

Development and validation of a simple spectrophotometric method for the determination of methyl dopa in both bulk and marketed dosage formulations

Paulo Roberto da Silva Ribeiro*, Reginária Morais Duarte

Centre for Social Science, Health and Technology, Federal University of Maranhão, Imperatriz, MA, Brazil

A simple, precise, sensitive, rapid, specific and economical spectrophotometric method was developed to determine methyl dopa (MTD) content in bulk and pharmaceutical dosage formulations. The proposed method was based on the formation of a colored product from the nitrosation reaction of MTD with sodium nitrite in an acid medium. The resultant nitroso derivative species reacts further with sodium hydroxide and is converted into a more stable compound. This yellow nitrosation product exhibited an absorption maximum at 430 nm. Beer's Law was obeyed in a concentration range of 6.37 to 82.81 $\mu\text{g mL}^{-1}$ MTD with an excellent coefficient of determination ($R^2 = 0.9998$). No interference was observed from common excipients in formulations. The results showed the method to be simple, accurate and readily applied for the determination of MTD in pure form and in pharmaceutical preparations. The analytical results obtained for these products using the proposed method are in agreement with those of the Brazilian Pharmacopoeia procedure at a 95% confidence level.

Uniterms: Methyl dopa/spectrophotometric determination. Pharmaceutical formulations/quality control. Spectrophotometry/qualitative analysis.

Desenvolveu-se método espectrofotométrico simples, preciso, sensível, rápido, específico e econômico para a determinação do teor de metildopa (MTD) em matéria-prima e em formulações farmacêuticas. O método proposto baseia-se na formação de um produto colorido resultante da reação de nitrosação da MTD com nitrito de sódio em meio ácido. A espécie resultante (nitroso derivado) reage com hidróxido de sódio e é convertida a um composto mais estável de cor amarela. Este produto exibiu máximo de absorção a 430 nm. A lei de Beer foi obedecida na faixa de concentração de 6,37 a 82,81 $\mu\text{g mL}^{-1}$ de MTD com excelente coeficiente de determinação ($R^2 = 0,9998$). Não se observou interferência de excipientes comumente encontrados em formulações farmacêuticas comerciais. Os resultados demonstraram que o método proposto apresenta simplicidade, excelentes precisão e exatidão e pode ser aplicado para a determinação de MTD na sua forma pura e em preparações farmacêuticas. Os resultados analíticos obtidos pelo método proposto estão de acordo com aqueles obtidos pelo método oficial descrito na Farmacopéia Brasileira, a um nível de confiança de 95%.

Unitermos: Metildopa/determinação espectrofotométrica. Formulações farmacêuticas/controle de qualidade. Espectrofotometria/análise qualitativa.

INTRODUCTION

Methyl dopa (MTD), chemically known as α -methyl-3,4-dihydroxyphenylalanine (Figure 1), is a catechol derivative (catecholamine) widely used as an antihypertensive agent. MTD is a centrally acting α_2 -

adrenoreceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure (Hoffman, 2005).

Several types of analytical procedures have been

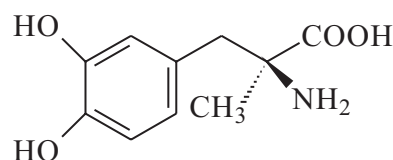


FIGURE 1 - Chemical structure of methyl dopa.

*Correspondence: P. R. S. Ribeiro. Centre for Social Science, Health and Technology, Universidade Federal de Maranhão. Rua Urbano Santos, s/n - Centro, 65900-000 - Imperatriz - MA, Brasil. E-mail: pauloufma@ufma.br

employed for the analysis of catechol derivatives in pharmaceutical formulations and/or biological specimens. These procedures include: titrimetric determination (Walash, Abououf, Salem, 1982; Mohamed, Salem, 1984; Amin, 1986; Salem, 1987; Salem, 1993a; Farmacopeia Brasileira, 2010; USP, 2013), gas chromatography (Lee, Hong-You, Fowlie, 1989; Sharma *et al.*, 1996), kinetic methods (Martinez-Lozano *et al.*, 1991), fluorimetry (Salem, 1993b), chemiluminescence (Nozaki *et al.*, 1996; Nozaki *et al.*, 1999), amperometry (Garrido, Lima, Delerue-Mattos, 1997), high-performance liquid chromatography (Tsuchiya *et al.*, 1997; Parsons, Kerr, Weiss, 1998), voltammetric determination (Kozminski *et al.*, 1998) and diffuse reflectance spectroscopy (Ribeiro, Pezza, Pezza, 2006). Some of these methods are complex for routine analysis and either require expensive or sophisticated instruments or involve procedures entailing rigorous control of the experimental conditions. Most of the titrimetric methods reported were indirect titrations and based on reduction reactions, which suffer interferences from unsaturated organic compounds. The official method reported in the Brazilian Pharmacopoeia (Farmacopeia brasileira, 2010) and in USP (2013) describes a nonaqueous titration for the assay of MTD.

Spectrophotometric methods have been proposed for the determination of catecholamines, such as MTD. A differential UV spectrophotometric procedure has also been used for the determination of MTD in pharmaceutical formulations in the presence of germanium dioxide at 292 nm (Davidson, 1984). MTD has been determined in the visible region after reaction with 2,3,5-triphenyltetrazolium chloride (El-Rabbat, Omar, 1978), potassium bromate (Mohamed, Salem, 1984), vanillin (Salem, 1985), *p*-dimethylaminocinnamaldehyde (Walash, Abououf, Salem, 1985), molybdophosphoric acid in sulphuric acid medium (Issopoulos, 1989), Fe(III), *o*-phenanthroline (Issopoulos, 1990), ferric chloride (Zivanovic, Vasiljevic, Radulovic, 1991), neotetrazolium chloride (Issopoulos, Economou, 1993), metaperiodate (Nevado, Gallego, Laguna, 1995), barbituric acid (Aman *et al.*, 1998), isoniazid in the presence of *N*-bromosuccinimide (Nagaraja *et al.*, 1998), polyphenol oxidase enzyme (Vieira, Fatibello Filho, 1998), diazotized sulfanilamide in the presence of molybdate (Nagaraja, Vasantha, Sunitha, 2001a), semicarbazide hydrochloride in the presence of potassium persulfate (Nagaraja *et al.*, 2001b), ammonium molybdate (Ribeiro *et al.*, 2005a; Ribeiro, Pezza, Pezza, 2005b), 2,2-diphenyl-picrylhydrazyl (Matos, Silva, Ribeiro, 2012) and ferric chloride/nitroso-R-salt (Al Abachi, Hadi, 2013). However, most of these methods suffer from several

disadvantages, such as long-waiting times or a heating step for reaction development, instability of the colored species, complex procedure, requirement for nonaqueous media, poor detection limit, or lack of previous application to pharmaceutical formulations.

The present study reports a simple, precise, sensitive, rapid, specific and economical spectrophotometric method developed to determine the (MTD) content in bulk and pharmaceutical dosage formulations. The proposed method was based on the formation of a nitrous derivative of MTD. The yellow-colored product had maximum absorption at 430 nm. The proposed method has none of the disadvantages of interference from the excipients normally found with MTD in tablet dosage formulations and involves no extraction or heating steps. The method was used to determine MTD in pharmaceutical formulations. The results obtained by applying the proposed method showed relatively good agreement with those obtained using the standard procedure reported in the Brazilian Pharmacopoeia (Farmacopeia Brasileira, 2010) at a 95% confidence level.

MATERIAL AND METHODS

Apparatus

A Femto Model CIRRUS 80ST spectrophotometer with 1 cm matched silica cells was used for all absorbance measurements. Volume measurements were made with a plunger-operated pipetter (100-1000 μ L) and Metrohm model 665 automatic burettes. All experiments were performed in a thermostatically-controlled room (25 ± 1) °C. A Hanna Model Pack pH 21 digital pH-meter, calibrated with standard buffer solutions, was used for pH measurements.

Chemicals and reagents

All of the reagents used were of analytical reagent grade. Deionized water was used throughout the experiments.

A stock solution (MTD_s – 318.4 μ g ml⁻¹) of MTD standard (Pharma Nostra – São Paulo, Brazil, purity grade > 99.2%) was prepared daily by dissolving 15.9 mg of the reference substance in water and diluting to the mark in a 50 ml volumetric flask. Working standard solutions were obtained by appropriate dilution of this stock solution with the same solvent and were standardized using the standard procedure reported in the Brazilian Pharmacopoeia (Farmacopeia Brasileira, 2010).

Aqueous solutions of sodium nitrite [0.6 mol L⁻¹]

(Merck), sodium hydroxide [$5.0 \times 10^{-2} \text{ mol L}^{-1}$] (Merck), and hydrochloric acid [$1.6 \times 10^{-2} \text{ mol L}^{-1}$] (Merck) were prepared and used.

Pharmaceutical formulations (tablets) of four commercial brands were analyzed. These tablets were purchased from local drugstores and all were tested prior to their respective listed expiry dates. All pharmaceuticals studied were package labeled to contain 250 or 500 mg of MTD per tablet.

The excipients used in the interference study were of pharmaceutical grade. Thus, sucrose, glucose, talc, fructose, lactose, polyethylene glycol, microcrystalline cellulose, croscarmellose sodium, starch, polyvinylpyrrolidone and magnesium stearate were purchased from Sigma (St. Louis, MO, p. a.).

Methodology

Determination of wavelength of maximum absorption

A reference substance stock solution (MTD_s) ($50.94 \mu\text{g mL}^{-1}$ of MTD) was transferred into a 5 mL calibrated flask. In this flask, 1.0 mL of hydrochloric acid ($1.6 \times 10^{-2} \text{ mol L}^{-1}$) and 1.0 mL of sodium nitrite (0.6 mol L^{-1}) were added and kept aside for 5 min; then 1.0 mL of sodium hydroxide ($5.0 \times 10^{-2} \text{ mol L}^{-1}$) was added to the solution. After five minutes, the volume was then made up with deionized water. A spectroscopic scan (400–800 nm) was carried out with this solution to determine the λ_{max} for the detection of MTD, against the corresponding reagent blank.

Linearity and range

Aliquots of reference substance stock solution ranging from 6.37 to 82.81 $\mu\text{g mL}^{-1}$ MTD were transferred to a series of 5.0 mL volumetric flasks. To each flask, 1.0 mL of hydrochloric acid ($1.6 \times 10^{-2} \text{ mol L}^{-1}$) and 1.0 mL of sodium nitrite (0.6 mol L^{-1}) were added and kept aside for 5 min; then 1.0 mL of sodium hydroxide ($5.0 \times 10^{-2} \text{ mol L}^{-1}$) was added to the solution. After five minutes, the volume was then made up with deionized water. The absorbance was measured at 430 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 were used to convert absorbance into MTD concentration, for any given analyzed sample.

Stoichiometric relationship

Job's method (Foster, 1969) was applied by placing 0.50 to 2.25 mL of $318.4 \mu\text{g mL}^{-1}$ MTD solution into a series of 10.0 mL volume flasks; this was followed by

placing 2.25 to 0.50 mL of $1.5 \times 10^{-3} \text{ mol L}^{-1}$ sodium nitrite, and 1.0 mL of hydrochloric acid ($1.6 \times 10^{-2} \text{ mol L}^{-1}$) into the flasks which were kept aside for 5 min; then 1.0 mL of sodium hydroxide ($5.0 \times 10^{-2} \text{ mol L}^{-1}$) was added to the solution. After five minutes, the volume was then made up with deionized water. The absorbance was measured at 430 nm against the corresponding reagent blank. The results were plotted as shown in Figure 5, indicating the existence of 1:1 (MTD: sodium nitrite).

Stability study

The stability of the product formed under the above-mentioned optimum conditions was investigated. For this study, a reference substance stock solution ($50.94 \mu\text{g mL}^{-1}$ of MTD) was transferred into a 5 mL calibrated flask and analyzed according to the recommended procedure for the calibration curve. This solution was kept for 24 h at room temperature and subsequently analyzed to test for short-term stability.

Intra-day precision (repeatability) and inter-day precision (intermediate precision) studies

MTD tablets were finely powdered and a sample stock solution (MTD_p) of $318.4 \mu\text{g mL}^{-1}$ was prepared following the same dilution pattern of MTD_s. Three different aliquots of MTD_p were then diluted to obtain the concentrations of 31.84, 44.58 and $57.31 \mu\text{g mL}^{-1}$ and analyzed according to the recommended procedure for the calibration curve. The quantity per tablet was calculated from the standard calibration graph. This procedure was repeated on the two preceding days.

Specificity in the presence of excipients

To assess the usefulness of the proposed method, the effect of the common components (additives, adjuvants and excipients) which often accompany MTD in tablet dosage formulations (silicon dioxide, sodium lauryl sulfate, mannitol, sodium metabisulfite, polyethylene glycol, citric acid, lactose, microcrystalline cellulose, croscarmellose sodium, talc, starch and magnesium stearate) were investigated using the developed method. The ratios of the concentrations of MTD ($44.58 \mu\text{g mL}^{-1}$) to the excipient substances were fixed at 1.0 and 10.0.

Procedure for the assay of MTD in pharmaceutical samples

Four market brands of MTD tablets purchased from local drugstores were randomly selected and analyzed using the newly developed and validated method. For the determination of MTD in pharmaceutical samples, the average tablet weight was calculated from the

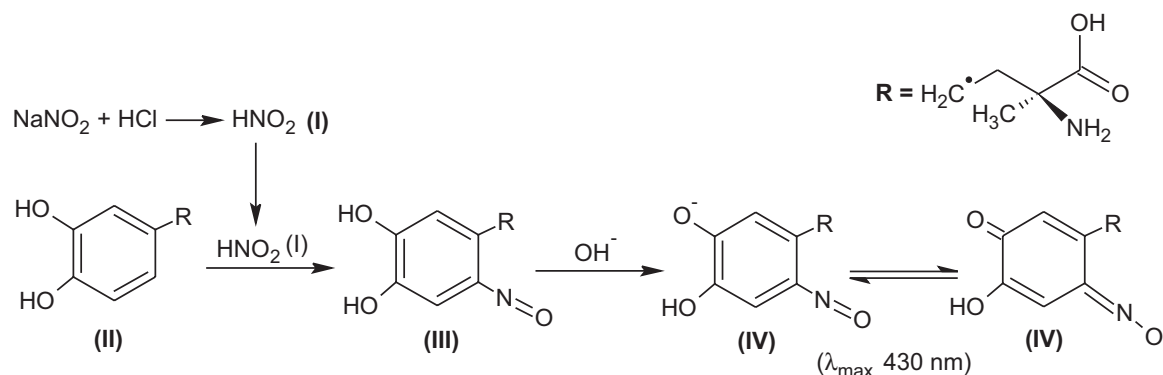


FIGURE 2 - Suggested mechanism of nitration reaction of MTD.

contents of 20 tablets that were finely powdered and weighed. A portion of this powder, equivalent to ca. 15.8 mg of MTD was accurately weighed and dissolved in 25 ml of deionized water by shaking for 15 min in a mechanical shaker. The resulting mixture was filtered and transferred into 50.0 mL graduated flasks, and the volume completed with deionized water. Aliquots from this solution, containing the equivalent of $44.58 \mu\text{g mL}^{-1}$ of MTD, were transferred into 5.0 mL graduated flasks and analyzed according to the recommended procedure for the calibration curve. The quantity per tablet was calculated from the standard calibration graph.

Accuracy/recovery studies

To study the accuracy of the proposed method and determine the interference from the excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts (17.19; 19.10; 21.01 and $22.92 \mu\text{g mL}^{-1}$, corresponding to levels of 90; 100; 110 and 120%, respectively) of the standard substance (pure drug – MTD) to a known concentration of the previously analyzed commercial tablets (samples A, B, C and D). The resulting mixtures were analyzed according to the recommended procedure for the calibration curve. The recovery of drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug.

RESULTS AND DISCUSSION

Sodium nitrite and hydrochloric acid react to produce nitrous acid (Figure 2, compound I). Owing to the presence of phenolic hydroxy groups in compounds (such as MTD – Figure 1), the reaction of this acid leads to the formation of nitroso derivatives (yellow-colored products – Figure 2, compound III) which are unstable (Suarez, Vieira, Fatibello Filho, 2005). The products

formed in acid media are unstable but can be stabilized by the interaction with an alkaline reagent to form the highly stable compounds (Figure 2, compound IV). This indicates that the alkalisation of the medium gives rise to the formation of a bathochromic shift, together with a hyperchromic effect (Belal *et al.*, 1979).

The absorption spectrum of the colored product shows maximum absorption at 430 nm (Figure 3). Thus, the probable mechanism for the reaction between MTD (catecholamine) and the sodium nitrite in an acid medium involves the formation of nitroso derivatives as shown in Figure 2. This type of formation is probably due to the contribution of an extra pair of unshared electrons in the interaction with the aromatic nucleus (Daveu *et al.*, 1997; d'Ischia, Costantini, 1995).

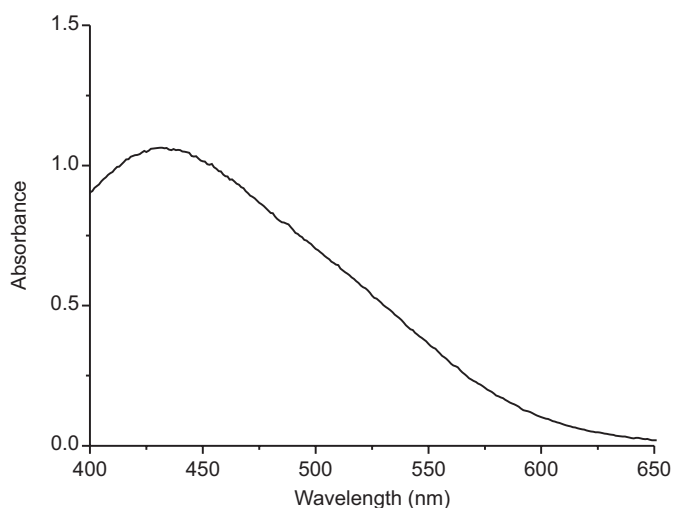


FIGURE 3 - Absorption spectrum of nitroso derivative of MTD in alkaline medium.

Method development and optimization

The effect of different volumes of the reagent used was investigated to obtain optimum results for the

determination of MTD. The optimum conditions were established based on the development of maximum color intensity and stability upon variation of parameters affecting the nitrosation reaction of MTD by the sodium nitrite in an acid medium.

Using different volumes of HCl and of NaOH, it was found that maximum color intensity and stability were obtained by developing the reactions in 1.0 mL of hydrochloric acid ($1.6 \times 10^{-2} \text{ mol L}^{-1}$) and 1.0 mL of sodium hydroxide ($5.0 \times 10^{-2} \text{ mol L}^{-1}$), as described in the recommended procedure. The adopted concentration of sodium nitrite (0.6 mol L^{-1}) was found to be sufficient for providing maximum and repeatable color intensity, but when the concentration of reagent fell below this concentration the absorbance decreased while at higher concentrations the absorbance was not increased.

The order of addition of the reactants recommended in the general procedure produced quantitative results. Any other order was found to produce deviant results and reduced color intensity.

Linearity and range

The analytical curve (Figure 4) was obtained by the method of least squares from seven points, each of which was the average of three determinations. The proposed analytical method was validated by evaluating linear dynamic range, precision, limit of detection (LOD), and limit of quantitation (LOQ). Under the experimental described, Beer's law was obeyed in the concentration range from 6.37 to 82.81 $\mu\text{g mL}^{-1}$ MTD with an excellent coefficient of determination ($R^2 = 0.9998$). The absorbance values for this concentration range were adjusted by the

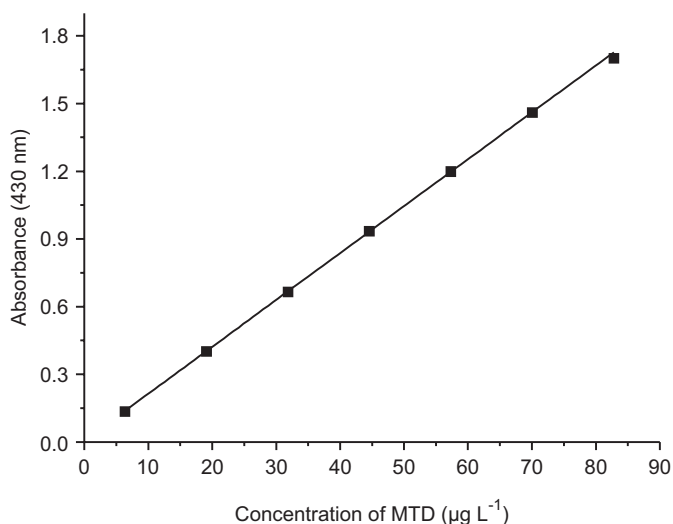


FIGURE 4 - Analytical curve for determination of MTD.

equation: $A = 5.92 \times 10^{-3} + 20.79 \times 10^{-3} \text{ } ^\circ\text{C}$, where C is the concentration of MTD in $\mu\text{g mL}^{-1}$ (molar absorptivity (ϵ): $4.423 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The LOD ($3 \cdot \text{SD}_{\text{blank}}/\text{slope}$ of curve) and LOQ ($10 \cdot \text{SD}_{\text{blank}}/\text{slope}$ of curve) were $2.52 \times 10^{-2} \mu\text{g mL}^{-1}$ and $7.68 \times 10^{-2} \mu\text{g mL}^{-1}$ of MTD, respectively (ICH, 2005).

Determination of stoichiometric relationships

Job's method of continuous variations (Foster, 1969) was employed for the determination of stoichiometric relationships: equimolar concentrations of MTD and sodium nitrite was found to be 1:1 (Figure 5).

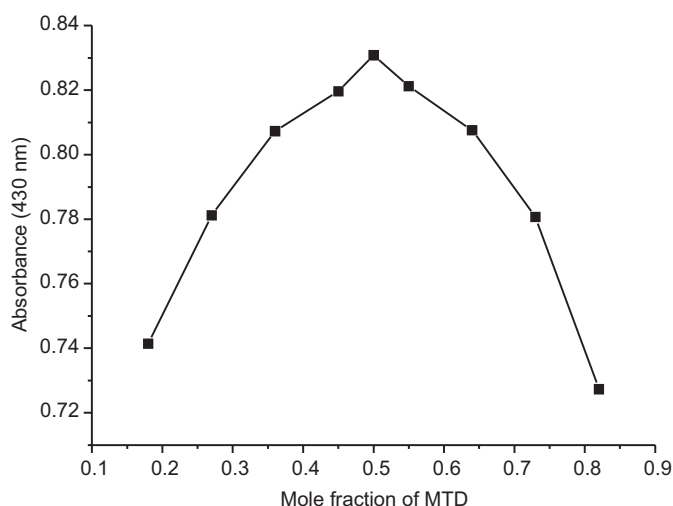


FIGURE 5 - Continuous variation plot for MTD with sodium nitrite.

Intra-day and inter-day precision

Assay precision was defined by determining intra-day and inter-day precision, expressed as relative standard deviation (RSD). The inter-day variation was evaluated over 3 days. The intra-day and inter-day precision studies (Table I) of the developed method confirmed adequate sample stability and method reliability, where all the RSDs were $< 2.0\%$.

Stability

The stability of the product formed under the above-mentioned optimum conditions was investigated. The data given in Table II show that full color development is immediate at room temperature (25°C) and the values of absorbance of the product formed were found to remain unchanged after standing for 24 hours at room temperature. This product was stable in the temperature

TABLE I - Intra-day and inter-day precision determined for three different concentrations of MTD ($n = 3$)

Concentration ($\mu\text{g mL}^{-1}$)	Intra-day precision			Inter-day precision		
	Absorbance measured (Mean \pm SD)	RSD (%)	Average potency (%)	Absorbance measured (Mean \pm SD)	RSD (%)	Average potency (%)
31.84	0.6636 \pm 0.0015	0.2260	99.77	0.6720 \pm 0.0038	0.5655	101.04
44.58	0.9239 \pm 0.0033	0.3572	98.92	0.9261 \pm 0.0054	0.5831	99.15
57.31	1.2084 \pm 0.0099	0.8293	100.81	1.2101 \pm 0.0087	0.7189	100.95

TABLE II - Optical stability of the reaction product at room temperature (25 °C)^a

Time (min)	Absorbance ^b
0	1.0630
5	1.0624
10	1.0622
15	1.0622
20	1.0635
25	1.0643
30	1.0633
35 ^c	1.0619

^aMTD concentration: 50.94 $\mu\text{g mL}^{-1}$. ^bMeasurements taken at 430 nm against the reagent blank for reactants at room temperature (25 °C), as described in the recommended procedure. ^cAbsorbance remains unchanged after standing for 24 hours at 25 °C.

range of 20–60 °C. However, a temperature of 25 °C was chosen for the absorbance measurements.

Specificity in the presence of excipients

Since the aim of this study was to determine MTD in pharmaceuticals, the effects of the most commonly used excipients were carefully examined. The excipients studied were silicon dioxide, sodium laureth sulfate, mannitol, sodium metabisulfite, polyethylene glycol, citric acid, lactose, microcrystalline cellulose, croscarmellose sodium, talc, starch and magnesium stearate. For this study, solutions containing MTD and each of the excipients taken separately at concentrations greater than or equal to 10 times that of MTD, were stirred with water in a magnetic mixer for 15 minutes, diluted, filtered when necessary, and analyzed under the same conditions described in the preparation of analytical curve.

The effect of each excipient was considered interference when the absorbance signal showed an error greater than or equal to 3.0% in the determination of the

drug. The percentage of MTD found in these solutions ranged from 99.0 to 100.5%, with coefficient of variation values of less than 2.0% for three replicates, indicating that no interferences were observed under the studied conditions.

Content of MTD in marketed brands

The samples were prepared using the developed method. The proposed method was successfully applied for the determination of MTD in four tablet formulations. The results given in Table III compared favorably with the official method (Farmacopeia Brasileira, 2010), at 95% confidence level, confirming the applicability of the proposed method for the determination of MTD in pharmaceutical dosage forms (tablets).

The results were subjected to a paired comparison test (Miller, Miller, 1993); the data of t and F ratios show no significant differences between the results of the proposed and the official methods, at a 95% confidence level. The RSD values obtained for the samples ranged from 0.7 to 3.8%, as shown in Table III. According to Horwitz (1982), the maximum RSD value acceptable for the working level of the analyte (48.58 $\mu\text{g mL}^{-1}$) is 8.0%. The AOAC (1993) set the maximum acceptable RSD value at 5.3% for the same analyte level. Therefore, the spectrophotometric method for determination of MTD in pharmaceutical formulations reported in this paper is precise, accurate, and suitable for use in routine analysis.

Accuracy/recovery and repeatability studies

In order to investigate the presence of matrix effects and to check the accuracy and precision of the developed method a recovery study was also carried out. The results of the recovery tests are given in Table IV. The mean recovery values for all samples within the 100.0–101.0% range and RSD within 0.9–1.6% confirm an accurate and precise method for application to pharmaceutical dosage

TABLE III - Determination of MTD in commercial pharmaceutical preparations

Sample	Label value ^a	Proposed method				Official method	
		Found ^b	RSD (%) ^c	<i>t</i> -value (2.78) ^d	<i>F</i> -value (19.00) ^d	Found ^b	RSD (%) ^c
A	250.0	274.2 ± 10.4	3.8	1.87	3.00	261.2 ± 6.0	2.3
B	250.0	255.6 ± 5.2	2.0	1.33	1.13	250.1 ± 4.9	2.0
C	500.0	501.0 ± 4.5	0.9	2.65	2.99	493.0 ± 2.6	0.5
D	500.0	499.7 ± 3.5	0.7	2.41	3.78	505.2 ± 1.8	1.4

^aLabel for content in tablets: mg unit⁻¹. ^bAverage value ± standard deviation (SD) of three determinations. ^cRelative standard deviation (RSD) of three determinations. ^dFigures between parentheses are the theoretical values of *t* and *F* at *P* = 0.05.

TABLE IV - Recovery/accuracy data of the proposed method for the pharmaceutical formulations spiked with four different concentrations of MTD

Sample	Added (µg mL ⁻¹)	Found (µg mL ⁻¹)	Recovery (%) ^a
A	17.19	17.51	101.9
	19.10	19.04	99.7
	21.01	21.20	100.9
	22.92	23.22	101.3
			μ ^a = 101.0 ± 0.9
B	17.19	17.10	99.5
	19.10	19.46	101.9
	21.01	21.18	100.8
	22.92	22.85	99.7
			μ ^a = 100.5 ± 1.1
C	17.19	17.53	102.0
	19.10	19.27	100.9
	21.01	21.37	101.7
	22.92	22.76	99.3
			μ ^a = 100.9 ± 1.2
D	17.19	17.07	99.3
	19.10	19.33	101.2
	21.01	20.61	98.1
	22.92	23.26	101.5
			μ ^a = 100.0 ± 1.6

^aAverage ± relative standard deviation (RSD) of three determinations.

forms. Thus, the results indicate no interference from any of the excipients present in tablets.

In the repeatability study, the RSD was 2.0; 1.1; 1.4 and 0.9% for solution (samples A, B, C and D, respectively) containing an equivalent of 44.58 µg mL⁻¹ of MTD (*n* = 10). These results reveal good precision of the proposed method.

CONCLUSION

The proposed method represents a very simple, cheap, rapid, precise, accurate, highly sensitive and environmentally-friendly (low consumption of reagents/

solvents) analytical method for determining MTD in commercial pharmaceutical preparations with satisfactory recovery. Additionally, the approach fulfils all the main requirements of routine analysis as it is robust, has a low instrumentation and operational cost in comparison to chromatographic methods, and involves no pre-treatment of the sample.

When applied to the assay of various tablet dosage forms, it offers the advantage of not requiring removal of usual excipients since these did not interfere with the determination of MTD. Therefore, the method can be useful in routine quality control analysis of MTD.

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