A Simple Flow Injection Spectrophotometric Method for the Determination of α-Methyldopa in Pharmaceutical Preparations

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Abstract

An accurate, precise and simple flow injection quantitative method for the determination of α-methyldopa in pharmaceutical formulations is proposed. The method is based on the reaction between α-methyldopa and *p*-aminophenol in strong alkaline medium. The reaction product is determined spectrophotometrically at 608 nm. Five pharmaceutical preparations purchased in the local market were analyzed. The results were compared with those obtained with the spectrophotometric United States Pharmacopoeia method. The statistical Student´s *t*-test was applied to compare the results obtained with the two independent methods. In all cases complete agreement was observed, in a confidence level of 95% (α=0.05). Considering five samples analyzed using the proposed method and three determinations for each sample, the observed mean relative standard deviation, RSD, is about 1.7 %. The working analytical range, considering the final solution, is between 2×10^{-4} mol L^{-1} and 3×10^{-3} mol L^{-1}. The correlation coefficient R of the analytical quadratic curve is 0.99998. The detection limit is about 0.3×10^{-4} mol L^{-1} in the final solution introduced in the flow system (3×SD of the blank) and 48 determinations can be performed per hour.

Keywords: flow injection; α-methyldopa; pharmaceutical preparations; spectrophotometry; remedies.

1. Introduction

α–Methyldopa (L-α-methyl-3,4-dihydroxyphenylalanine) is a widely used substance to treat hypertension, inclusive when complicated with renal disease. Its hypertensive properties mainly occur due to the action on the central nervous system (CNS), probably by the stimulation of the α–adrenergic central receptors through the metabolite α-methylnorepinephrine. α-methyldopa enters CNS where it is decarboxylated and β–hydroxylated to form α-methylnorepinephrine. When liberated, α-methylnorepinephrine stimulates the α2-receptors inhibiting the sympathetic transmission to the heart, kidneys and peripheric nervous system. Independently if administrated via oral or parenteral it can provoke, as secondary effects, sedation, vertigo, psychic depression, dry mouth, nasal congestion, gastrointestinal disturbs, edemas and sexual impotency [1].

Several methods are cited in the literature for the determination of α-methyldopa in medicines and biological fluids due to the wide use of this drug in numerous cardiac and circulatory diseases. Besides, it is necessary to control the quality of the produced medicines. The analytical methods include several procedures: titrimetric [2], potentiometric [3], spectrophotometric [4-16], HPLC [17-19] and NMR [20].

As observed, spectrophotometry is a very useful technique for the determination of α-methyldopa in pharmaceutical preparations. As examples can be cited the reaction with metaperiodate in acid solution at 665 nm [15] and with metaperiodate associated to aminophenols at 450 – 520 nm [4]; the reaction with barbituric acid gives a red color with maximum at 540 nm [9]; the use of sodium bismutate that oxidizes the vicinal di-hydroxy group giving a yellow product with a maximum absorption at 420-429 nm [11]; the reaction with cerium IV nitrate, in acid medium, forming a compound with maximum absorption at 550 nm [8]; the heating of the α-methyldopa in sodium hydroxide solution that promotes its oxidation allowing the determination at 360 nm [6]; the reaction with polyphenol oxidase with detection at 470 - 480 nm [7]; the reaction with diazotised *p*-nitroaniline [13] or diazotised sulphanilamide [16] in the presence of molybdate in acid medium; the reaction with iron III in the presence of 1,10-phenanthroline or 2,2'-bipyridyne [12] or 2,4,6-tris(2-pyridyl)-1,3,5-triazine [14]; the reaction using isoniazid in the presence of N-bromosuccinimide [10]. FI methods associated with spectrophotometric detection had also been proposed [4-7].

Despite the fact that spectrophotometric methods are relatively of low cost, when compared with other methods such as chromatographic and spectrofluorimetric, some of the spectrophotometric procedures show some disadvantages as, for example, low sensibility, long time to develop color, necessity of extraction or of heating, etc. [12].

The main of this work is to develop a simple, sensitive, rapid, low cost and reliable spectrophotometric flow injection method for the determination of α-methyldopa in pharmaceutical preparations.

2. Experimental

The proposed method is based on the spectrophotometric detection of the deep blue compound formed in the reaction of *p*-aminophenol and α-methyldopa, a cathecol derivative, in basic aqueous solution, leading to the formation of an indophenol blue dye [21]

The results obtained with the proposed method when applied to commercial pharmaceutical preparations were compared with those obtained with the spectrophotometric procedure recommended by the United States Pharmacopeia [22], using the statistical Student's *t*-test method [23].

2.1 Reagents and solutions

All the chemicals used in this work were of analytical grade, except when specified. The water used for the solutions was distilled in a glass distiller and then deionized in a Milli Q Plus

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deionizer. Pharmaceutical preparations containing α-methyldopa were purchased in the local market.

Ethanol solutions, *hydrochloridric acid* solution 0.1 mol L^{-1}, *sulfuric acid* solution 0.05 mol L^{-1} and sodium hydroxide solutions were prepared in the usual way. *p-aminophenol solution 4.6 × 10⁴ mol L⁻¹:* 0.0050 g of *p*-aminophenol were dissolved with a 0.1 mol L^{-1} hydrochloric acid solution in a 100 mL volumetric flask and the volume was completed to the mark with the acid solution. *Standard α-methyldopa solutions:* a pharmaceutical grade certificated α-methyldopa (88.6 %) was gently provided by a pharmaceutical industry. 0.70444 g of the product was dried at 130 °C in an oven to constant weight. The remaining mass (0.62452 g) corresponded to 88.65 %, which agrees with the content of α–methyldopa of the pharmaceutical product and indicates that the 11.35 % eliminated at 130 °C probably corresponds to the amount of water vaporized. Aliquots of the same product were submitted to differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA) techniques. The infrared spectra obtained from 4000 cm^{-1} to 400 cm^{-1}, of the original and that of the product dried in the oven, indicated, together with DSC and TGA results, that only water is eliminated when the hydrated α-methyldopa is heated at 130 °C. Two stock solutions were prepared: (4.74×10^{-3} mol L^{-1}) 0.1130 g of the pharmaceutical product was dissolved in 40 mL of the 0.05 mol L^{-1} sulfuric acid solution and the volume was completed with the same acid solution to 100 mL in a volumetric flask; (9.48×10^{-3} mol L^{-1}) 0.2260 g was dissolved in 40 mL of the 0.05 mol L^{-1} sulfuric acid solution and the volume was completed to 100 mL in a volumetric flask with the same acid solution. From these solutions, through adequate dilutions, were obtained the concentrations: 2.37×10^{-4} mol L^{-1}, 4.74×10^{-4} mol L^{-1}, 1.18×10^{-3} mol L^{-1}, 2.37×10^{-3} mol L^{-1} and 3.55×10^{-3} mol L^{-1}.

Sample treatment: ten tablets were triturated and a quantity, corresponding to the mass of one tablet, was carefully weighed and dissolved in 40 mL of the 0.05 mol L^{-1} sulfuric acid solution, at room temperature. The volume was completed to 100 mL in a volumetric flask with the acid solution. This solution was filtered and 10.0 mL were diluted to 100 mL with water in a volumetric flask. The final concentrations were expected to be about 1.2×10^{-3} mol L^{-1} (250 mg tablets) or 2.4×10^{-3} mol L^{-1} (500 mg tablets).

2.2 Apparatus

Peristaltic pump: Ismatec mp13 GJ4. *Spectrophotometers*: Single-beam Femto model 600 for FI experiments; Hewlett-Packard 8452 A diode array for the spectra. *Chart recorder:* Cole Parmer Series 8375. *Flow cuvette*: 1.00 cm path length quartz cuvette. *Sampling valve*: This sampling valve has been described previously in detail [24,25]. *Pumping tubes*: Ismatec two-stop tubes, blue-blue, Tygon®, internal diameter 1.65 mm. *Conducting tubes*: Polyethylene tubes, internal diameter 1 mm. *Infrared spectrophotometer:* FTIR Bomem MB Series, model B100. *Differential scanning calorimeter*: Du Pont Instruments model 910. *Thermogravimetric analyzer*: TA Instruments model 2050.

2.2.1. The flow injection system

The determination of α-methyldopa was done through the detection of the colored compound formed in its reaction with *p*-aminophenol in basic medium. The flow injection system used is depicted in Fig.1. The sample is introduced in the system through the valve **V** and is transported by an ethanol/water solution (7.5 % v/v) flow $\mathbf{A_3}$ to the mixing zone **M,** where it is mixed with the 2.5 % w/v sodium hydroxide solution $\mathbf{A_1}$ and with the *p*-aminofenol 4.6×10^{-4} mol L^{-1} solution $\mathbf{A_2}$. This final mixture, where the blue color is developed, flows to the spectrophotometer **S.** The absorbance is monitored at 608 nm. The signal is registered on a chart recorder **R**.

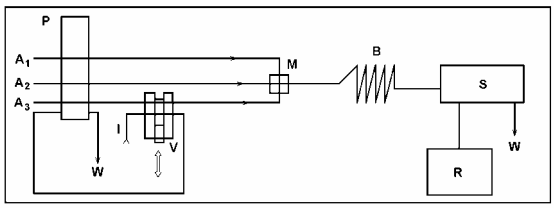

Fig. 1 Scheme of the employed flow system. $\mathbf{A_1}$ = 2.5 % w/v sodium hydroxide aqueous solution. $\mathbf{A_2}$ = 4.6×10^{-4} mol L^{-1} *p*-aminophenol aqueous solution (HCl 0.1 mol L^{-1}). $\mathbf{A_3}$ = ethanol 7.5 % v/v aqueous solution (carrier). **B** = reaction bobbin. **I**=sample introduction (volume 300 μL). **M** = mixer. **P**=peristaltic pump. **R** = potentiometric chart recorder. **S**=spectrophotometer. **V** = sampling valve. **W** = waste. Flow rates $\mathbf{A_1} = \mathbf{A_2} = \mathbf{A_3}$.

3. Results and discussion

Fig. 2 shows the visible spectrum of the product formed in the reaction of α-methyldopa with *p*-aminophenol, with maximum absorption at 608 nm.

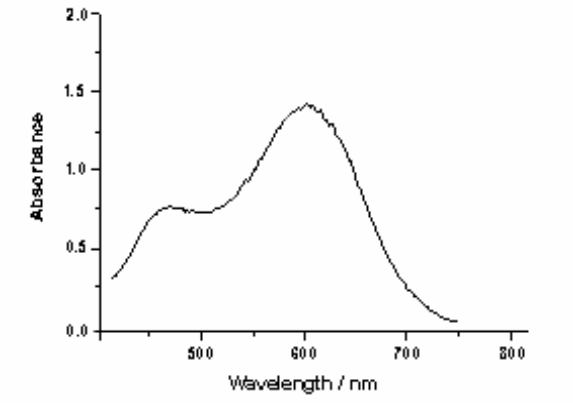

Fig. 2 Visible spectrum of the blue dye formed in the reaction of α-methyldopa with *p*-aminophenol, with maximum at 608 nm.

The best conditions were established concerning the total flow rate (1.8 mL min^{-1}) maintaining equal the three independent flows, *p*-aminophenol concentration (4.6×10^{-4} mol L^{-1}), sodium hydroxide concentration (2.5 % w/v), ethanol content of the carrier (7.5 % v/v), sampling volume (300 μL) and reaction bobbin 100 cm. Under such conditions about 48 determinations per hour can be performed.

The influence of the ethanol content in the carrier was studied from 0 to 10% w/v. At 7.5% of ethanol the maximum influence was observed. The signal increases about 7% in comparison with the signal related to the solution without ethanol. Therefore this ethanol concentration was adopted. The influence of the ethanol concentration in the sample solution was also studied in the range from 0 to 10% w/v. No significant effect was observed and then no ethanol was added to sample

solutions.

The influence of sodium hydroxide concentration was studied from 0.5 to 3.0 % w/v. Higher concentrations generated base line instability, probably related to gradients in the refraction index in the spectrophotometric cell, due to incomplete mixture, and were not considered. The observed results can be seen in Fig. 3. It can be observed that the signal increases until 2.5 % w/v in NaOH remaining constant beyond this concentration. Therefore, the 2.5 % w/v concentration was selected.

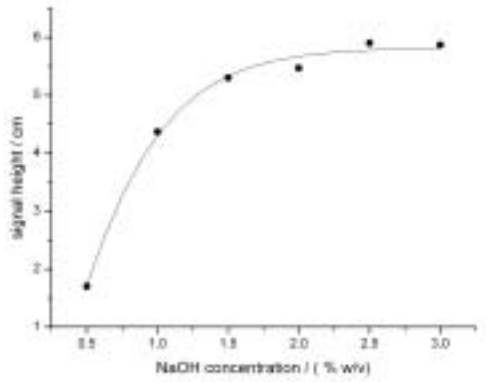

Fig. 3. Influence of NaOH concentration on the analytical signal. **h** is the signal height in cm. 1 cm is equal to 0.084 absorbance. Flux rate 1.8 mL min^{-1}. α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. *p*-aminophenol concentration 2.30×10^{-3} mol L^{-1}. Sample volume 180 μL. Reaction bobbin length 50 cm. Wavelength 608 nm.

The influence of the concentration of *p*-aminophenol was studied in the range from 9.0×10^{-5} mol L^{-1} to 4.6×10^{-4} mol L^{-1}. Fig. 4 depicts the obtained results. The signal increases until the *p*-aminophenol concentration near to 4×10^{-4} mol L^{-1} remaining constant beyond this value. The concentration 4.6×10^{-3} mol L^{-1} was adopted for the method.

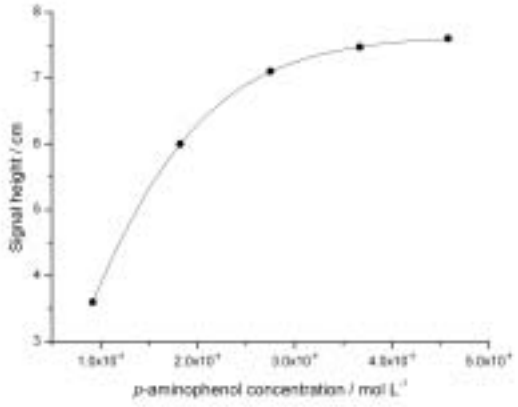

Fig. 4 Influence of *p*-aminophenol concentration on the analytical signal. **h** is the signal height in cm. 1 cm is equal to 0.084 absorbance. Flux rate 1.8 mL min^{-1}. α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. NaOH concentration 2.5 % w/v. Sample volume 180 μL. Reaction bobbin length 50 cm. Wavelength 608 nm.

Fig. 5 shows the influence of reaction bobbin length on the analytical signal. It can be observed a great increase going from 15 cm to 100 cm. No significant increase was observed in longer bobbins. For analytical purposes, the length of 100 cm was adopted.

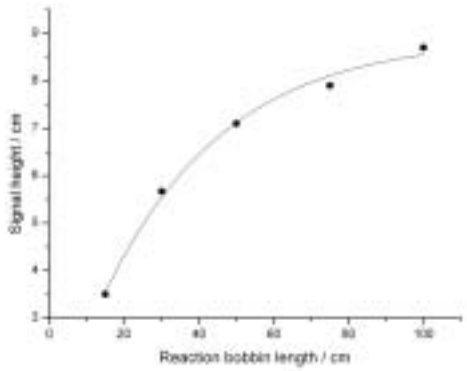

Fig. 5 Influence of the reaction bobbin length on the analytical signal height. **h** is the signal height in cm (1 cm is equal to 0.084 absorbance). Flux rate 1.8 mL min^{-1}. α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. *p*-aminophenol concentration 4.6×10^{-4} mol L^{-1} NaOH concentration 2.5 % w/v. Sample volume 180 μL. Wavelength 608 nm.

The study of the dependence of the sampling volume is shown in Fig. 6. The volume of 300 μL was selected for the procedure.

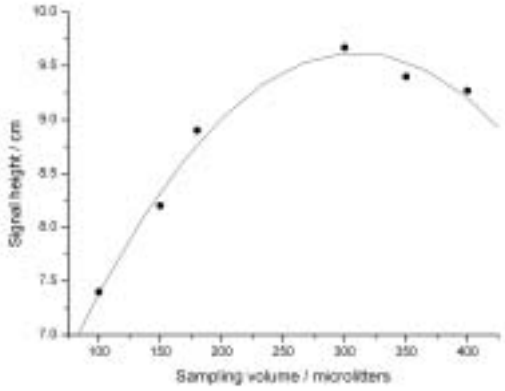

Fig. 6 Influence of the sampling volume on the analytical signal height. **h** is the signal height in cm (1 cm is equal to 0.084 absorbance). Flow rate 1.8 mL min^{-1}. α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. *p*-aminophenol concentration 4.6×10^{-4} mol L^{-1} NaOH concentration 2.5 % w/v. Reaction bobbin length 100 cm. Wavelength 608 nm.

The study of the influence of the total flow rate is depicted in Fig. 7. The signal decreases almost linearly with the flow rate increase. As adequate value was selected the flow rate of 1.8 mL min^{-1} that allows good signal and adequate analytical frequency.

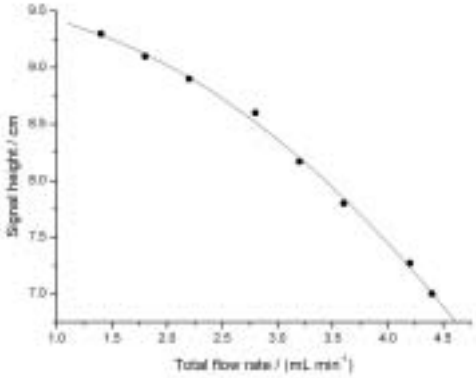

Fig. 7 The influence of the total flow rate on the analytical signal. The three independent flows were maintained equal. Influence of the sampling volume on the analytical signal height. **h** is the signal height in cm (1 cm is equal to 0.084 absorbance). α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. *p*-aminophenol concentration 4.6×10^{-4} mol L^{-1}. NaOH concentration 2.5 % w/v. Reaction bobbin length 100 cm. Sampling volume 300 μL. Wavelength 608 nm.

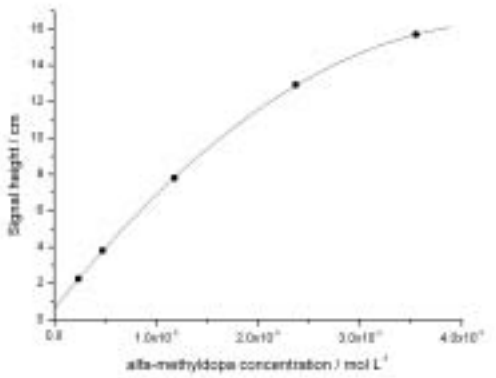

Fig. 8 Calibration curve. **h** is the signal height in cm (1 cm is equal to 0.084 absorbance). Flow rate 1.8 mL min^{-1}. α-Methyldopa concentration 1.0×10^{-2} mol L^{-1}. *p*-aminophenol concentration 4.6×10^{-4} mol L^{-1} NaOH concentration 2.5 % w/v. Sampling volume 300 μL. Reaction bobbin length 100 cm. Wavelength 608 nm.

The calibration curve is shown in Fig. 8. It was done using five standard solutions of α-methyldopa in the range from 2.0×10^{-4} mol L^{-1} to 3.5×10^{-3} mol L^{-1} and is described by the equation $\mathbf{h} = 0.62 + 70.5 \times 10^{2}\,C - 78.8 \times 10^{4}\,\mathbf{C}^2$, where **h**= peak height in cm (1cm = 0.084 absorbance) and **C** is the analyte concentration in mol L^{-1}. The observed correlation coefficient, R, is 0.99998.

Five different samples were analyzed using the proposed method and the spectrophotometric method with ferrous tartrate established in USP [22]. Three determinations were performed with each sample. The obtained data were compared through the statistical Student's *t*-test [23]. The results are shown in Table 1. It can be observed that there is a complete agreement between the two methods at 95% confidence level and degree of freedom equal to 4.

Table 1 Comparison, using the statistical Student's *t*-test, between the results obtained by the proposed FI method and the method recommended by the Pharmacopeia [22]. Tabled $t = 2.776$ for degree of freedom $\nu = 4$ ($n_1 + n_2 - 2 = 4$) and confidence coefficient $(1-\alpha) = 0.95$ (95% confidence level) [30]; $n_1 = n_2 = 3$ in this instance.

Sample	Nominal content / mg	Proposed method / mg	RSD / %	USP method /mg	RSD / %	Calculated *t*
1[a]	250	257.4 ± 4.2	1.6	249.2 ± 3.5	1.4	2.121
2[b]	250	252.0 ± 6.5	2.6	248.7 ± 8.6	3.4	0.433
3[c]	500	504.1 ± 3.2	0.6	505.0 ± 6.6	1.3	0.174
4[d]	500	504.8 ± 9.7	1.9	510.1 ± 6.4	1.3	0.645
5[b]	500	515.3 ± 9.7	1.9	514.3 ± 6.6	1.3	0.121
Mean	RSD		1.7		1.7	

[a] Sample 1. Also contains: disodium edetate dihydrate, citric acid, polyvidone, crospovidone, magnesium stearate, polymer methacrylic acid, polyethyleneglycol, titanium dioxide, red iron oxide, yellow dye, talc.
[b] Samples 2 and 5. Also contain: citric acid, disodium edetate of calcium, ethyl cellulose, silicon dioxide, magnesium stearate, guar gum, hydroxyl propyl methyl cellulose, titanium dioxide, polyethyleneglycol, cellulose, alcohol anhydrous, yellow and red dye.
[c] Sample 3. Also contains: citric acid, disodium edetate, cellulose microcrystalline, polyvinylpyrrolidone, sodium croscarmelose, silicon dioxide, magnesium stearate, hydroxyl propyl methylcellulose, titanium dioxide, polyethyleneglycol, yellow dye.
[d] Sample 4. Also contains: citric acid, disodium edetate of calcium, cellulose microcrystalline, polyvidone, sodium croscarmelose, silicon dioxide, magnesium stearate, macrogol, hypromelose, titanium dioxide, yellow dye.

4. Conclusion

The method proposed in this work can be considered of very simple procedure compared with other flow methods. The results obtained with the analytical method proposed in this work, applied to five different commercial pharmaceutical preparations, agree with the results obtained through the spectrophotometric analytical USP procedure [22] for the same samples. The methods were compared through the statistical Student's *t*-test [23]. The observed precision of two methods (RSD) is about the same (1.7 %) but the proposed procedure presents higher analytical frequency. The working analytical range, considering the final solution, is between 2×10^{-4} mol L^{-1} and 3×10^{-3} mol L^{1}. The correlation coefficient, R, of the analytical curve, is 0.99998.

The detection limit is about 0.3 $\times 10^{-4}$ mol L^{-1} in the final solution introduced in the flow system (3×SD of the blank) and 48 determinations can be performed per hour.

Considering the reliability, the simplicity, the analytical frequency and the low cost of the method, it can be recommended for the analysis of α-methyldopa in pharmaceutical preparations.

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6. References

[1] J.A. Oates, in: J.G. Hardman, L.E. Limbird (Eds.), Goodman & Gilman´s: *"The pharmacological basis of therapeutics"*, 9th ed., Mac Graw-Hill, New York, 1996, p. 780.
[2] F.B. Salem, *Talanta*, **34**, 810 (1987).
[3] S.S. Badawy, Y.M. Issa, A.S. TagEldin, *Electroanal.*, **8**,1060 (1996).
[4] J.J.B. Nevado, J.M.L. Gallego, P.B. Laguna, *Fresen. J. Anal. Chem.*, **353**, 221 (1995).
[5] J.M. Calatayud, *"Flow injection analysis of pharmaceuticals – automation in the laboratory"*, Taylor & Francis, New York, 1996.
[6] J.J. Berzas, J.M. Lemus, P.Buitrago, P., *Anal. Lett.*, **30**, 1109 (1997).
[7] I.C. Vieira, O. Fatibello-Filho, *Talanta*, **46**, 559 (1998).
[8] M.I.H. Helaleh, N. Rahman, E. S. M. Abu-Nameh, *Anal. Sci.*, **13**, 1007 (1997).
[9] T. Aman, I.U. Khan, N. Aslam, I. Ahmad, *Anal. Lett.*, **31**, 1007 (1998).
[10] P. Nagaraja, K.C.S. Murthy, K.S. Rangappa, N.M.M. Gowda, *Talanta*, **46,** 39 (1998).
[11] A.G. Sajjan, M.B. Melwanki, J. Seetharamappa, *J. Anal. Chem.*, **56**, 827 (2001).
[12] B.S. Nagaralli, J. Seetharamappa, M.B. Melwanki, K.C. Ramesh, J. Keshavayya, *J. AOAC Int.*, **85**, 1288 (2002).
[13] P. Nagaraja, R.S.Vasantha, K. R. Sunitha, *J. Pharmac. Biomed.*, **25**, 417 (2001).
[14] P.B. Issopoulos, S.E. Salta, *Fármaco*, **51**, 673 (1996).
[15] A. Afkhami, H A. Khatami, *J. Anal. Chem.*, **58**, 135 (2003).
[16] P. Nagaraja, R. A. Vasantha, K. R. Sunitha, *Talanta*, **55**, 1039 (2001).
[17] C. H. Oliveira, R. E. Barrientos-Astigarra, M. Sucupira, G. S. Graudenz, M. N. Musacara, G. De Nucci, *J. Chromatogr. B*, **768**, 341 (2002).
[18] M. Zecevic, L. Zivanovic, S. Agatonovic-Kustrin, *J. Pharm. Biom. Anal.*, **24**, 1019 (2001).
[19] K. Rona, K. Ary, B. Gachalyi, I. Klebovich, *J. Chromatogr. A*, **730**, 125 (1996).
[20] Z. Talebpour, S. Haghgoo, M. Shamsipur, *Anal. Chim. Acta*, **506**, 97 (2004).
[21] K. C. Brown, J.F. Corbett, and R. Labinson, *J.C.S. Perkin II*, 1292 (1978).
[22] The United States Pharmacopeia. The National Formulary, USP XXIV. United States Pharmacopeial Convention, Rockville, 2000, p. 1081.
[23] K. Eckschlager, *"Errors, measurement and results in chemical analysis"*, Van Nostrand Reinhold, London 1972, p. 107.
[24] M. Tubino, F. G. Barros, *Quím. Nova*, **14,** 49 (1991).
[25] T.C. Rodrigues, M. Tubino, O.E.S. Godinho, G. Oliveira Neto, *Anal. Sci.*, **17**, 629 (2001).

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