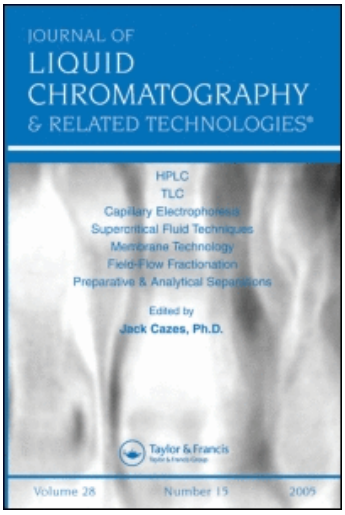

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Development and Validation of an RP-HPLC Method for the Dissolution Studies of Bisoprolol in Pharmaceutical Dosage Forms

Clarice Madalena Bueno Rolim[a]; Liberato Brum Jr.[a]; Marcio Fronza[a]; Marcelo Donadel Malesuik[a]; Lisiane Bajerski[a]; Sérgio Luiz Dalmora[a]
[a] Department of Industrial Pharmacy, Health Science Center, Federal University of Santa Maria, Santa Maria, RS, Brazil

To cite this Article Rolim, Clarice Madalena Bueno , Brum Jr., Liberato , Fronza, Marcio , Malesuik, Marcelo Donadel , Bajerski, Lisiane and Dalmora, Sérgio Luiz(2005) 'Development and Validation of an RP-HPLC Method for the Dissolution Studies of Bisoprolol in Pharmaceutical Dosage Forms', Journal of Liquid Chromatography & Related Technologies, 28: 3, 477 — 486
To link to this Article: DOI: 10.1081/JLC-200044531
URL: http://dx.doi.org/10.1081/JLC-200044531

Journal of Liquid Chromatography & Related Technologies®, 28: 477–486, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200044531

Development and Validation of an RP-HPLC Method for the Dissolution Studies of Bisoprolol in Pharmaceutical Dosage Forms

Clarice Madalena Bueno Rolim, Liberato Brum, Jr., Marcio Fronza, Marcelo Donadel Malesuik, Lisiane Bajerski, and Sérgio Luiz Dalmora
Department of Industrial Pharmacy, Health Science Center, Federal University of Santa Maria, Santa Maria, RS, Brazil

Abstract: An RP-HPLC procedure was developed for the dissolution rate studies of bisoprolol in solid dosage formulations. The HPLC system was operated isocratically at controlled-ambient temperature with reversed phase C_{18} column (150 mm × 4.6 mm i.d.; 5 μm particle size), using a mobile phase methanol : phosphate buffer (pH 3.5; 0.01 M) (55 : 45, v/v) at a flow rate of 1.0 mL min^{-1}. Detection was achieved with a photodiode array (PDA) detector at 225 nm. Method validation investigated parameters such as the range, linearity ($r^2 = 0.9999$), precision, accuracy, and robustness. The dissolution test conditions and the medium were chosen as 0.1 M HCl at a stirring rate of 50 rpm, and the methodology was applied to bisoprolol fumarate tablets giving similar dissolution profiles compared by the difference and similarity factor (f_1, f_2), obtaining values lower than 7.22 and higher than 72.28, respectively.

Keywords: Bisoprolol, dissolution, RP-HPLC, validation

INTRODUCTION

Bisoprolol, (5) l-(4- (2-isopropylethoxy) methylphenoxy)-3-isopropylamino-2-propanol hemifumarate is a highly selective β_1-adrenoreceptor antagonist

Address correspondence to Clarice Madalena Bueno Rolim, Department of Industrial Pharmacy, Health Science Center, Federal University of Santa Maria, 97.105-900, Santa Maria, RS, Brazil. E-mail: crolim@smail.ufsm.br

lacking intrinsic sympathomimetic activity and with low anaesthetic potency.[1,2]

Drug dissolution testing is an integral part of pharmaceutical development and in routine, quality control monitoring the drug release characteristics. The importance of the test is based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be solubilized. Therefore, dissolution tests are used not only for quality control of finished products, to assess batch-to-batch consistency of drug release from solid dosage forms, but also they are essential in the stages of formulation development, for screening and proper assessment of different formulations. For curves to be considered similar, f_1 values should be up to 15 (0–15) and f_2 values greater than 50 (50–100), which means that an average difference of no more than 10% at the sample time points, ensure equivalence of the two curves and, thus, of the performance of the test and reference products.[3–6]

The in vitro dissolution studies are relevant to the prediction of in vivo performance of the products and the procedures and guidelines are described by the literature.[7,8] Studies on the bioavailability of drugs from a given dosage form revealed, that in many situations, various dosage forms with the same content of the active compound did not give the same therapeutic effect. This is ascribed to differences in physical characteristics of the active compound, in formulation factors or in technological processes used by different manufacturers, therefore, resulting in different bioavailability profiles.[9]

High performance liquid chromatography with fluorescence detection was developed for the measurement of bisoprolol enantiomers in human plasma and urine.[10–12] The RP-HPLC method with UV detection was also described for the determination of bisoprolol and potential impurities in drug substances and human plasma, as well as for bisoprolol enantiomers separation.[3–15]

The aim of the present paper was to develop and investigate a dissolution medium for bisoprolol tablet dosage forms and validate a sensitive RP-HPLC method to be applied for the in vitro dissolution rate profiles studies.

EXPERIMENTAL

Chemical and Reagents

HPLC-grade acetonitrile and orthophosphoric acid were purchased from Tedia (Fairfield, USA). Analytical grade hydrochloric acid and potassium dihydrogen phosphate monobasic were obtained from Merck (Darmstadt, Germany). The bisoprolol fumarate reference standard (Lot: F0B038) was obtained from the United States Pharmacopeia (Rockville, USA). Samples of bisoprolol fumarate containing 5 and 10 mg per tablet were obtained

from commercial sources and used within their shelf life period. All chemicals used were of pharmaceutical or special analytical grade. For all analyses, double-distilled water filtered through a 0.45 μm membrane filter was used.

Instrument and Chromatographic Conditions

A Vankel VK7010 (VanKel Technology Group, Cary, NC), a paddle-stirrer type of apparatus, was used integrated with a VK 8000 dissolution sampling station, VK type bidirectional peristaltic pump.

The HPLC system consisted of a Shimadzu (Kyoto, Japan) equipped with an SCL-10A_{VP} system controller, LC-10 AD_{VP} pump, DGU-14A degasser, SIL-10AD_{VP} autosampler, and an SPD-M10A_{VP} photodiode array (PDA) detector. The detector was set at 225 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP® software program. The experiments were carried out on a reversed phase Shimadzu (Kyoto, Japan) C_{18} Shim-Pack CLC-ODS column (150 mm × 4.6 mm i.d.; 5 μm particle size). A CLC G-ODS (10 mm × 4.0 mm i.d.; 5 μm particle size) guard column was used to protect the analytical column. The HPLC system was operated isocratically at controlled-ambient temperature using methanol : phosphate buffer (pH 3.5; 0.01 M) (55 : 45, v/v) as mobile phase, filtered through a 0.45 μm membrane filter (Millipore) and run at a flow rate of 1.0 mL min^{-1}. The injection volume of 60 μL was used for both standard and samples, and all determinations were carried out in three to five replicates.

Procedure

Standard Solutions

Working standard solutions of bisoprolol were prepared daily by diluting to an appropriate concentration in methanol.

Validation of the RP-HPLC Method

The method was validated by the determination of the following parameters: linearity, range, precision, accuracy, robustness, limit of detection (LOD), and limit of quantitation (LOQ), following the ICH guidelines.[16]

Linearity and Range

Linearity was determined by constructing three independent calibration curves. The stock solution of bisoprolol standard was prepared in methanol

and used for further dilutions obtaining the following seven concentrations in the range: 0.5, 1.5, 3.0, 5.0, 10.0, 15.0, and 20.0 $\mu g\,mL^{-1}$ used for the construction of the calibration curves. To verify the reproducibility of the detector response at each concentration level all the 60 μL injections were made in triplicate.

The peak areas of the RP-HPLC chromatograms were plotted, respectively, against each one of the seven concentrations of bisoprolol to obtain the calibration curve, as well as for the regression analysis calculating the calibration equation and the correlation coefficients.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was performed over three concentration levels of the bisoprolol standard covering the specified range, and carrying out 15 determinations, each one in five replicates, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on five different days (inter-day).

Accuracy

To confirm the accuracy of the proposed method, a total of 15 determinations were performed with a minimum of three concentration levels covering the specified range.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters. The robustness of the method was assessed by altering the following experimental conditions, such as by changing the flow rate from 0.6 to 1.2 $mL\,min^{-1}$, the mobile phase composition with methanol : phosphate buffer pH 3.5 (50 : 50; 45 : 55; 55 : 45), the pH of the mobile phase to 2.5 and 4.5, the wavelength in the range of 200–280 nm, and the temperature of the column between 25°C and 35°C.

LOQ and LOD

LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified, and the LOQ was defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

In vitro Dissolution Studies

The dissolution rate studies of bisoprolol from tablets were performed on a paddle-stirrer type of apparatus by a semi-automated system. Drug release tests were carried out according to conventional dissolution procedures recommended for single-entity products[4,6] in 900 mL of different media of HCl 0.1 and 0.01 M (50 and 75 rpm), phosphate buffer pH 4.5 and 6.8 (50 rpm), and distilled water (50 and 75 rpm), for 45 min. The temperature of the cell was maintained at $37 \pm 0.5^{\circ}C$ by using a thermostatic bath. At each sample time interval, an exact volume of the sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium to maintain a dissolution sink condition. At predetermined time intervals (0, 5, 15, 25, 30, and 45 min) for the development of the methodology and (0, 1, 3, 5, 7, 10, 15, 25, and 45 min) for the dissolution studies, the concentrations of bisoprolol in the dissolution medium were determined by the proposed RP-HPLC method. The cumulative percentage of drug released was plotted against time in order to obtain the release profile and to calculate the in vitro dissolution data ($n = 12$) by the linear regression equation. The dissolution data were subjected to the pairwise approach, determining a "difference factor, f_1" and "similarity factor, f_2", using the mean percentage released values.[4]

RESULTS AND DISCUSSION

The calibration curves for bisoprolol fumarate were constructed by plotting the area of the peaks vs. concentration; this was found to be linear in the range of $0.5-20.0\ \mu g\ mL^{-1}$. A linear regression by the least squares method was then applied. The calculated value for the determination coefficient ($r^2 = 0.9999$) indicated significant linearity of the calibration curve of the method.

Accuracy and precision of the proposed method were assessed by performing five replicate analyses of the standard solutions. Three different concentrations, diluted in the mobile phase, were prepared in the linear range of the calibration curve and analyzed to determine intra-day and inter-day variability and accuracy. The within and between-day precision were calculated as the RSD%. The results and the mean values were shown in Table 1 demonstrating good precision and accuracy.

The robustness of the method was determined by analyzing the same samples under a variety of conditions including the flow rate, composition, and pH of the mobile phase, the wavelength, and the column temperature. The retention time was affected by the changes in the mobile phase composition. No one significant change in the chromatographic pattern was observed when the other mentioned modifications were introduced in

Table 1. Intra-day and inter-day accuracy and precision data of RP-HPLC for bisoprolol

Theoretical concentration ($\mu g\,mL^{-1}$)	Intra-day[a]		Inter-day[a]	
	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
3	97.2	0.39	97.1	1.80
5	97.7	0.13	97.5	0.72
15	98.4	0.19	98.1	0.67

[a]Mean of five determinations for each concentration.

experimental conditions, thus showing the method to be robust. The optimized conditions were 1.0 mL min^{-1} for the flow rate, pH = 3.5 for the mobile phase and composition methanol : phosphate buffer 3.5 (55 : 45), and the wavelength 225 nm.

The LOD and LOQ limit of quantitation were obtained using the slope and standard deviation of the intercept from three curves and determined by the linear regression line. The LOD and LOQ obtained were 0.05 and 0.12 $\mu g\,mL^{-1}$, respectively. These values were also used in an experimental assay confirming the calculation.

A typical chromatogram obtained from the analysis of a sample collected from the dissolution medium by the proposed RP-HPLC method, with the resolution of the peaks corresponding probably to the excipients and bisoprolol, is shown in Figure 1.

The in vitro studies were performed using eight different dissolution conditions. The drug release profile curves were obtained plotting the time against the drug released percentage. As shown in Figure 2, the best dissolution rate profile was achieved with the medium of 0.1 M HCl with a paddle rotating at 50 rpm, that was chosen to carry out the dissolution test of the batches of bisoprolol containing 5 and 10 mg, as previously analyzed, giving dosage values of 100.62% and 101.50%, respectively. The concentrations of bisoprolol in the dissolution medium was evaluated by the proposed RP-HPLC method and the coefficients of variation ranged from 3.67 to 19.02 ($n = 12$).

To allow the use of mean data, it was observed that the coefficient of variation calculated was not more than 20% at the earlier time points (e.g., 15 min) and not more than 10% at other time points. The f_1 calculated using the mean dissolution profiles showed values lower than 7.22 and the f_2 values higher than 72.28, so that the release profiles from both formulations can be considered similar (Table 2).

The dissolution rate profiles obtained for the two tablets using the chosen conditions were shown in Figure 3.

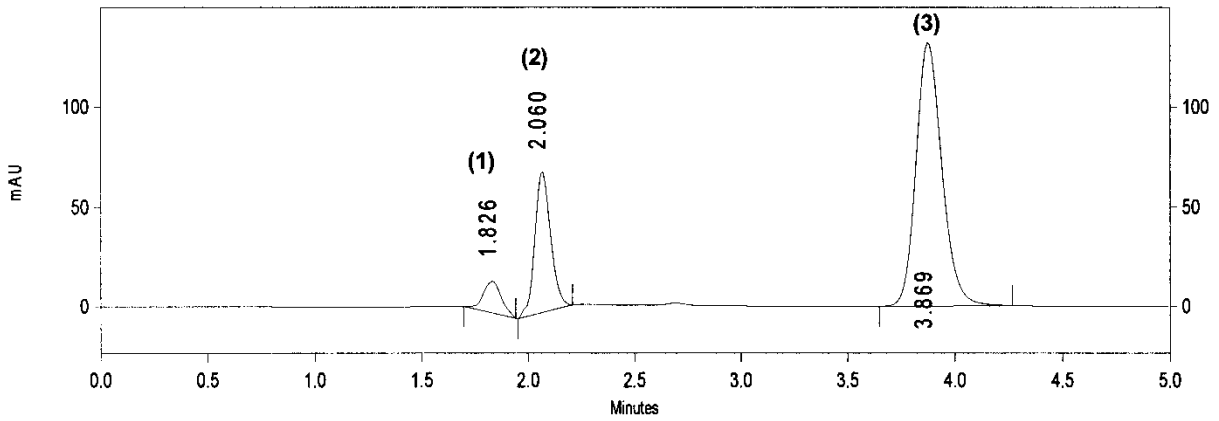

Figure 1. RP-HPLC chromatogram of bisoprolol collected from the dissolution medium after the dissolution time of 45 min of the tablet containing 5 mg: peaks 1 and 2: excipient; peak 3: bisoprolol.

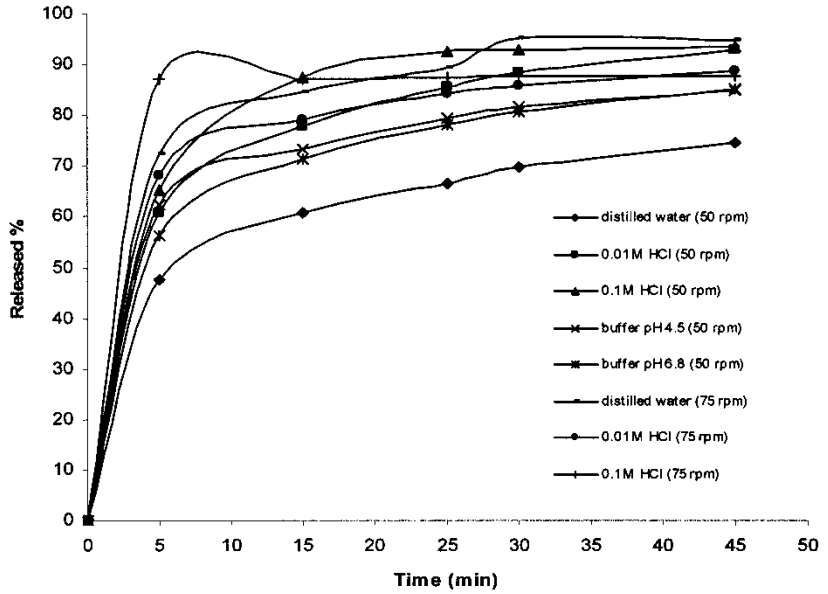

Figure 2. Comparison of dissolution profiles of bisoprolol tablets in different dissolution media and paddle rotating conditions analyzed by RP-HPLC.

CONCLUSION

The data validation shows that the RP-HPLC method is accurate, robust, and possesses excellent linearity and precision characteristics. This method has been successfully used on a routine basis and allowed the quantitation of

Table 2. Comparison of release profiles between the two dosage of bisoprolol tablets with 10 and 5 mg, by the difference and similarity factors

Time (min)	10 mg (%)	5 mg (%)	f_1	f_2
1	14.66	14.84	1.18	99.68
3	51.61	56.22	7.22	73.37
5	66.87	70.65	6.43	72.28
7	75.68	78.26	5.34	73.43
10	83.88	84.28	3.94	75.58
15	89.51	87.19	3.63	76.20
25	92.32	91.17	—	—
45	93.70	92.90	—	—

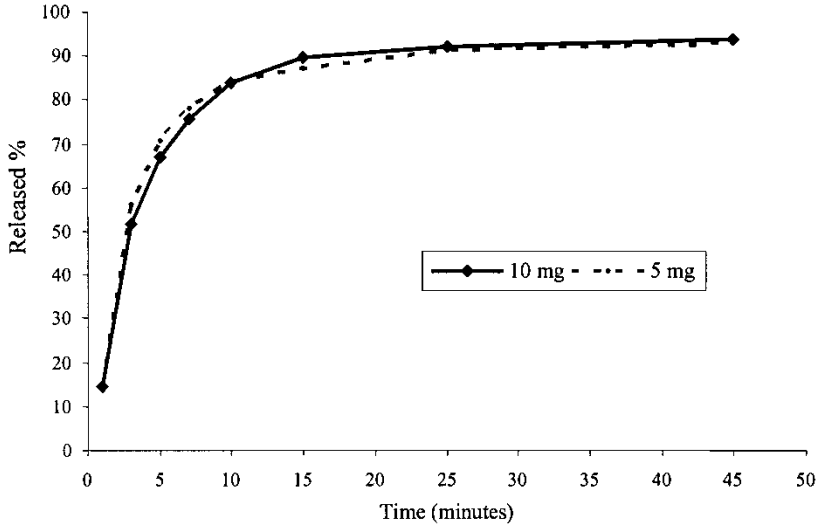

Figure 3. Comparison of dissolution profiles using difference and similarity factors (f_1; f_2) for the two tablet dosage forms of bisoprolol fumarate (5 and 10 mg) in 900 mL of 0.1 M HCl ($n = 12$).

bisoprolol fumarate from dissolution studies of pharmaceutical formulations. On the basis of f_1 difference factor and f_2 similarity factor, it is demonstrated that the similar dissolution profiles of the two tablet dosage forms that can be considered are not pharmaceutically different.

ACKNOWLEDGMENT

The authors wish to thank FAPERGS for financial support.

REFERENCES

1. Deroubaix, X.; Lins, R.L.; Lens, S.; Deblon, C.; Jeanbaptiste, B.; Poelaert, D.; Stockis, A. Comparative bioavailability of a metoprolol controlled release formulation and a bisoprolol release tablet after single oral dose administration in healthy volunteers. Int. J. Clin. Pharmacol. Therapeut. **1996**, *34* (2), 61–70.
2. Pousset, F.; Copie, X.; Lechat, P.; Jaillon, P.; Boissel, J.P.; Hetzel, M.; Fillete, F.; Remme, W.; Guize, L.; Le Heuzei, J.Y. Effects of bisoprolol on heart rate variability in heart failure. Am. J. Cardiol. **1996**, *77* (8), 612–617.
3. Carrico, C.K. Workshop report—AAPS:USP workshop on dissolution calibration and testing. Pharm. Res. **1996**, *13*, 6–9.

4. Moore, J.W.; Flanner, H.H. Mathematical comparison of curves with an emphasis on *in vitro* dissolution profiles. Pharm. Technol. **1996**, *20* (6), 64–74.
5. Shah, V.P.; Lesko, L.J.; Fan, J.; Fleischer, N.; Handerson, J.; Malinowski, H.; Makary, M.; Ouderkirk, L.; Roy, S.; Sathe, P.; Singh, G.J.P.; Tillman, L.; Tsong, Y.; Williams, R.L. FDA Guidance for industry: dissolution testing of immediate release solid oral dosage forms. Dissol. Technol. **1997**, *4*, 15–22.
6. Shah, V.P.; Tsong, Y.; Sathe, P.; Williams, R.L. Dissolution profile comparison using similarity factor, f_2. Dissol. Technol. **1999**, *6* (3), 15.
7. FDA 1997a, Center for Drug Evaluation and Research, Guidance for Industry: Dissolution testing of immediate release solid oral dosage forms, August 1997.
8. *United States Pharmacopeia (USP 27)*; United Pharmacopeial Convention Inc.: Rockville, USA, 2003; 2303–2304.
9. Banakar, U.V. *Pharmaceutical Dissolution Testing*, 1st Ed.; Marcel Dekker Inc.: New York, USA, 1992.
10. Bühring, K.U.; Garbe, A. Determination of the new β-blocker bisoprolol and of metoprolol, atenolol, and propranolol in plasma and urine by high-performance liquid chromatography. J. Chromatogr. **1986**, *382*, 215–224.
11. Poirier, J.; Perez, M.; Nd Cheymol, G. Rapid and sensitive high-performance liquid chromatographic determination of bisoprolol in plasma and urine. J. Chromatogr. **1988**, *426*, 431–437.
12. Suzuki, T.; Horikiri, Y.; Mizobe, M.; Noda, K. Sensitive determination of bisoprolol enantiomers in plasma and urine by high-performance liquid chromatography using fluorescence detection, and application to preliminary study in humans. J. Chromatogr. **1993**, *619*, 267–273.
13. Agapova, N.N. High performance liquid chromatographic method for the determination of bisoprolol and potential impurities. J. Chromatogr. A. **1993**, *654*, 299–302.
14. Zhang, X.; Ouyang, J.; Baeyens, W.R.G.; Zhai, S.; Yang, Y.; Huang, G. Enantiomeric separation of β-blockers by HPLC using (*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid as chiral stationary phase. J. Pharm. Biomed. Anal. **2003**, *31*, 1047–1057.
15. Caudron, E.; Laurent, S.; Billaud, E.M.; Prognon, P. Simultaneous determination of the acid/base antihypertensive drugs celiprolol, bisoprolol and irbesartan in human plasma by liquid chromatography. J. Chromatogr. B. **2004**, *801*, 339–345.
16. 1–8 November 1996, International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use, Validation of Analytical Procedures: Methodology (ICH-Q2B).

Received September 21, 2004
Accepted October 20, 2004
Manuscript 6499