Eclética Química
ISSN: 0100-4670
atadorno@iq.unesp.br
Universidade Estadual Paulista Júlio de Mesquita Filho
Brasil

Gotardo, M. A.; Sequinel, R.; Pezza, L.; Pezza, H. R.
Determination of atenolol in pharmaceutical formulations by diffuse reflectance spectroscopy
Eclética Química, vol. 33, núm. 4, octubre-diciembre, 2008, pp. 7-12
Universidade Estadual Paulista Júlio de Mesquita Filho
Araraquara, Brasil

ECLÉTICA Química
www.scielo.br/eq
www.ecletica.iq.unesp.br
Volume 33, número 4, 2008

Determination of atenolol in pharmaceutical formulations by diffuse reflectance spectroscopy

*M. A. Gotardo, R. Sequinel, L. Pezza, and H. R. Pezza**
Sao Paulo State University - UNESP, Chemistry Institute of Araraquara, P.O. Box 355, 14801-970, Araraquara, SP, Brazil
**hrpezza@iq.unesp.br*

Abstract: A simple analytical method for quantification of atenolol in pharmaceutical formulations by diffuse reflectance spectroscopy is described. The method is based on the reaction, on the filter paper surface, between the drug and *p*-chloranil producing a colored compound. The best reaction conditions were obtained with 20 μL of atenolol solution and 20 μL of *p*-chloranil. All reflectance measurements were carried out at 550 nm and the linear range was from 1.13×10^{-2} to 7.88×10^{-2} mol L^{-1} (r = 0.9992). The limit of detection was 2.80×10^{-3} mol L^{-1}. The proposed method was successfully applied to analysis of different commercial brands of pharmaceutical formulations and the results obtained by the proposed method were in good agreement with those obtained using the British Pharmacopoeia method.

Keywords: diffuse reflectance spectroscopy; atenolol; *p*-chloranil, pharmaceutical formulations.

Introduction

Nowadays, in the development of new analytical procedures, care about the toxicity and danger of the reagents used and the wastes produced are as important as any other analytical feature. Hence, there is a urgent necessity to develop methods which are less harmful to human and to the environment according to 12 principles of Green Chemistry [1,2]. Other great problem in the whole world is the falsification and adulteration of pharmaceuticals consumed by the population [3]. The use of these pharmaceuticals represents a risk for people's health. In Brazil, this problem was detected in 1998, when a variety of pharmaceuticals such as contraceptive, antibiotic, anticarcinogenic, and antipyretic were falsified with serious consequences [3]. So, the analysis of pharmaceutical formulations seeks not only the industrial quality control but also the product idoneousness proof [4]. In addition, in the Green Chemistry context it is evident the requirement for efficient methods to control the amount of drug in pharmaceutical formulations.

Atenolol, 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide is a β_1-selective (cardioselective) adrenoreceptor antagonist drug commonly used for management of hypertension, prevention of heart diseases as angina pectoris and control of some forms of cardiac arrithymia [5].

Several analytical methods have been reported for the determination of atenolol in pharmaceutical formulations. The United States Pharmacopeia (2003) describes a method that uses high performance liquid chromatography (HPLC) with UV detection for assay of atenolol tablets [6]. The method recommended by British Pharmacopoeia (2001) involves UV spectrophotometry [7].

In Brazilian Pharmacopeia, however, a method for assay of atenolol was not found. Other methods reported in the literature for the determination of atenolol in pharmaceutical formulations include visible spectrophotometry [8-13], UV derivative spectrophotometry [14,15], HPLC [16], high performed thin layer chromatography [17,18], potentiometry [19-21], capillary electrophoresis [22-24], and voltametry [25,26]. Nevertheless, most of these techniques are time-consuming, involving the use of large volumes of organic solvents or require expensive and sophisticated instruments. On the other hand, the combined spot test-diffuse reflectance spectroscopy offers advantages over other methods, such as simplicity and extremely low consumption of reagents. Moreover, quantitative spot test procedures can be performed in locus using a very simple homemade reflectometer or a portable diffuse reflectance spectrophotometer, which are small, lightweight, inexpensive and battery operated, characteristics highly attractive for many applications in any location by nearly everyone.

Up to now, the diffuse reflectance spectroscopy using spot tests has been used with success in quantitative analysis [27-32]. The diffuse reflectance spectroscopy associated to solid phase extraction (SPE) was proposed for the quantification of aliphatic amines [31] and any metals [27]. The use of silica gel loaded with analytical reagents as adsorbent has also been associated to diffuse reflectance spectroscopy for the determination of several metals [33,34]. A portable optic fiber sensor was described by Matias *et al*. for analysis of diesel engine smoke [35]. The sensor works by sampling the smoke on the surface of a white adhesive tape and then measuring the diffuse reflectance from the stained tape. Another portable device employing the diffuse reflectance spectroscopy was developed for determining Ni (II) in catalysts [29]. This method was based on the classical reaction of Ni (II) with dimethylglyoxime, in alkaline medium (NH_4OH) and the detection was carried out from LDR (Light Detector Resistor) measured by a multimeter, which was correlated with the reflectance.

The diffuse reflectance spectroscopy using spot test reactions on filter paper has been used for the determination of few drugs [32,36,37]. The objective of this study is to show the convenience of the diffuse reflectance spectroscopy as an attractive technique for analysis of atenolol in pharmaceutical formulations, emphasizing its simplicity, the extremely low reagents/solvents consumption and the possibility to perform quick, precise and accurate measurements.

Experimental

Instruments

The reflectance measurements were made in a handheld integrating sphere (USP-REF, Ocean Optics, Dunedin, USA) connected to a fiber optic minispectrometer (USB2000, Ocean Optics). The USB2000 minispectrometer is equipped with a 2048 pixels Sony ILX511 CCD array detector. Software Spectra Suite (Ocean Optics) was used for acquisition and storage of spectra. Eppendorf (10 to 100 μL) micropipette was used to measure smaller volumes in the experiment.

Materials, chemicals and solutions

Whatman 42 filter paper was used as solid support. The excipients used in the interference study were of pharmaceutical grade. Solvents used were dioxane (analytical reagent grade) (Tedia, Fairfield, EUA) and methanol HPLC grade (J.T. Baker, Phillipsburg, EUA). p-Chloranil (Sigma, St Louis, EUA) was used to prepare a 4.00×10^{-2} mol L^{-1} solution in dioxane. A stock standard solution of atenolol (Purifarma – São Paulo, Brazil, purity grade > 99.9%) in methanol (2.25 x 10-1 mol L-1) was prepared and, the working solutions were prepared by convenient dilutions with methanol. All solutions were daily prepared.

Pharmaceutical formulations

Pharmaceutical formulations (tablets) of five commercial brands containing nominal values of 25, 50 and 100 mg of atenolol per tablet were analyzed. These pharmaceuticals were purchased from local drugstores and all were tested prior to the listed expiration date.

Spot test reaction

For the spot test, solutions were spotted onto 2.0 cm x 2.0 cm squares filter papers (Whatman 42). Firstly 20 μL of the drug solution were spotted, fol-

lowed by the addition of 20 μL of p-chloranil solution (4.00 x 10^{-2} mol L^{-1}). In order to dispense the solutions on the center of the filter paper a micropipette fixed in a holder according to the procedure described by Tubino et al. was used [38]. In the sequence, the reflectance measurements were obtained as a function of AR (optical intensity for reflectance measurements – where AR = log 1/R) at 550 nm. Blank with 20 μL of methanol and 20 μL of reagent solution was used as reference.

Sample preparation

Twenty tablets of each commercial brand of pharmaceutical were separately weighed and finely powdered. The value of one tablet mass was expressed as the mean of 20 determinations, with variation lower than 2%. A portion of powered sample, equivalent to 120 mg of atenolol, was weighed and shaking with methanol in a magnetic mixer for 10 minutes. This solution was transferred into a 10.00 mL volumetric flask and then an aliquot of this solution was taken for the spot test reaction and analyzed by diffuse reflectance spectroscopy at 550 nm.

Study of interferences

Since the aim of this study was determine atenolol in tablets, the effect of the most commonly used excipients was carefully examined. The excipients studied were starch, lactose, talc, magnesium stearate, sodic croscarmelose, ethylcellulose, microcristalina cellulose, sodium lauril sulphate and silicon dioxide. For this study, 120 mg of atenolol and each one of the excipients (in equal amount) were shaken with methanol in a magnetic mixer for 10 minutes and, then quantitatively transferred to 10.00 mL volumetric flask which was completed with the same solvent. The procedure was repeated using quantities of excipients four times greater than atenolol. Next, an aliquot of these solutions was used for the spot test reaction and analyzed by diffuse reflectance spectroscopy at 550 nm, according to procedure described in the previous section.

Standard Addition

The standard addition was carried out in order to evaluate possible interferences from the matrix (pharmaceutical formulation) on the analysis. For this study, amounts corresponding to 30, 60, 90 and 120 mg of standard atenolol were added over samples containing 60 mg equivalent mass of atenolol in a beaker with methanol. These solutions were shaken in a magnetic stirrer for 10 minutes and transferred into a 10.00 mL volumetric flask and, afterward filtered in Whatman 42 filter paper. An aliquot of this solution was taken for the spot test reaction and analyzed by diffuse reflectance at 550 nm.

Results and Discussion

Spot test reaction

p-Chloranil (2,3,5,6-tetrachloro-*p*-benzoquinone) has been used as chromogenic reagent for determination of several drugs, such as isoprenaline sulphate and metyldopa [39], fluoxetine and sertraline [40] and other antidepressives [41], salbutamol [42] and β-blockers [43], including atenolol. These methods are based on the interaction between electron donors (drugs) and *p*-chloranil, which acts as π-acceptor, producing colored charge transfer complexes [44].

In the present work the reaction between atenolol and *p*-chloranil was carried out directly on the filter paper surface, resulting in the immediate appearance of the colored complex that was used in the development of the quantitative spot tests. Fig. 1 shows the reflectance spectrum with maximum of A_R at 550 nm.

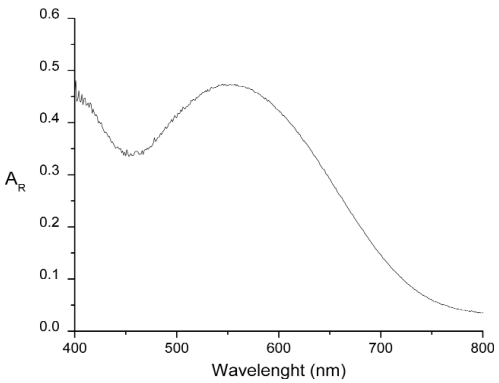

Figure 1. Reflectance spectrum obtained from the spot test reaction between atenolol and *p*-chloranil. Maximum value of A_R: 550 nm. Concentration of atenolol solution: 7.88 x 10^{-1} mol L^{-1}.

Effect of p-chloranil concentration on the spot test

The effect of *p*-chloranil concentration on the spot test was studied in order to reach the highest reflectance value at 550 nm. A univariate procedure was carried out and the studied *p*-chloranil concentration range was from 2.0 x 10^{-2} to 1.4 x 10^{-1} mol L^{-1}. *p*-Chloranil solutions at concentrations higher than 1.4 x 10^{-1} mol L^{-1} were not studied due to its low solubility in dioxane. In this study, the atenolol concentration was set at 7.88 x 10^{-2} mol L^{-1}.

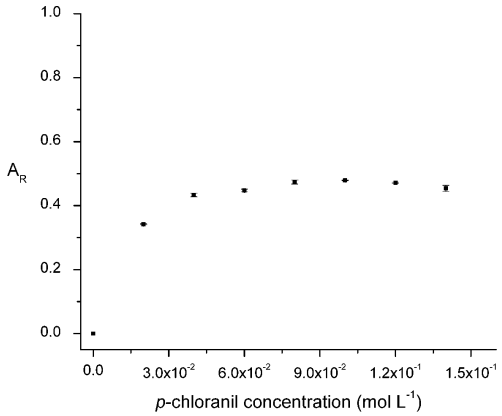

Figure 2. Study of the effect of p-chloranil concentration on the reflectance response. The bars represent the standard deviations for 3 replicates.

As observed in the Fig. 2 the addition of atenolol standard solution without *p*-chloranil (first point of the graph) do not showed reflectance response, indicating that atenolol do not react with components of the paper. Also, it can be observed that the reflectance values increased according to the increase of *p*-chloranil concentration up to 4.00 x 10^{-2} mol L^{-1}. The addition of *p*-chloranil solutions at concentrations higher than 4.00 x 10^{-2} mol L^{-1} do not exerted significant effect on the reflectance signal. Thus, the results obtained indicated that the *p*-chloranil solution at 4.00 x 10^{-2} mol L^{-1} was the lower concentration that gave the highest reflectance values and then, the chosen concentration.

Stability

In order to evaluate the stability of the product of the spot test reaction between atenolol and *p*-chloranil on the filter paper was carried out kinetic monitoring of the reflectance value at 550 nm, in zero time up to 60 minutes, each 2 minutes. The results demonstrated that there is not significance difference on the A_R values obtained along the different periods of time.

Study of Interferences

The effect of each excipient (starch, lactose, talc, magnesium stearate, cellullose microcristalina, lauril sulfato de sódio e dióxido de silício) was considered as interference when the reflectance signal shows an error equal or greater than 3% in the determination of drug. The atenolol percentage found in the added solutions ranged from 99 - 101% with variation coefficients lower than 3% for three repetitions. So, no interferences were observed from these excipients under the studied conditions.

Analytical Curve

The analytical curve was constructed using atenolol standard solutions in the concentration range from 1.13 x 10^{-2} to 7.88 x 10^{-2} mol L^{-1}. The correlation coefficient (r = 0.9992) was obtained plotting A_R versus log of the concentration (mol L^{-1}). A_R values for the concentration range were fitted by the equation: $A_R = 0.13469 + 0.32613\ C$, where C = log [atenolol] x 10^2 (mol L^{-1}). The factor 10^2 was used for adjust the analytical curve to log values higher than zero. The limit of detection was 2.80 x 10^{-3} mol L^{-1} [45].

Standard addition

In order to evaluate possible interferences from of whole tablet matrix, the addition standard method was carried out using 3 samples of different commercial brands tablets. For all samples the recovery average values (n=3) ranged from 96.1 to 101.9%, proving the absence of significant matrix effect.

Application of the proposed method and comparison with the reference method

The results obtained from the proposed method were statistically compared with the

pharmacopoeia method for determining atenolol in tablets. According the pharmacopoeia analysis the quantitative determination is performed by UV spectrophotometry at 275 nm and involves successive dilutions with methanol, and heating at 60 ºC under shaking for 15 minutes. The proposed method was applied to some pharmaceutical tablets containing atenolol commercially available and the results obtained showed good agreement with those obtained by the pharmacopoeia method (Table 1). For all formulations assayed, the results obtained by both methods were compared by application of the *F* test and *t* test at 95% confidence level. In all cases, the calculated *F* and *t* values did not exceed the theoretical values, indicating that there is no significant difference between both methods in concerning precision and accuracy.

Table 1. Determination of atenolol in commercial pharmaceutical formulations.

Samples	Label to content (mg/tablet)	Proposedmethod		Reference Method	
		Found value[b] (mg/tablet)	t value (2.78)	F value (19.00)	Found value[b] (mg/tablet)
A	100	98.6±1.3	1.23	6.76	99.5±0.5
B	100	98.4±1.5	0.21	9.00	98.6±0.5
C	50	47.8±1.3	0.25	10.56	48.0±0.4
D	50	49.1±1.6	0.01	3.16	49.1±0.9
E	25	24.1±0.4	0.87	5.06	24.6±0.9

[a] Average ± standard deviation (SD), n = 3.

Conclusions

This paper presents a reflectometric method for the quantitative determination of atenolol in tablets, exposing the advantages as regards to simplicity, rapidity and very low consumption of reagents/solvents. The method based on spot test/diffuse reflectance spectroscopy was successfully applied to determine atenolol in tablet samples of different commercial brands, with good precision and accuracy when compared to the pharmacopoeia procedure.

Acknowledgements

We would like to thank Capes, CNPq and FAPESP foundations (Brazil) for financial support.

Received May 21 2008
Accepted July 01 2008

A. A. Gotardo, R. Sequinel, L. Pezza e H. R. Pezza. Determinação de atenolol em formulações farmacêuticas por espectroscopia de reflectância difusa

Resumo: Este trabalho descreve um método analítico simples para a quantificação de atenolol, em formulações farmacêuticas, por espectroscopia de reflectância difusa. O método é baseado na reação, sobre uma superfície de papel de filtro, entre atenolol e o reagente *p*-cloranil, produzindo um composto colorido. As melhores condições para a reação foram obtidas com 20 µL de atenolol e 20 µL de *p*-cloranil. As medidas de reflectância foram realizadas em 550 nm, para obtenção de uma faixa linear que variou de $1.13x10^{-2}$ to $7.88x10^{-2}$ mol L^{-1}, com excelente coeficiente de correlação (r = 0.9992). O limite de detecção foi de 2.80×10^{-3} mol L^{-1}. O método proposto foi aplicado com sucesso na análise de diferentes marcas de formulações farmacêuticas comerciais e os resultados obtidos pelo método proposto estiveram em boa concordância com aqueles obtidos pelo método da Farmacopéia Britânica.

Palavras-chave: espectroscopia de reflectância difusa; atenolol; *p*-cloranil; formulações farmacêuticas.

References

[1] E. C. Vidotti, W. F. Costa, and C. C. Oliveira, Talanta, 68 (2005) 516.
[2] P. T. Anasta, Crit. Rev. Anal. Chem., 29 (1999) 167.
[3] K. Pastore, Revista Veja, 31 (1998) 40.
[4] L. Pezza, M. Tubino, C. B. Melios, and H. R. Pezza, Anal. Sci., 16 (2000) 313.
[5] B. B. Hoffman, in: J. G. Hardman, L. E. Limbird, A. G. Gilman (eds), Goodman & Goodman¥s The Pharmacological Basis of Therapeutics, tenth ed., MacGraw-Hill, Rio de Janeiro, v. 10, 1987, p. 1011-1021.
[6] United States Pharmacopeia: USP 26 the national formulary USP 26-NF1, twenty sixth ed. Rockville, MD, 2003.
[7] British Pharmacopoeia Londom: The Stationer Office, v. 2, 2001.
[8] Y. K. Agrawal, K. Raman, S. Raiput, and S. K. Menon, Anal. Lett., 12 (1992) 1503.
[9] A. Gülcü, C. Yücesoy, and S. Serin, Farmaco, 59 (2004) 487.
[10] H. Salem, J. Pharm. Biomed. Anal., 29 (2002) 527.
[11] S. M. Al-Ghannam and F. Belal, J. AOAC Int., 85 (2002) 817.
[12] A. S. Amin, G. H. Ragab, and H. Saleh, J. Pharm. Biomed. Anal., 30 (2002) 1347.
[13] S. M. Al-Ghannam, J. Pharm. Biomed. Anal., 40 (2006) 151.
[14] M. C. F. Ferraro, P. M. Castellano, and T. S. Kaufman, J. Pharm. Biomed. Anal., 34 (2004) 305.
[15] D. Bonazzi, R. Gotti, V. Andrisano, and V. Cavrini, Farmaco, 51 (1996) 733.
[16] I. R. Martinez, M. C. G. A. Coque, and R. M. V. Camanas, J. Chromatogr., A, 765 (1997) 221.
[17] A. P. Argekar, and J. G. Sawant, J. Liq. Chromatogr. Rel. Technol., 22 (1999) 1571.
[18] A. P. Argekar, and S.G. Powar, Farmaco, 21 (2000) 1137.
[19] D. P. Nikolelis, S. E. Petropoulou, and M. V. Mitrokotsa, Bioelectrochemistry, 58 (2002) 107.
[20] S. S. M. Hassan, M. M. Abou-Sekkina, M. A. El-Ries, and A. A. Wassel, J. Pharm. Biomed. Anal., 32 (2003) 175.
[21] M. Shamsipur, and F. Jalali, Anal. Lett., 38 (2005) 401.
[22] P. S. Bonato, A. C. C. Briguenti, Drug Dev. Ind. Pharm., 31 (2005) 209.
[23] A. Shafaati and B. J. Clark, Anal. Lett., 14 (1996) 1547.
[24] M. I. Maguregui, R. M. Jimenez, and R. M. Alonso, J. Chromatogr. Sci., 36 (1998) 516.
[25] R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, Electrochem. Commun., 8 (2006) 65.
[26] R. N. Goyal, and S. P. Singh, Talanta, 39 (2006) 932.
[27] M. P. Arena, M. D. Porter, J. S. Fritz, Anal. Chim. Acta, 482 (2003) 197.
[28] S. G. Dmitrienko, O. A. Sviridova, L. N. Pyatkova, V. M. Senyavin, Anal. Bioanal. Chem. 374 (2002) 361.
[29] F. A. A. Matias, M. M. D. C. Vila, M. Tubino, Sens. Actuators B, 88 (2003) 60.
[30] A. Ghauch, C. Turnar, C. Fachinger, J. Rima, A. Charef, J. Suptil, M. Martin-Bouyer, Chemosphere, 40 (2000) 1327.
[31] Y. Moliner-Martinez, P. Campíns-Falcó, Talanta, 65 (2005) 217.
[32] M. Tubino, R. L. Souza, Talanta, 68 (2006) 776.
[33] O. A. Zaporozhets, O. Gawer, V. Sukhan, Talanta, 46 (1998) 1387.
[34] O. A. Zaporozhets, L. Tsyukalo, Talanta, 58 (2002) 861.
[35] F. A. A. Matias, W. A. Oliveira, E. Moschim, Sens. Actuators B, 41 (1997) 159.
[36] M. A. Gotardo, A. C. Gigante, L. Pezza, H. R. Pezza, Talanta, 64 (2004) 361.
[37] M. A. Gotardo, L. Pezza, H. R. Pezza, Eclet. Quim., 30 (2005) 17.
[38] M. Tubino, A. V. Rossi, M. E. A. Magalhães, Anal. Lett., 30 (1997) 271.
[39] M. A. Korany, A. A. M. Wahbi, Analyst, 104 (1979) 146.
[40] L. I. Bebawy, N. El-Kousy, J. K. Suddik, and M. Shokry, J. Pharm. Biomed. Anal., 21 (1999) 33.
[41] E. A. Ibrahim, A. S. Issa, M. A. A. Salam, and M. S. Mahrous, Talanta, 30 (1983) 531.
[42] R. S. Bakry, O. A. Razak, A. F. M. El-Walily, and S. F. Belal, J. Pharm. Biomed. Anal., 14 (1996) 357.
[43] H. Salem, J. Pharm. Biomed. Anal., 29 (2002) 527.
[44] R. Foster, Organic Charge-Transfer Complexes, 2nd ed., Academic Press, London, 1968, p. 470.
[45] D. A. Skoog, F. J. Holler, T. A. Nieman, Principles of Instrumental Analysis, fifth ed., Harcourt Brace Company, Philadelphia, 1998, p. 27.