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Spectrophotometric determination of methyldopa in pharmaceutical formulations

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Abstract: A new, simple, precise, rapid and low-cost spectrophotometric method for methyldopa determination in pharmaceutical preparations is described. This method is based on the complexation reaction of methyldopa with molybdate. Absorbance of the resulting yellow coloured product is measured at 410 nm. Beer's Law is obeyed in a concentration range of 50 – 200 μg ml^{-1} methyldopa with an excellent correlation coefficient (r = 0.9999). No interference was observed from common excipients in formulations. The results show a simple, accurate, fast and readily applied method to the determination of methyldopa in pharmaceutical products. The analytical results obtained for these products by the proposed method are in agreement with those of the Brazilian Pharmacopoeia procedure at 95% confidence level.

Keywords: methyldopa; spectrophotometric determination; pharmaceuticals formulations; ammonium molybdate.

Introduction

Methyldopa (MTD), chemically known as α-methyl-3,4-dihydroxyphenylalanine (Figure 1), is a catechol derivative (catecholamine) widely used as an antihypertensive agent. The MTD is a centrally acting $alpha_2$-adrenoreceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure [1].

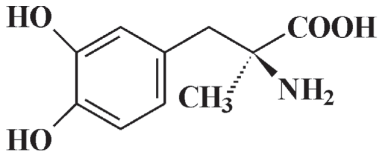

Figure 1. Chemical structure of methyldopa.

Several types of analytical procedures have been employed for the analysis of catechol derivatives in pharmaceuticals formulations and/or biological specimens. These procedures include titrimetric determination [2-7], fluorimetry [8], kinetic methods [9], amperometry [10], gas chromatography [11, 12], high-performance liquid chromatography (HPLC) [13, 14], chemiluminescence [15, 16] and voltammetric determination [17]. Some of theses methods are not simple for routine analysis and require expensive or sophisticated instruments or involve procedures with rigorous control of the experimental conditions. Most of the titrimetric methods reported [3 – 7] were indirect titrations and based in reduction reactions, which present interferences of unsaturated organic compounds. The official method reported in USP [2] describes a nonaqueous titration for the assay of MTD.

Many spectrophotometric methods have been proposed for the determination of catecholamines, such as MTD [4-7, 18-32]. A differential UV spectrophotometric procedure has been used for the determination of MTD in pharmaceutical formulations in the presence of germanium dioxide at 292 nm [18]. MTD has been determined in the visible region after reaction with potassium bromate [5], vanillin [19], 2,3,5-

triphenyltetrazolium chloride [20], ferric chloride [21], semicarbazide hydrochloride in the presence of potassium perssulfate [22], Fe(III)-*o*-phenanthroline [23], barbituric acid [24], metaperiodate [25], isoniazid in presence of *N*-bromosuccinamide [26], polyphenol oxidase enzyme [27], neotetrazolium chloride [28], *p*-dimethylaminocinnamaldehyde [29], diazotized sulphanilamide in the presence of molybdate [30] and molibdofosforic acid in sulphuric acid medium [31]. However, most of these methods suffer from several disadvantages such as the need of the long waiting times [5 – 7, 18 – 24] or heating step [23 – 25] for the reaction development, instability of the coloured species [26], complex procedure [27], require nonaqueous media [28, 29], poor detection limit [5] or has not been applied to pharmaceutical formulations [31].

Molybdate can react with catechol to form colored complexes [32, 33]. The cathecolate functionalities on the MTD (Fig. 1) suggest that it is capable of binding at available coordination sites on Mo(VI) center to produce species analogous to the well-known bis(catecholate)complex [33].

The present communication reports a new spectrophotometric method for the determination of methyldopa based on its reaction with molybdate producing a highly stable colored complex. The proposed method is free from the disadvantages of interference of the excipients normally found along with MTD in tablet dosage formulations and does not involve any extraction or heating steps. It was used to determine methyldopa in pharmaceutical formulations. The results obtained by applying the proposed method agreed fairly well with those obtained by the Brazilian Pharmacopoeia standard procedure [34] at 95% confidence level.

Experimental

Apparatus

A Hewlett Packard Model HP8453 spectrophotometer with 1 cm matched silica cells was used for all absorbance measurements. Volume measurements were made with plunger-operated pipetter (100 – 1000 μL) and Metrohm model 665 automatic burettes. All experiments were performed in a thermostatically controlled room (25±1) °C. A Micronal Model B375 digital pH-meter, calibrated with standard buffer solutions, was used for pH measurements.

Reagents and solutions

For the preparation of the solutions and samples, deionised water and grade A glassware were used throughout. Analytical reagent or pharmaceutical grade chemicals were used.

Stock 1000 μg ml^{-1} MTD (Sigma, St. Louis, MO, USA, 99.95%) solution was prepared daily by dissolving 50.0 mg of the drug in 50.0 ml of deionised water. Using a mechanical shaker, the powder is completely disintegrated after shaking 15 minutes. Working standard solutions were obtained by appropriate dilution of this stock solution with the same solvent and were standardized by the reported method [34].

Ammonium molybdate [$(NH_4)_6.Mo_7.O_{24}.4H_2O$)] was purchased from Merck (Darmstadt, Germany, p. a.). The ammonium molybdate aqueous solution 1.0% (m v^{-1}) was prepared daily.

Sucrose, glucose, talc, fructose, lactose, poly(ethylene glycol), microcrystalline cellulose, croscarmellose sodium, starch, polyvinylpyrrolidone and magnesium stearate were purchased from Sigma (St. Louis, MO, p. a.). The commercial dosages of MTD (250 and 500 mg) were purchased from local drugstores.

General procedure

Procedure for the analytical curve

1.000 ml of MTD working standard solutions were transferred into each series of 5.0 ml standard flasks, comprising 50 – 200 μg ml^{-1} of the drug. 1.000 ml 1.0% ammonium molybdate was added to each graduated flask and the volume completed with deionised water. The absorbance was measured at 410 nm against the corresponding reagent blank. Calibration graphs were prepared by plotting absorbance against drug concentration. These graphs or the corresponding linear least squares equations are used to convert absorbance into MTD concentration, for any analyzed sample.

Procedure for the assay of MTD in pharmaceutical samples

The average tablet weight was calculated from the contents of 20 tablets that been finely

powdered and weighed. A portion of this powder, equivalent to ca. 100.0 mg of MTD was accurately weighed and dissolved in 60 ml of water by shaking for 15 min in a mechanical shaker. The resulting mixture was transferred into 100.0 ml graduated flasks, the volume completed with deionised water. This solution was clarified by passing it through a cotton column filter [35], rejecting the first 20 ml. Aliquots containing equivalent to 750 μg ml^{-1} were transferred into 5.0 ml graduated flasks and were analyzed according to the recommended procedure for the calibration curve. The quantity per tablet was calculated from the standard calibration graph.

Results and Discussion

The proposed method involves the reaction of MTD with molybdate ions to produce a coloured water soluble complex. The absorption spectrum of the reaction product shows that the best analytical wavelength is located at 410 nm.

Investigations were carried out to establish the optimum conditions for complex formation. Thus, the influence of the molybdate concentration and of the pH on the reaction was studied in order to achieve maximum absorbance, repeatability, stability and obedience to Beer's Law.

The effect of molybdate concentration on complex formation was studied. The solutions of this reagent were evaluated in the following concentrations: 2.5 x 10^{-2}, 5.0 x 10^{-2}, 1.0 x 10^{-1}, 2.5 x 10^{-1}, 5.0 x 10^{-1}, 1.0, 2.0 and 4.0% (m v^{-1}). The 1.0% ammonium molybdate solution was found to be sufficient for providing maximum and reproducible colour intensity.

The effect of pH on the formation and on the stability of the complex was studied over the range 1.0 – 9.0. The absorbance of the product formed was found to remain unchanged at pH 3.5 – 7.5. All the absorbance measurements were obtained in solutions contained standards or samples with pH in the range 4.5 – 6.5.

The stability of the product formed in the optimum conditions above mentioned was investigated. The data given in Table 1 shows that full colour development is immediate at room temperature (25±1 °C) and the values of absorbances of the product formed were found to remain unchanged after standing for 24 hours at room temperature. This product was stable in the temperature range of 20 – 60 °C. However, a temperature of 25 °C was choice for the absorbance measurements.

Table 1. Optical stability of the reaction product at room temperature (25±1 °C) [a]

Time (min)	Absorbance [b]
0	0.70989
5	0.70929
10	0.70910
15	0.70909
20	0.70907
25	0.71122
30	0.71017
35 [c]	0.70897

[a] MTD concentration: 150 μg ml^{-1}.
[b] Measurements taken at 410 nm against the reagent blank for reactants at room temperature (25±1 °C), as described in the recommended procedure.
[c] The absorbance remains unchanged after standing for 24 hours at 25 °C.

Analytical curves

Beer's law is obeyed in the concentration range of 50 – 200 μg ml^{-1} of drug, in the final solution with an excellent correlation coefficient (r = 0.9999; slope = $1.134 \times 10^3 \pm 0.002 \times 10^3$ l mol^{-1} cm^{-1} (n = 8) and intercept zero). The limit of detection ($3.SD^{blank}$/slope of curve) and limit of quantification ($10.SD^{blank}$/slope of curve) were 1.9 mg l^{-1} and 6.3 mg l^{-1} MTD, respectively.

Effected of interferents, recovery and repeatability studies

To assess the usefulness of the proposed method, the effect of the common excipients which often accompany MTD in tablet dosage formulations, such as sucrose, glucose, talc, fructose, lactose, poly(ethylene glycol), microcrystalline cellulose, croscarmellose sodium, starch, polyvinylpyrrolidone and magnesium stearate were evaluated using the developed method. The ratios of the concentrations of MTD to those excipient substances were fixed at 1.0 and 10.0. No interferences were observed in the presence of the substances tested.

For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed method. The results presented in Table 2 show that the average recoveries obtained ranged from 100.1 to 101.8% of MTD from four pharmaceutical formulations samples (n = 4); the relative standard deviation (R.S.D.) were within 0.5 – 1.2%. The recovery studies were carried out after adding known quantities (25.0, 50.0, 75.0 and 100.0 μg ml^{-1}) of the standard substance (pure drug) to the each sample. These results point out the very good accuracy and precision of the method and the absence of significant matrix effects on spectrophotometric measurements.

Table 2. Recovery data for MTD spiked pharmaceuticals

Pharmaceutical Formulations	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)
A	0.0	50.5	----
	25.0	76.9	102.5
	50.0	101.9	101.9
	75.0	126.7	101.4
	100.0	151.9	101.3
			$\mu^a = 101.8 \pm 0.6$
B	0.0	51.0	----
	25.0	75.8	101.0
	50.0	99.5	99.5
	75.0	125.8	100.6
	100.0	150.9	100.5
			$\mu^a = 100.4 \pm 0.6$
C	0.0	50.9	----
	25.0	76.0	101.3
	50.0	100.2	100.2
	75.0	123.1	98.5
	100.0	150.8	100.5
			$\mu^a = 100.1 \pm 1.2$
D	0.0	50.6	----
	25.0	76.1	101.5
	50.0	100.9	100.9
	75.0	127.2	101.8
	100.0	151.0	100.7
			$\mu^a = 101.2 \pm 0.5$

μ^a Average ± relative standard deviation (RSD) of four determinations.

In the repeatability study, the R.S.D. was 1.6% for solution (sample C) containing equivalent to 150 μg ml^{-1} of MTD (n = 10). These results reveal good precision of the proposed method.

Analytical applications

The samples were prepared using the developed method. The proposed method was successfully applied for determination of MTD in four tablet formulations. The results, presented in Table 3, compare favorably with the official method of the Brazilian Pharmacopoeia [34], which testifies to the applicability of the proposed method to the determination of MTD in pharmaceutical dosage. The results were subjected to a paired comparison test [36], the data of t and F ratios show no significant differences between the results of the proposed and the official methods at 95% confidence level. Moreover, the spectrophotometric method for determination of MTD in pharmaceutical formulations reported in this paper is simple, fast, inexpensive, precise, accurate and it may be suitable for routine analysis.

Table 3. Determination of MTD in commercial pharmaceutical preparations

Sample	Label value [a]	Proposed method				Official method [34]	
		Found [b]	RSD (%) [c]	t-value (2.45) [d]	F-value (9.28) [d]	Found [b]	RSD (%) [c]
A	250.0	252.7 ± 2.3	0.9	2.01	6.53	256.3 ± 0.9	0.4
B	250.0	258.0 ± 1.3	0.5	0.03	1.51	258.3 ± 1.6	0.6
C	500.0	509.4 ± 2.4	0.5	0.65	1.78	510.3 ± 1.8	0.4
D	500.0	506.6 ± 4.1	0.6	1.34	4.82	513.9 ± 9.0	1.7

[a] Label to content for tablets: mg unit $^{-1}$.
[b] Average value ± standard deviation (SD) of four determinations.
[c] Relative standard deviation (RSD) of four determinations.
[d] The figures between parentheses are the theoretical values of t and F at P = 0.05.

Conclusions

The proposed method results in a simple, fast, inexpensive, precise and accurate analytical technique to determine MTD in commercial pharmaceutical preparations with satisfactory recoveries. Additionally, it fulfils all the main demands of routine analysis as it is robust, has low instrumentation and operational cost in comparison to chromatographic methods and does not involve any pre-treatment of the sample.

When applied to the assay of various tablet dosage forms, its advantage is that it does not require the removal of usual excipients since they were found not to interfere with the determination of MTD.

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Resumo: Um novo método espectrofotométrico, simples, preciso, rápido e de baixo custo para determinação de metildopa em formulações farmacêuticas é descrito neste trabalho. Este método é baseado na reação de complexação do metildopa com molibdato. A absorbância do produto colorido resultante foi medida a 410 nm. A Lei de Beer foi obedecida em um intervalo de concentração de 50 – 200 mg L^{-1} de metildopa com um excelente coeficiente de correlação (r = 0,9999). Os excipientes comumente usados como aditivos em formulações farmacêuticas contendo metildopa não interferem no método proposto. Os resultados mostram um método simples, exato, rápido e que pode ser aplicado para a determinação rotineira de metildopa em produtos farmacêuticos. Os resultados obtidos a partir da análise destes produtos pelo método proposto concordaram muito bem com aqueles obtidos a partir do método oficial descrito na Farmacopéia Brasileira a um nível de confiança de 95%.

Palavras-chave: metildopa; espectrofotometria; medicamentos; molibdato de amônio.

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