Journal of Chromatography A, 654 (1993) 299–302
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 301

Short Communication

High-performance liquid chromatographic method for the determination of bisoprolol and potential impurities

Nelly N. Agapova*

National Drug Institute, Bul. "Ianko Sakasov" 26, Sofia (Bulgaria)

Elissaveta Vasileva

Chemical Pharmaceutical Research Institute, Sofia (Bulgaria)

(First received February 1st, 1993; revised manuscript received May 5th, 1993)

ABSTRACT

An HPLC method capable of determining bisoprolol in bulk substance in the presence of its synthetic intermediates, which are potential impurities, is described. To choose the optimum conditions the behaviour of the compounds on octadecylsilica columns with acetonitrile–phosphate buffer mobile phases was studied. The influence of the acetonitrile and buffer concentrations and the pH of the mobile phase on retention was investigated. The results indicate that hydrophobic and silanophilic interactions contribute to the retention of the compounds.

INTRODUCTION

Bisoprolol, (±)-1-[4-(isopropylethoxy)methylphenoxy] - 3 - isopropylamino - 2 - propanol, is a highly selective β-adrenoceptor antagonist lacking intrinsic sympathomimetic activity with low anaesthetic potency [1–4]. A previously reported technique employing high-performance liquid chromatography (HPLC) [5,6] has been restricted to the determination of bisoprolol in biological samples. Bisoprolol is not described in any of the Pharmacopoeias and there are no official methods for the determination of chromatographic purity and assay of the compound.

This paper describes an HPLC method for the separation and determination of bisoprolol and potential impurities in drug substances.

EXPERIMENTAL

Chemicals

The molecular formulae of bisoprolol hemifumarate and potential impurities are shown in Fig. 1. They were all synthesized at the Chemical Pharmaceutical Research Institute (Sofia, Bulgaria) and had a purity of >98%, as determined by HPLC at 226 nm. Their identities were established by mass and NMR spectrometry. The organic solvents were of HPLC grade and all the reagents were of analytical-reagent grade.

* Corresponding author.

0021-9673/93/$06.00

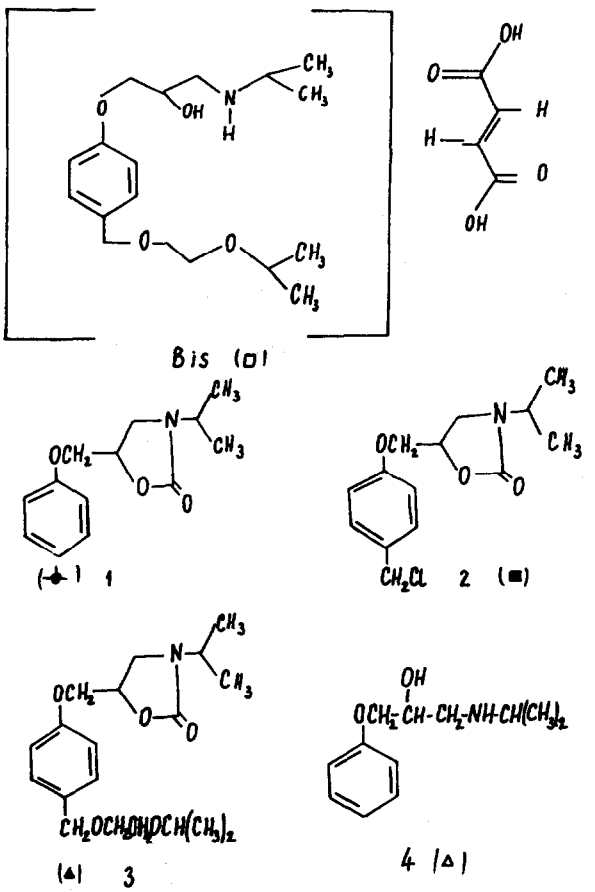

Fig. 1. Structures of bisoprolol fumarate (Bis) and conceivable impurities (1–4). The symbols are those used in Figs. 2 and 3. Amines are indicated by open symbols.

Equipment

The HPLC equipment consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 3B chromatograph linked to a Perkin-Elmer LC 75 spectrophotometric detector with autocontrol and a Waters (Milford, MA, USA) Model 740 Data Module. The UV detector was set at 226 nm. A Rheodyne (Cotati, CA, USA) Model 7120 injection valve (20-μl sample loop) was used. Prepacked LiChrosorb RP-18 columns (25 cm × 4 mm I.D.), particle size 10 μm, were obtained from Merck (Darmstadt, Germany). A Radelkis (Budapest, Hungary) Model OP-211/1 pH meter equipped with a glass electrode and a calomel reference electrode was used.

Mobile phase

The mobile phases were prepared from acetonitrile and aqueous phosphate buffer. Acetonitrile and buffer solutions were filtered, mixed in the desired volume ratios and degassed ultrasonically. The pH value stated was measured in the buffer before mixing in the final eluent. The buffer was prepared from diammonium hydrogenphosphate [$(NH_4)_2HPO_4$] by adding 5 *M* orthophosphoric acid to give the desired pH, followed by dilution to the final concentration with water. The flow-rate was maintained at 1.5 ml/min. The retention time of an unretained compound, t_0, was determined using potassium nitrate.

Preparation of solutions

For the determination of chromatographic purity, about 50 mg of bisoprolol fumarate were transferred into a 50-ml volumetric flask and dissolved in and diluted to volume with the mobile phase.

For the assay, a standard solution was prepared by transferring about 25 mg of bisoprolol fumarate reference standard into a 100 ml volumetric flask and dissolving it in and diluting to volume with the mobile phase. A sample solution was prepared in the same way but using a bisoprolol fumarate sample instead of the reference standard.

RESULTS AND DISCUSSION

The influence of the mobile phase conditions (concentrations of the organic solvent and buffer and pH) on retention was studied.

Effect of acetonitrile content in the mobile phase

Fig. 2 illustrates the effect of the acetonitrile concentration in the mobile phase on the capacity factors (k') of the components investigated. It is clear that 50% acetonitrile is the optimum concentration. The elution order is advantageous for the determination of minor components in the presence of a large excess of bisoprolol. A comparison of the retention behaviours indicates that the capacity factors of compounds containing oxazolidin in the molecule (**1**, **2** and **3**) depend more strongly than those of components

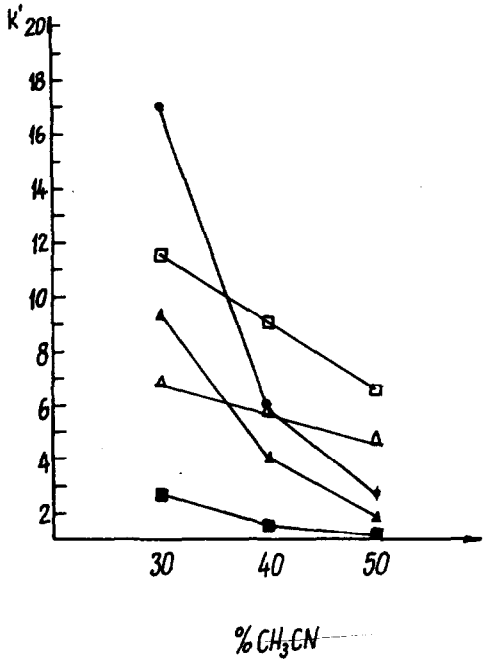

Fig. 2. Effect of acetonitrile content in the mobile phase on k'. Column, LiChrosorb RP-18, 10 μm; mobile phase, mixtures of acetonitrile and 0.050 M ammonium phosphate buffer (pH 7.0); flow-rate 1.5 ml/min. Symbols as in Fig. 1.

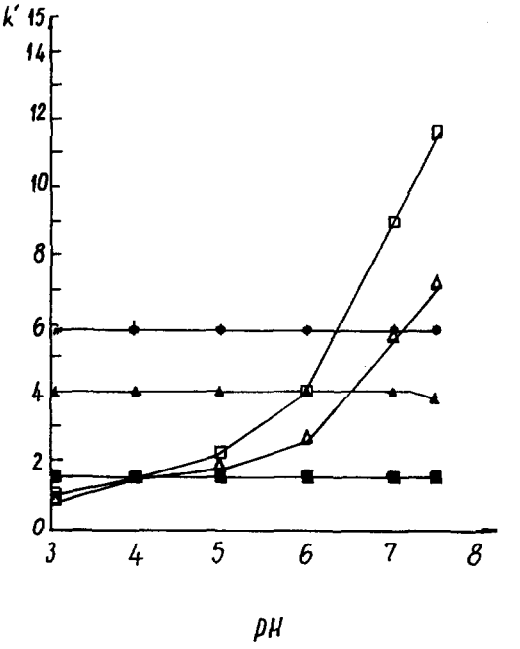

Fig. 3. pH dependence of k'. Mobile phase, acetonitrile–0.050 M ammonium phosphate buffer (4:6). Symbols as in Fig. 1.

containing a secondary amino group (bisoprolol and **4**) on the acetonitrile content of the mobile phase.

The difference in selectivity between acetonitrile and tetrahydrofuran (THF) was investigated using mixtures with different proportions of the two solvents in the mobile phase. Bisoprolol showed a decrease in retention time and tailing, but no effect on selectivity was observed and the elution order of all the compounds was maintained.

Effect of pH of the mobile phase

Fig. 3 shows the dependence of k' of the compounds investigated when using 40% acetonitrile as the mobile phase and 0.05 M ammonium phosphate buffer solution. The effect of pH on k' differs for the different compounds. A strong dependence of k' on pH was found for bisoprolol and **4**, which have secondary amino groups. At lower pH the proportion of protonated species increased and the retention of bisoprolol and **4** decreased. At pH 7.0 the retention of bisoprolol and **4** increased. This is due mostly to ionic interactions between ionized silanol groups and protonated bases. Compounds **1–3** do not have such ionizable moieties like bisoprolol and **4** in the pH range studied and pH had no effect on their retention. The pH dependence of k' was used for the separation of bisoprolol and **1–4**. It can be seen that a changed elution order of bisoprolol and **3** was obtained when the pH was increased from 3 to 7. pH 7.0 was chosen because one of the essential considerations is the order of elution of the separated peaks.

Effect of buffer concentration in the mobile phase

Capacity factors were determined for bisoprolol and **1–4** using mobile phases consisting of 40% of acetonitrile and 60% of an aqueous solution of diammonium hydrogenphosphate of concentrations 0.025, 0.050, 0.075 and 0.100 M.

The pH of the buffer was maintained at 7.0. An increased concentration of NH_4^+ cations led to decreased retention owing to ion exchange of protonated bases, whereas the retention of the other compounds was unaffected.

Based on the reported results, an HPLC method is recommended, that gives complete separation of bisoprolol from its related compounds. The chromatographic conditions are summarized in Fig. 4, which shows a typical chromatogram for a mixture of all five compounds.

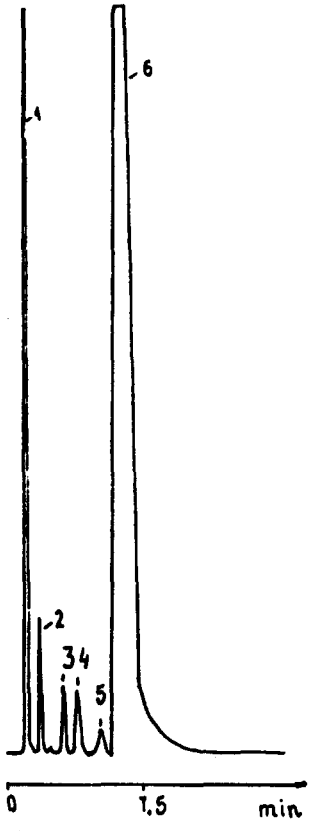

Fig. 4. Separation of 20 μg of bisoprolol fumarate and 40 ng each of the other solutes by use of the recommended method. Mobile phase, acetonitrile–0.050 *M* ammonium phosphate buffer (pH 7.0) (1:1); flow-rate, 1.5 ml/min; column, LiChrosorb RP-18, 10 μm (250 × 4.6 mm I.D.); UV detection at 226 nm (0.16 a.u.f.s.). Peaks: 1 = fumaric acid; 2 = **2**; 3 = **1**; 4 = **3**; 5 = **4**; 6 = bisoprolol.

To test the linearity of the assay procedure, a series of five standard solutions of known concentration with volumes from 1 to 10 μl injected were analysed. The regression coefficient of the linearity test was 0.9996. The precision of the system was determined by making five replicate injections of a bisoprolol standard solution. The relative standard deviation (R.S.D.) of the peak-area measurements was 0.3%. The precision of the method was determined by analysing the same sample of bisoprolol on six different days, with two replicate determinations on each day. The R.S.D. was 0.7%. To test the column-to-column variability, the results were checked on a similar, second column from the same manufacturer.

Detection limits, expressed as the amount of substance injected corresponding to a peak height equal to three times the noise, were in the range 5–20 ng for all solutes.

REFERENCES

1 A.S. Manalan, H.R. Besch and A.M. Watanale, *Circ. Res.*, 49 (1981) 326.
2 H.J. Schliep and J. Harting, *J. Cardiovasc. Pharmacol.*, 6 (1984) 1156.
3 A.E. Tatersfield, D.J. Cragg and R.J. Bacon, *Br. J. Clin. Pharmacol.*, 18 (1984) 343.
4 B. Kramer, J. Balser, K. Stubbing, G. Kramerand and W. Kuber, *J. Cardiovasc. Pharmacol.*, 8 (1986) 546.
5 K.U. Buhring and A. Garbe, *J. Chromatogr.*, 382 (1986) 215.
6 J.M. Poirier, M. Perez, G. Cheymol and P. Jailon, *J. Chromatogr.*, 426 (1988) 431.
7 W.R. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 111.