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Flow-injection spectrophotometric determination of methyldopa in pharmaceutical formulations

P.R.S. Ribeiro, J.A. Gomes Neto, L. Pezza, H.R. Pezza *

Instituto de Química–UNESP, P.O. Box 355, CEP 14801-970, Araraquara, SP, Brazil

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Abstract

A flow-injection spectrophotometric procedure is proposed for methyldopa determination in pharmaceutical preparations. The determination is based on formation of a yellow product (measured at 410 nm) after complexation of methyldopa with molybdate. Under optimal conditions, Beer's law is obeyed in a concentration range of 50–200 mg l^{-1} methyldopa. Typical correlation between absorbance and analyte concentration was 0.9999. Usual excipients used as additives in pharmaceuticals do not interfere with the proposed method. The analytical frequency was 210 h^{-1} and the relative standard deviation (R.S.D.) was ≤2% for sample solution containing 150 mg l^{-1} methyldopa ($n = 11$). The analytical results obtained in commercial formulations by applying the proposed FIA method were in good agreement with labeled values and those obtained by the Brazilian Pharmacopoeia procedure at 95% confidence level.

Keywords: Flow-injection analysis; Methyldopa; Molybdate; Pharmaceutical formulations

1. Introduction

Methyldopa (α-methyl-3,4-dihydroxyphenylalanine, MTD, Fig. 1) is a catechol derivative (catecholamine) widely used as antihypertensive agent. It is a centrally acting α_2-adrenoreceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure [1].

Several types of analytical procedures have been employed for the analysis of catechol derivatives in pharmaceutical formulations and or biological samples. Among techniques used in several procedures most are based on titrimetry [2–7], fluorimetry [8], kinetic measurements [9], amperometry [10], gas chromatography [11,12], high-performance liquid chromatography [13,14], chemiluminescence [15,16] and voltammetry [17]. Some of methods described above are not simple for direct application in a large-scale routine analysis and require expensive or sophisticated instruments or involve procedures with rigorous control of the experimental conditions. Most of the titrimetric methods reported were indirect titration and based on reduction reactions which present disadvantages such as interferences of unsaturated organic compounds [3–7]. The official method reported in USP [2] describes a non-aqueous titration for the assay of methyldopa.

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], diazotised sulphanilamide in the presence of molybdate [30] and ferrous tartrate at pH 8.5 [31]. The spectrophotometric method for the determination of pure MTD in the visible region, after reaction with

* Corresponding author. Fax: +55 16 3322 7932.
E-mail address: hrpezza@iq.unesp.br (H.R. Pezza).

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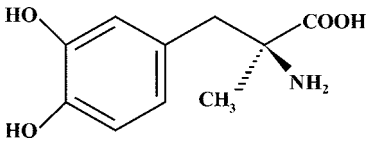

Fig. 1. Chemical structure of methyldopa.

molybdofosforic acid is cited in the literature [32]. Most of the spectrophotometric methods reported present some disadvantages such as require long waiting times [5–7,18–24] or heating step [23–25] for the reaction development, instability of the coloured product at higher temperature [26], complex procedure [27], and require non-aqueous media [28,29] or poor detection limits [5].

In recent years, more strict regulation related to the quality control of pharmaceuticals led to increasing demands on automation of the analytical assays carried out in appropriate control laboratories. The flow-injection analysis (FIA) procedure became a versatile instrumental tool that contributed substantially to the development of automation in pharmaceutical analysis due to its simplicity, low cost and relatively short analysis time. As flow-injection analysis is a tool for solution management [33] and its coupling to spectrophotometry for on-line sample preparation are already found in the literature [34–37]. So far, little attention has been given to the use of a FIA system for on-line preparation of pharmaceutical samples for direct determination of methyldopa. To the best of our knowledge, there is a single report on the use of FI spectrophotometric method for the determination of methyldopa [25]. This method is based on the oxidation reaction with metaperiodate in a moderate acid medium, which require heating step for the reaction development.

It has long been known that molybdate can react with catechol to form colored complexes [38,39]. The catecholate functionalities on the ligand in Fig. 1 suggest that it is capable of binding at available coordination sites on a *cis*-dioxo Mo(VI) center to produce species analogous to the well-known bis(catecholate)complex, $MoO_2(cat)_2^{2-}$ (H_2cat = catechol) [39].

In the present work, an FI spectrophotometric procedure for the determination of methyldopa based on the complexation of this drug with molybdate ions is described. The performance of the proposed procedure was checked after analyzing commercial pharmaceutical formulations. This procedure is simple, rapid, inexpensive, does not involve any pretreatment procedure or heating steps and has smaller sample consumption and a higher sample analysis frequency than the existing FI spectrophotometric procedure [25].

2. Experimental

2.1. Apparatus

The flow system comprised an Ismatec (Zurich, Switzerland) IPC-8 multi-channel peristaltic pump equipped with

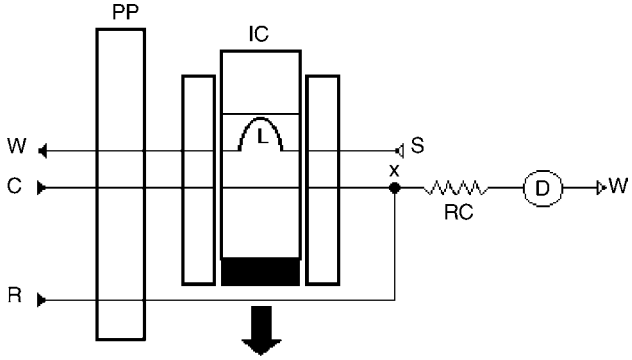

Fig. 2. Flow diagram of the FIA system used for MTD determination. IC: injector–commutator; L: sampling loop (57 μL); S: sample or analytical solutions (1.4 ml min^{-1}); R: 0.5% (m/v) $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ solution (1.7 ml min^{-1}); C: water (4.6 ml min^{-1}); W: wastes; RC: coiled reactor (150 cm × 0.7 mm i.d.); *x*: confluence point; PP: peristaltic pump. D: detector (410 nm). Black area: next position of the injector–commutator. Black arrow downwards indicates the movement of the central part of the IC.

Tygon® pumping tubes, a manual injector–commutator [40], a 111 Kipp and Zonen strip chart recorder, a Femto (São Paulo, Brazil) 482 spectrophotometer with a U-shaped flow cell (10 mm optical path; ≈100 μl lighted volume), polyethylene tubing (i.d. 0.7 mm), coiled reactors and accessories. The selected wavelength was 410 nm. The flow diagram of the system is shown in Fig. 2. In the position specified of the figure, a sample volume (S) of 57 μl is selected by the sampling loop (L). After loop-based injection, the methyldopa zone merges at the confluent point *x* with ammonium molybdate solution (R) and reacts inside reactor RC (150 cm) forming the yellow product. Passage of the colored compound through the flow cell of spectrophotometer (410 nm) results in a transient absorbance that is recorded as a peak with height proportional to the methyldopa content in the sample. After peak maximum measurement, the injector–commutator is switched back to the initial position starting another cycle.

2.2. Reagents, analytical solutions and samples preparation

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.

A 1000 mg l^{-1} stock solution of MTD (Sigma, St. Louis, MO, USA, 99.95%) was prepared daily by dissolving 50.0 mg of the drug in ca. 40 ml deionised water (using mechanical shaker the powdered sample is completely disintegrated after 15 min shaking) and completing the volume up to 50 ml with water. This solution was standardized according to the literature [31]. Working standard solutions (0.0, 50.0, 75.0, 100.0, 125.0, 150.0, 175.0 and 200.0 mg l^{-1} MTD) were obtained by appropriate dilution of the MTD stock solution in water.

Ammonium molybdate $[(NH_4)_6{\cdot}Mo_7{\cdot}O_{24}{\cdot}4H_2O)]$ was purchased form Merck (Darmstadt, Germany, p.a.). The ammonium molybdate aqueous solution 0.5% (m/v) 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.).

Four commercial samples (A–D) of MTD containing 250 or 500 mg MTD were purchased from local drugstores in Araraquara city, Brazil. Twenty tablets of each sample were weighted (to calculate the average weight of tablet), finely powdered and homogenized. A mass of 20 mg (samples A and C) or 40 mg (samples B and D) was accurately weighed and dissolved in 200 ml of water. With mechanical shaker, it takes 15 min for complete dissolution. The resulting solution was transferred to 250 ml flasks and the volume completed with water. This solution was injected into the FIA system and analyte determined using standard calibration graph.

For validation of the proposed method, the samples were also analyzed by the Brazilian Pharmacopoeia standard procedure [31]. In this method, the MTD is determined at 520 nm after reaction with ferrous tartrate at pH 8.5.

2.3. Procedure

The main parameters such as carrier and reagent flow rates (2.3–7.7 ml min^{-1}), length of the reaction coil (10–30 cm), reagent concentration 0.5–4.0% (m/v) and sample volume (38–114 μl) were investigated. The following solutions were tested for selectivity evaluation: 200 and 2000 mg l^{-1} of sucrose, glucose, talc, fructose, lactose, poly(ethylene glycol), microcrystalline cellulose, croscarmellose sodium, starch, polyvinylpyrrolidone and magnesium stearate.

After the parameters had been selected, the proposed procedure was applied to MTD determination in pharmaceutical samples. In addition, MTD recovery tests were also carried out on same type of samples.

3. Results and discussion

3.1. Flow-injection parameters

The main parameters related to the performance of the FIA system such as sample carrier and reagent flow rates, length of the reaction coil, reagent concentration and sample volume were studied. The values chosen as optimum were those that resulted in the best compromise between transient absorbance signal, repeatability and sample throughout.

Regarding the effect of reagent concentration on signal, the ammonium molybdate was evaluated within the 0.5, 1.0, 2.0 and 4.0% (m/v) concentration interval. The peak height remained constant with increasing reagent concentration up to 2.0%, above which it decreased slightly. So, the optimum sensitivity, repeatability and baseline stability was attained for 0.5% (m/v) reagent, the selected concentration for further experiments.

The influence of the sample volume on the transient peak was studied in the 38–114 μl by changing the length of sampling loop from 10 to 30 cm. The absorbance signal of a solution 150 mg l^{-1} MTD was measured at a flow rate of 1.4 ml min^{-1} and R = 0.5% (m/v) ammonium molybdate. The signals increased steeply with injected volume up to 57 μl, above which they became constant. So, as a compromise between sensitivity, sampling rate and linear correlation, 57 μl was the sample volume selected.

The flow rate of the carrier stream C was studied by varying the rotation speed of peristaltic pump. This parameter was optimized by using univariant approach. When the flow rate of the carrier stream C was increased from 2.3 to 7.7 ml min^{-1}, the slope of calibration curve increased steeply with flow rate up to 4.6 ml min^{-1}, above which it showed a slight decrease. As a compromise among sensibility, reagent consumption and analytical frequency, the flow rate of the carrier C selected for subsequent experiments was 4.6 ml min^{-1}.

With regards to the length of the reaction coil, the analytical signals did not vary significantly when it was varied from 50 to 200 cm. As a compromise among analyte dispersion, sensitivity, reagent consumption and sampling rate, the reaction coil selected for subsequent experiments was 150 cm.

3.2. Analytical curve

Under optimized experimental conditions, the analytical solutions were injected in triplicate to verify the correlation between absorbance (peak height) and analyte concentration in the 50.0–200.0 mg l^{-1}. Calibration curves (slope = $1.400 \times 10^{-3} \pm 0.004 \times 10^{-3}$ l mg^{-1} cm^{-1} ($n = 8$) and intercept = $-1.320 \times 10^{-3} \pm 0.001 \times 10^{-3}$) with good linearity ($r = 0.9999$) were consistently obtained. The limit of detection (3 S.D.^{blank}/slope of curve) and limit of

Table 1
Results of the addition-recovery experiments

Sample	Added (mg l^{-1})	Found (mg l^{-1})	Recovery (%)
	25.0	24.6	98.5
	50.0	49.9	99.9
A	75.0	74.8	99.8
	100.0	99.7	99.7
			$\mu^a = 99.5 \pm 0.7$
	25.0	24.8	99.1
	50.0	49.8	99.5
B	75.0	75.5	100.7
	100.0	99.4	99.4
			$\mu^a = 99.7 \pm 0.7$
	25.0	25.3	101.2
	50.0	49.6	99.3
C	75.0	74.5	99.3
	100.0	99.2	99.2
			$\mu^a = 99.8 \pm 1.0$
	25.0	24.7	98.8
	50.0	49.2	98.5
D	75.0	74.6	99.4
	100.0	98.7	98.7
			$\mu^a = 98.9 \pm 0.4$

μ^a = Average ± R.S.D. for the four determinations.

Table 2
MTD contents in commercial pharmaceutical samples determined by the proposed procedure and by the official method

Sample	Label value[a]	Proposed FI method				Official method [31]	
		Found [b] (mg unit^{-1})	R.S.D. (%)[c]	*t*-value (2.45)[d]	*F*-value (9.28)[d]	Found[b] (mg unit^{-1})	R.S.D. (%)[c]
A	250.0	247.2 ± 0.7	0.2	1.51	3.70	256.3 ± 0.9	0.4
B	250.0	248.1 ± 1.6	0.6	1.97	1.13	258.3 ± 1.6	0.6
C	500.0	493.9 ± 1.2	0.2	1.10	4.73	510.3 ± 1.8	0.4
D	500.0	488.0 ± 1.3	0.2	2.13	1.49	513.9 ± 9.0	1.7

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

quantification (10 S.D.^{blank}/slope of curve) were 2.1 mg l^{-1} and 7.0 mg l^{-1} MTD, respectively.

3.3. Interferences and recovery studies

The influence of excipients that can commonly accompany MTD in pharmaceutical formulations was studied. Then, the selectivity of the flow-injection procedure was investigated using solutions containing 150 mg l^{-1} MTD added to excipients that are commonly found in MTD tablets. No interference in the proposed FIA procedure was observed up to ca. 10-fold excess of sucrose, glucose, talc, fructose, lactose, poly(ethylene glycol), microcrystalline cellulose, croscarmellose sodium, starch, polyvinylpyrrolidone and magnesium stearate. It should be emphasized that this last analyte/concomitant concentration ratio studied is much higher than those normally found in the commercial pharmaceutical products. Moreover, no interferences were performed in the absorbance measurements by presence of pigment in some samples, which could be attributed to the low volume of the sample solution injected.

To study the recovery of the MTD from pharmaceuticals formulations, four commercial samples were used. The recovery of MTD was examined by adding MTD reference solution at five levels (25.0, 50.0, 75.0 and 100.0 mg l^{-1}) to the samples and the results obtained (Table 1) were compared with the added concentrations. Recoveries varied from 98.9 to 99.8%, showing the minimum matrix effects. These results were confirmed by the official procedure [31] and it is in good agreement with those obtained by the proposed method.

3.4. Analytical applications

Analyzing some of the commercially available pharmaceutical preparations led to a further evaluation of the proposed FIA spectrophotometric procedure in pharmaceutical analysis. As shown in Table 2, the results obtained by proposed method are excellent for all tablets analyzed, proving the potential of this method in pharmaceutical analysis. Statistical analysis of the results obtained by the proposed and official methods using *t*-test and *F*-test [41] showed no significant difference between the performance of these methods, for 95% confidence level, regarding accuracy and precision. Then, the results obtained by the proposed flow procedure are in good agreement with those obtained by the official method,

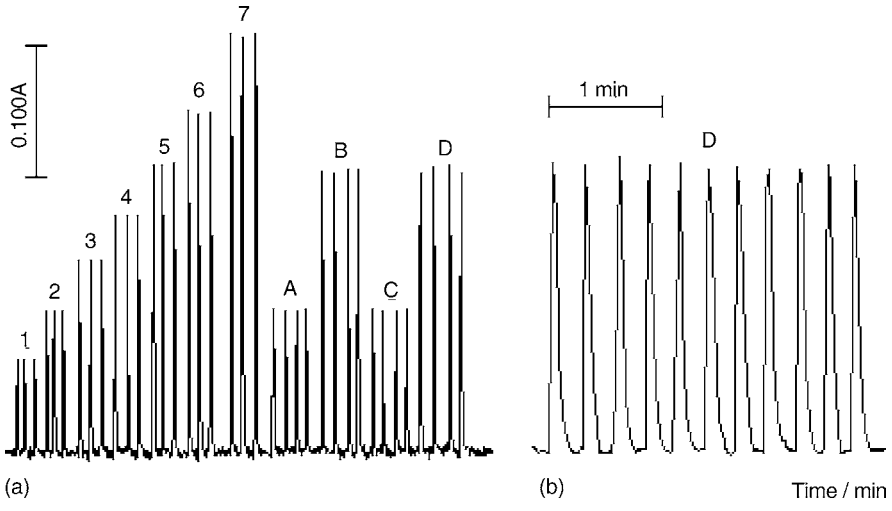

Fig. 3. Part of the recorder tracing related to MTD determination. The results related to the system in Fig. 2. From the left (a), recorded peaks refer to a seven analytical solutions (50, 75, 100, 125, 150, 175 and 200 mg l^{-1} MTD) plus four samples (A, B, C and D) processed four times and a sample (D) processed eleven times.

showing that the flow procedure can be satisfactorily applied for the determination of MTD in pharmaceutical products.

The sampling rate was equivalent to ca. 210 determinations per hour. The typical transient signals, in triplicate, corresponding to a linear analytical curve for MTD and injections, in quadruplicate, of four samples of tablets were showed in Fig. 3(a). The repeatability of the proposed method was tested by repeated runs of a real sample solution (C) containing the equivalent at 150 mg l^{-1} of MTD. The R.S.D. was lower than 2% ($n = 11$). The transient signals of this study were showed in the Fig. 3(b).

4. Conclusions

In conclusion, the proposed FI spectrophotometric procedure can be used for the analysis of methyldopa in pharmaceutical preparations. This method is simple, fast, relatively inexpensive, precise, accurate, sensitive, using minimum number of reagents and reaction sequence. Then, the speed of analysis and the precision make this method also suitable for the quality control of formulations containing methyldopa replacing tedious, expensive, and slow official and chromatographic methods. Complex pre-treatment of the samples is not necessary because the preparation of the pharmaceutical formulations and reagents is done simply by dissolving in water, in this manner, it does not require the removal of usual excipients since they were found not to interfere with the determination of methyldopa. Therefore, this system is particularly useful for the implementation of routine analysis.

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