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Development and validation of liquid chromatography methods for the quantitation of propranolol, metoprolol, atenolol and bisoprolol: application in solution stability studies

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Abstract

Four analytical methods have been developed and validated for the quantitation of β-blockers (propranolol, metoprolol, atenolol and bisoprolol) using high-performance liquid chromatography (HPLC) with UV detection. Excellent proportionality, precision and accuracy were achieved by the assays. The use of only one kind of reverse-phase column and a mobile phase for all β-blockers permitted the analysis of a large number of samples in a short time. Their stability in solution was also studied. The results show that the β-blockers assayed are stable for at least 84 h at 90°C.

Keywords: Propranolol; Metoprolol; Atenolol; Bisoprolol; HPLC; Validation; Stability; Kinetic order

β-Blockers are frequently prescribed in the treatment of cardiovascular disorders in which long-term therapy is often required, and their efficacy is well established (Frishman, 1987; Opie, 1993; Yedinak, 1994). The β-blockers selected were propranolol hydrochloride, metoprolol tartrate, atenolol and bisoprolol fumarate.

It is not easy to find information about their stability (Gupta and Gupta, 1979; Alonso-Sedano et al., 1985; Henry et al., 1986; Gupta and Stewart, 1987). Therefore, the present study was undertaken first to develop and validate chromatographic conditions for the quantification of all four β-blockers selected by HPLC, and second to determine their stability in solution.

Propranolol hydrochoride and atenolol were obtained from ICI-Farma (Madrid, Spain); metoprolol tartrate from Ciba-Geigy (Barcelona, Spain) and bisoprolol fumarate from Merck-Igoda (Barcelona, Spain). All solvents and reagents were of analytical grade except acetonitrile and methanol, which were HPLC grade.

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Table 1
Chromatographic conditions of the β-blockers assayed

Drug	Mobile phase (% acetonitrile)	Injection volume (μl)	Wavelengh (nm)	Response time (min)
Propranolol	40	80	294	2.3
Metoprolol	25	40	227	2.9
Atenolol	10	20	225	2.7
Bisoprolol	30	40	225	3.4

The HPLC system consisted of a Kontron (mod. 420) (Kontron Instruments, Barcelona, Spain) equipped with an automatic sampling system, a UV-visible detector with variable wavelength (mod. 433) and a computerized integration system and data output (mod. MT-450). Liquid chromatographic analyses were performed on a 5-μm C18 Nucleosil column (125 × 4 mm i.d.) (Teknokroma, Barcelona, Spain).

Stock solutions of β-blockers were prepared at 0.25 mg ml^{-1} in phosphate buffer (pH 7.4; 0.067 M). The concentration ranges of the calibration curves were 25–0.78 μg ml^{-1}.

The mobile phase was composed of acetonitrile and phosphate buffer (pH 3.0; 0.067 M) with 0.2% (w/v) of triethylamine. Active principles were eluted isocratically at a flow rate of 0.8 ml min^{-1}.

Chromatographic conditions of the β-blockers assayed are summarized in Table 1. The quantitation limit was 0.098 μg ml^{-1} for propranolol and bisoprolol and 0.195 μg ml^{-1} for metoprolol and atenolol.

Validation of the HPLC methods was based on proportionality (linearity assay), precision (repeatability and reproducibility assay) and accuracy.

Linearity was assessed by the determination of the same concentration range as the calibration curve.

To evaluate precision and accuracy, three concentrations within the linear range (high, medium and low), were chosen. Five standard solutions of each concentration were prepared and analysed in triplicate (repeatability assay). This assay was repeated on five different days (reproducibility assay).

In the linearity assay, the coefficient of variation (C.V.) of the response factors were 4.05, 3.82, 2.24 and 2.49% for propranolol, metoprolol, atenolol and bisoprolol, respectively. The correlation coefficients were all above 0.999. Good linearity between the peak area and concentration was observed.

Maximum values (C.V.) for repeatability and reproducibility assays were respectively 2.27 and 1.16% for propranolol, 1.99 and 1.50% for metoprolol, 1.10 and 3.68% for atenolol and 1.93 and 1.18% for bisoprolol. After application of Student's t-test no significant differences ($P > 0.05$) appeared between the percentage of mean recovery and 100% in any active principle. These results proved these analytical methods had acceptable precision and accuracy in every case.

Accelerated studies of stability in solution were carried out by placing working solution aliquots in water baths at temperatures of 60, 70 and 90°C. Aliquots were withdrawn at specified times and frozen at −20°C until analysis. Each sample was analysed in triplicate (Cartwright, 1989).

Results of the percentage of unaltered concentration of each active principle at the temperatures of the study as a function of sampling times are shown in Fig. 1(a–d). These graphs indicated no apparent degradation of any active principle at any temperature for at least the 84 h of the solution stability studies. Nevertheless, we investigated if it was possible to fit experimental results of unaltered concentration versus time to hypothetical zero and first-order kinetics of degradation. Linear regression analysis was initially used in both cases.

In the case of propranolol and metoprolol correlation coefficients were lower than the discrimination coefficients to accept possible variations of

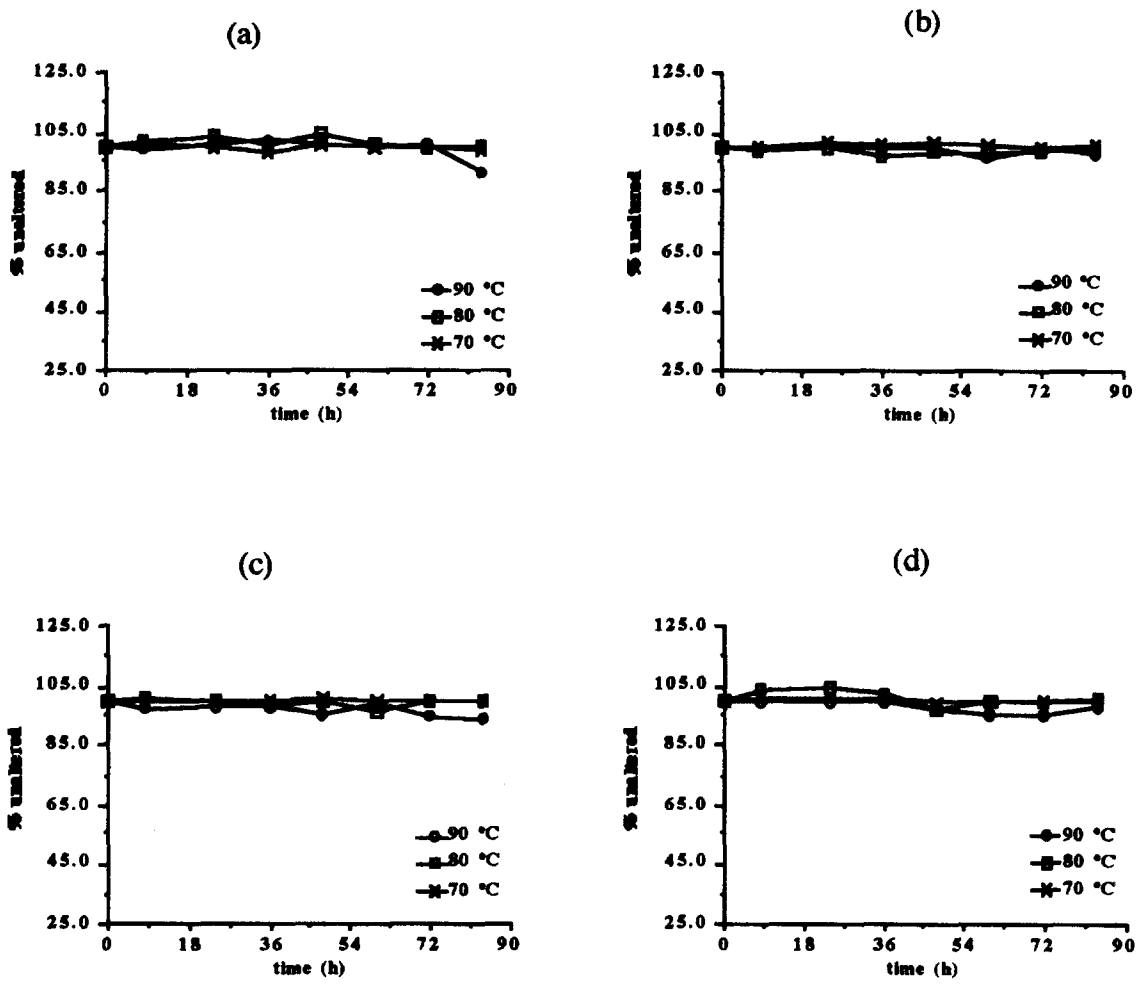

Fig. 1. Unaltered concentrations of propranolol (a), metoprolol (b), atenolol (c) and bisoprolol (d) versus time at 70, 80 and 90°C.

the concentration versus time at all three temperatures studied. Moreover, results of the analysis of variance of the linear regression demonstrated none of the kinetics of degradation.

With reference to atenolol and bisoprolol, results depended on the temperature. At the highest temperature (90°C) a relation between the percentage of unaltered concentration versus time was found. However, it was not possible to discriminate the kinetic order or to estimate the rate constant of the degradation process, since the percentages of degradation were too low (6.2% for atenolol and 2.2% for bisoprolol).

These limitations in the determination of drug stability contrast strongly with previous attempts to characterize other β-blockers in our laboratory. In particular reliable data were obtained for celiprolol and oxprenolol, since the percentage of unaltered active principle was between 20 and 30% (Modamio et al., 1994a; Modamio et al., 1994b).

Finally, a comparative study between unaltered concentrations and all three temperatures of the study was performed for each active principle. These data were subjected to the analysis of variance test. No significant differences in the stability of propranolol as a function of temperature were found, for at least 84 h. However, for metoprolol, atenolol and bisoprolol, although there was no apparent degradation, the analysis of variance showed significant differences between percentages of unaltered concentration related to the temperatures of the study. Peritz' F-test (Harper, 1984) was applied to determine which groups presented these differences. No significant differences were found for metoprolol between 80 and 90°C, and no significant differences were found for atenolol or bisoprolol between 70 and 80°C.

In conclusion, four HPLC methods for the determination and quantification of propranolol, metoprolol, atenolol and bisoprolol were developed and validated. These methods offer the advantage that a large number of samples can be processed daily, since only one kind of column and mobile phase for the determination of several active principles is used.

Accelerated stability studies in solution demonstrated first that, at 90°C and for the 84 h of the study, there was no apparent degradation in any of the active principles assayed and second that, in the remaining active principles other than propranolol, significant differences between the unaltered concentration which can be explained as a function of the temperature were found.

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