korea, labeled to contain 88.65% w/w anhyd. methyldopa. Pharmaceutical formulations containing methyldopa were obtained locally from different sources. The spectrophotometric measurement and the infra-red spectroscopy study were done using Jasco V-530 UV/vis spectrophotometers, Japan and Shimadzu FTIR model 8400, Kyoto, Japan, respectively. The chromogen reagent, 2,6-dichloroquinone-4-chlorimide (DCQ), was obtained from Merck, Darmstadt, Germany. The 0.125% w/v of the reagent was freshly prepared in absolute ethanol. Dimethylsulfoxide (DMSO) extra pure was obtained from Scharlau Chemie, S.A., Spain.

All chemicals and solvents were either analytical grade or general purpose reagents purchased from different sources.

2.2. Buffer solutions

Phosphate buffers of pH (3.0, 7.0, and 9.6) were prepared according to BP (2005) The British Pharmacopoeia, 2007; and sodium acetate buffer of pH 8.0 and strength of 1% w/v was prepared in water.

2.3. Stock solution of standard methyldopa

A mass containing about 0.08 g of anhyd. methyldopa was accurately weighed and transformed into a 100 ml volumetric flask, dissolved in water to 100 ml; then 1 ml of the resultant solution was diluted to 10 ml with water to give a final concentration of 80 μg/ml.

2.4. Stock solution of methyldopa tablets

Twenty tablets were accurately weighed and powdered. A mass containing an equivalent to 0.08 g of anhydrous methyldopa was accurately weighed and quantitatively transformed into a 100 ml volumetric flask. About 70 ml of water was added, and the mixture was shaken for about 10 min. The volume of the mixture was adjusted to 100 ml with water and filtered. Exactly 1 ml of the filtrate was diluted to 10 ml with water to give a nominal concentration of 80 μg/ml methyldopa.

3. Procedure

3.1. Calibration curve

Several dilutions were prepared from the standard methyldopa stock solution. Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml of the stock solution were transformed to 10 ml-volumetric flasks followed by addition of 2 ml, 1.5 ml, 1.0 ml, 0.5 ml and 0 ml water to the five 10 ml flasks in order. Finally 1 ml of sodium acetate buffer and 1 ml of freshly prepared DCQ were added to each flask. The solutions were allowed to stand for 1 h before their volumes were completed to 10 ml with water. A blank solution was prepared in the same way using 2.5 ml water instead of 2.5 ml standard stock solution of methyldopa.

The absorbance of each solution was measured at 400 nm against a blank. Linear regression analysis was performed and the linear equation for the best fitting line was calculated.

3.2. Assay of methyldopa tablets

To 1.5 ml of stock solution of methyldopa tablets, 1 ml of water, 1 ml of sodium acetate buffer and 1 ml of freshly prepared DCQ were added. The solution was allowed to stand for 1 h before the completion of volume to 10 ml with water. The concentration of the test solution can be computed from the calculated linear equation and accordingly the content of methyldopa per tablet can be worked out.

Alternatively the content of methyldopa per tablet can be found out from the following adopted expression (Gadkariem and Abounassif, 2000)

$$\text{Content of methyldopa/tablet} = \frac{A_{sm} \times C \times D \times W_1}{A_{std} \times W_2 \times 10^5}$$

where A_{sm} = absorbance of sample solution (i.e. test solution); A_{std} = absorbance of standard solution; C = concentration of standard solution (μg/ml); D = dilution factor for the sample; W_1 = average weight of the tablet; W_2 = weight of the powdered tablet taken.

4. Results and discussion

Methyldopa exhibits weak UV-absorption in the range 250–300 nm (λ_{max} is 280 in water and the molar absorbitivity "ε" is ~2789.4). Therefore, a suitable chromogen is needed to react with methyldopa to obtain a more light absorbing derivative that can be useful for a sensitive spectrophotometric determination of methyldopa in bulk from, dosage form and, if possible, in biological fluids.

Methyldopa has a catechol moiety (3,4-dihydroxyphenyl) and DCQ is known to react with phenolic compounds in alkaline media to give indophenols (colored species) (Pesez and Bartos, 1974). Based on this reported reaction we investigated the behavior of DCQ when allowed to react with methyldopa

under different conditions that can influence the color formation, intensity and stability. These factors include diluting solvent, the reagent concentration and volume, the reaction time, alkalinizing agent and temperature. Different diluting solvents with differrent dielectric constants (DE), namely water (DE 80), ethanol (DE 24.6), acetonitrile (DE 37.5), and DMSO (DE 46.7), and different alkalinizing agents, namely phosphate buffer of pH 3.7 and 9.6 and 1% w/v sodium acetate solution pH 8 were used. The reagent DCQ at a preliminary concentration of 0.25% w/v in ethanol at volume 1 ml was used to study the color formation with these solvents and buffers. The color was observed to develop well with all these solvents and buffers at different rates. UV–vis scanning between 300–750 nm of the developed color for each solution revealed the presence of two peaks at about 400 nm and at about 670 nm suggesting possible π–π^* and n–π^* transitions. Using water as the diluting solvent. The colored product was having an analytically useful maximum at 400 nm (possibly π–π^* transition) and a weak rather flat peak at about 670 nm (possibly n–π^* transition). A similar pattern of spectra was obtained when using ethanol as diluting solvent; however both peaks at 400 nm and 670 nm were clear and of definite shape and maxima. For acetonitrile, the peak at 670 nm was of higher intensity than the peak at 400 nm which was weak and rather flat. However the peak at 670 nm was of limited range of sensitivity (0.1–0.6 μg/ml, $r = 0.97$) and poor reproducibility. Solution diluted with DMSO also gave a high intensity peak at about 670 nm which was not consistent (wavelength shift between 635 and 670 nm) and a weak one at 400 nm. The blank solution in the case of DMSO tends to increase quickly and negative absorption values for the colored product at low concentrations were obtained. It is known that polar solvents enhance π–π^* transition and non-polar solvents enhance n–π^* transition. This is due to stabilization of the ground state through hydrogen bonding (Williams and Fleming, 1980). Thus these findings are in agreement with this statement. The influence of pH on the absorption value of the reaction product was evaluated using the above mentioned buffers. Maximum color intensity was obtained using 1% w/v sodium acetate (pH 8). Other buffers were either giving less sensitivity response or distorted undefined peak with high blank reading.

Attempts to accelerate the reaction by heating at different temperatures was not successful as inconsistent readings were obtained and even negative absorption values were obtained due to high colored blank and shifts in wavelength maxima.

To optimize the concentration and volume of the reagent 0.5 ml, 1.0 ml and 1.5 ml of the 0.25% w/v DCQ in ethanol were used for color development using water or ethanol as diluting solvents and 20 μg/ml solution of the methyldopa reference material. Absorption values of less than 3% variation were obtained for the different volumes. Therefore a volume of 1 ml of 0.125% w/v DCQ in ethanol was used for accurate reproducible volume use.

It was found that, the sequence of reagents addition, the diluting solvent and one hour waiting time were necessary for maximum color development. Either water or ethanol could be used as a diluting solvent; although ethanol gave a higher color intensity about 1.3 times that obtained with water; however, water being readily available, of less cost and hazards, is therefore analytically preferred. Fig. 1 shows a typical absorption spectra of methyldopa–DCQ derivative diluted with water overlaid with DCQ blank spectrum. The formed

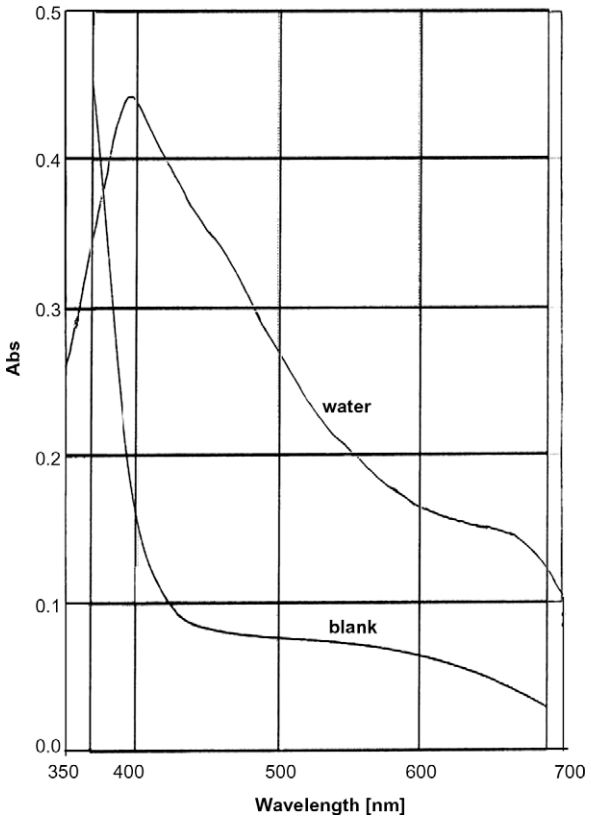


Figure 1 UV/vis spectrum of the reaction product and the DCQ blank using water as solvent (pH 8.0, conc. 16 μg/ml).

color was stable for at least two hours showing variations of less than ±2% absorption readings for a 16 μg/ml solution. Under these conditions calibration graphs were constructed using a reference sample of methyldopa. The graphs were linear with drug concentrations range 4–20 μg/ml and 3–15 μg/ml for the water and the ethanol solutions respectively.

Data for linearity of the reaction and the spectral data using water and ethanol are assembled in Table 1.

The accuracy of the procedure and freedom from interference by the tablet excipients were checked by recovery testing of added amounts of the reference drug to sample solutions in the ratio 1:1. The results showed a good recovery (101.7%) with a low RSD (±0.84%).

The repeatability of the method was investigated using 16 μg/ml – reference solution; the result obtained was 100.0 ± 1.7%. The reproducibility of the method was studied using 8 μg/ml, 12 μg/ml and 16 μg/ml reference solutions ($n = 3$); the results obtained were 102.2 ± 1.96, 101.88 ± 1.64 and 101.1 ± 1.8 for these solutions respectively. These results represent the accuracy and precision of the method.

Table 2 shows the results obtained for two brands of methyldopa tablets collected from local pharmacies using the developed method compared to the BP method.

The observed t-test and F-ratio values as compared to the corresponding tabulated values at 95% confidence level indicated that the calculated t-values and F-ratios are less than the tabulated ones; suggesting that there were no significant differences between the proposed method and the B.P method with regard to accuracy and precision (Miller and Miller, 2005).

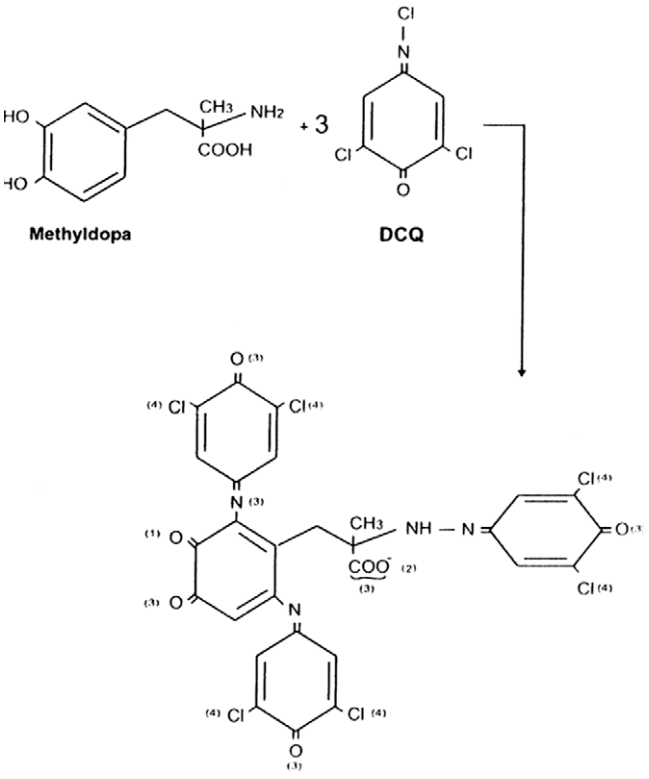
Table 1 Linear regression data for methyldopa–DCQ reaction.

Solvent	λ_{max} (nm)	Linearity range (μg/ml)	Intercept ± SD	Slope ± SD	Correlation coefficient (r)	Molar absorptivity (L mol^{-1} cm^{-1})
Water	400	4–20	−0.035 ± 0.011	0.0339 ± 0.01	0.997	6.42×10^3
Ethanol	400	3–15	−0.071 ± 0.0134	0.0469 ± 0.01	0.998	8.32×10^3

Table 2 Content (%) of methyldopa ± SD in tablets using proposed method compared to the BP method.

	The developed method	BP method	t_{cal} (tab)*	F_{cal} (tab)*
Brand (1)	101.91 ± 1.82 $n = 6$	99.90 ± 1.44 $n = 6$	2.12 (2.78)	1.60 (19.00)
Brand (2)	101.44 ± 0.96 $n = 3$	101.62 ± 0.24 $n = 3$	0.33 (2.78)	16.61 (19.00)

n = number of replicates; * = tab at 95% confidence level.



Scheme 1 Proposed reaction pathway between DCQ and methyldopa.

The limit of detection (LOD) and the limit of quantification (LOQ) for methyldopa using the proposed method were found to be 1.1 μg/ml and 3.21 μg/ml, respectively. The statistical calculation of these limits was performed using the following equation (Guidance for Industry, 2001):

$$\text{LOD} = 3.3\,\delta/S \quad \text{and} \quad \text{LOQ} = 10\,\delta/S$$

where δ = standard deviation of the intercept of the regression line; S = the slope of the calibration line.

No significant interference was observed due to the coexisting hydrochlorothiazide which is coformulated with methyldopa at the ratios 1:16 or 1:10 (hydrochlorthiazide:methyldopa). Investigation of the stoichiometry of the reaction using Job's method, molar ratio method and the slope ratio method revealed a 1:3 ratio (1 mol methyldopa:3 mol DCQ). Furthermore, the derivative was prepared according to the procedure described in the experimental part and isolated. A KBr-disc was made and the recorded IR-spectrum of the derivative, was compared with the IR-spectra of pure methyldopa, and pure DCQ.

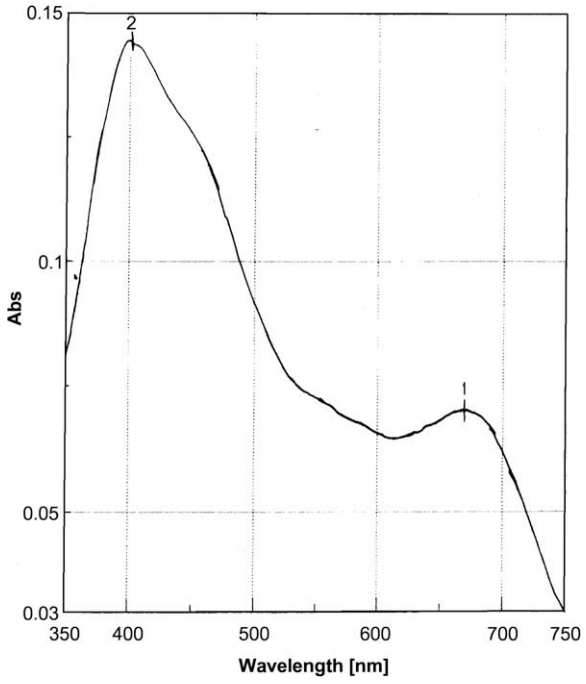


Figure 2 UV/vis spectrum of the reaction product using ethanol as solvent (pH 8.0, conc. 4 μg/ml).

A possible scheme for the reaction and structure of the formed derivative is shown in Scheme 1. Although the IR-spectrum of the derivative supports the proposed mechanism, no definite conclusion for the structure could be drawn. Further studies involving nuclear magnetic resonance, mass spectrometry and elemental analysis are therefore recommended.

The structure was proposed based on the disappearance of phenolic-OH stretch at about 3630 cm^{-1}, disappearance of

–OH stretch at about 3400 cm^{-1}, absorption at 1620–1720 cm^{-1} indicates the presence of carbonyls of ketones and carboxylates and C=N, presence of peaks at 800–600 cm^{-1} indicates the presence of C–CL stretch, and the presence of −NH stretch at about 3140 cm^{-1} and N–H bend at 1540 cm^{-1}.

Of interest was the observation that the derivative showed in ethanol two peaks at 400 nm (major) and at 670 nm (minor) Fig. 2. Beer's law was obeyed at the two wavelengths. Based on this finding, the absorption ratio A_{400}/A_{670} was found to be 1.9 ± 0.1, $n = 5$. It is therefore possible to use this value of the absorption ratio as an identification test for methyldopa.

Unlike the developed method, most of the reported methods for the assay of methyldopa require expensive or sophisticated instruments or involve procedures with rigorous control of experimental conditions. Therefore, it can be concluded that the developed method is suitable for routine analysis of methyldopa. Furthermore, the method is not susceptible to the interference by the coformulated hydrochlorothiazide.

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