ELSEVIER

Journal of Chromatography A, 765 (1997) 221–231

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatographic procedure for the evaluation of β-blockers in pharmaceuticals using hybrid micellar mobile phases

I. Rapado-Martínez, M.C. García-Alvarez-Coque*, R.M. Villanueva-Camañas

Departamento de Química Analítica, Facultad de Química, Universitat de Valencia, 46100 Burjassot (Valencia), Spain

Received 25 July 1996; revised 15 October 1996; accepted 25 October 1996

Abstract

A reversed-phase chromatographic procedure with a micellar eluent is proposed for the determination of several β-blockers (acebutolol, atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol, and timolol) in pharmaceutical formulations (tablets, capsules and ophthalmic solutions). A study is shown on the chromatographic behaviour of these drugs with mobile phases containing sodium dodecyl sulphate (0.075–0.15 *M*) and propanol (0–15%, v/v), at different pH values (3–7). The excellent correlation between log of the octanol–water partition coefficient and log of capacity factor, for the ten drugs in mobile phases of SDS and propanol, suggested that the difference in retention among them was mainly governed by their hydrophobicity. A mobile phase of 0.15 *M* SDS–15% propanol at pH 3 permitted the elution of the β-blockers in less than 15 min. The recoveries were usually in the 96–103% range, and the variation coefficients were below 2.5%. The results were compared with those obtained with hydro-organic eluents of methanol–phosphate buffer.

Keywords: Mobile phase composition; β-Blockers

1. Introduction

β-Blockers are used in the treatment of some neurological, neuropsychiatric and cardiovascular disorders, such as migraine, tremor, anxiety, hypertension, arrhythmia, coronary insufficiency and glaucoma. The compounds are isoprenaline derivatives and contain an alkanolamine side-chain terminating in a secondary amino group. Their simultaneous determination is difficult because of the wide range of hydrophobicity. The direct spectrophotometric and fluorometric determinations of β-blockers in pharmaceutical preparations suffer from lack of selectivity, which is usually overcome by formation of colored derivatives [1,2], through the direct extraction of the compounds [3,4] or extraction of colored ion-pairs [5–7]. Derivative spectrophotometry [8] and derivative synchronous spectrofluorometry [9] have also been used. High-performance liquid-chromatography is another interesting choice for these analysis. Hydro-organic mobile phases of diverse composition have been recommended for the determination of different β-blockers [3,4,10–16].

Several reversed-phase chromatographic procedures for the determination of drugs in pharmaceutical preparations, that make use of micellar mobile phases, have recently been reported. Some examples are the evaluation of paracetamol, pseudoephedrine chlorhydrate and chlorpheniramine

*Corresponding author.

PII S0021-9673(96)00919-3

maleate (Brij 35–sodium dodecyl sulphate, SDS, mobile phase at pH 2.2) [17], the diuretics hydrochlorothiazide, chlorthalidone and spironolactone (SDS–propanol at pH 7) [18], amiloride, bendroflumethiazide, chlorthalidone, spironolactone and triamterene (SDS–pentanol at pH 7) [19], steroids (SDS–pentanol at pH 7) [20], and catecholamines (SDS–propanol at pH 3) [21]. In some procedures, the drugs were derivatized previously to their injection in the chromatograph. Thus, sulphonamides were diazotized and coupled with *N*-(1-naphthyl-)ethylenediamine (SDS–pentanol mobile phase at pH 7) [22], and amino acid isoindoles were formed with *o*-phthalaldehyde and *N*-acetyl-L-cysteine (SDS–propanol at pH 3) [23].

Some interesting characteristics of micellar mobile phases are their lower cost and toxicity, and the higher biodegradability with respect to hydro-organic mobile phases. Compounds of different polarity may be eluted with the same mobile phase, due to the diverse interactions occuring in the chromatographic column, mainly of hydrophobic and electrostatic nature. Also, analytes and matrix are often easily solubilized in the micellar medium, which produces an important reduction in the time employed in the preparation of the sample.

There are some references in the literature on the possibility of using micellar liquid chromatography (MLC) for the determination of β-blockers. Cline Love and Fett [24] developed a procedure for the determination of propranolol in urine by direct injection of the sample into a C_{18} column, where the drug was eluted with a Brij-35 mobile phase. Vadillo et al. [25] compared the performance of MLC and capillary zone electrophoresis for the evaluation of atenolol and nadolol in urine. An SDS mobile phase and a C_{18} column were used. Haupt et al. [26] studied the use of a chiral-AGP column and a micellar mobile phase containing Tween(R) 20 and heptanoic acid for the enantiomeric resolution of propranolol.

In this work, the possibility of using micellar mobile phases in the analysis of pharmaceuticals containing β-blockers (acebutolol, atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol and timolol) is studied. It is demonstrated that these determinations can be performed in less than 15 min with a unique mobile phase of SDS and propanol. The results are compared with those obtained with hydro-organic eluents of methanol–phosphate buffer.

2. Experimental

2.1. Reagents and apparatus

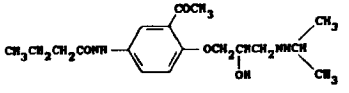
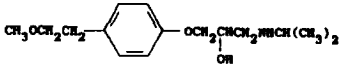
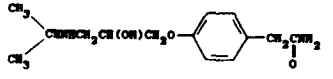
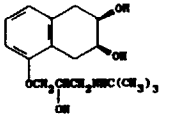
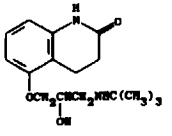
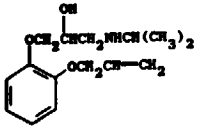
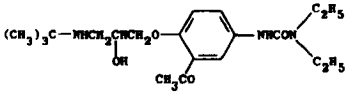
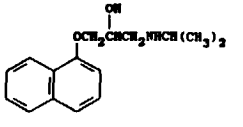
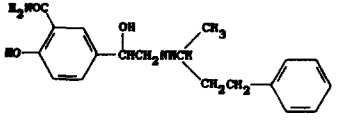
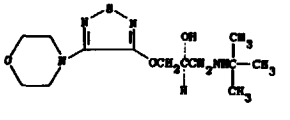
Sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany), sodium dihydrogenphosphate (for analysis, Scharlau, Barcelona, Spain), HCl (for analysis, Panreac, Barcelona, Spain), methanol (HPLC grade, Scharlau), *n*-propanol, *n*-butanol and *n*-pentanol (for analysis, Scharlau) were used. Acebutolol chlorhydrate, atenolol, carteolol chlorhydrate, celiprolol chlorhydrate, labetalol chlorhydrate, metoprolol tartrate, nadolol, oxprenolol chlorhydrate, propranolol chlorhydrate and timolol maleate were kindly donated by the manufacturers (see Table 1). Stock solutions containing 100 μg/ml of these drugs were prepared in a 0.05 *M* SDS medium.

The micellar mobile phase recommended in this work for the determination of the β-blockers contained 0.15 *M* SDS, 15% propanol and 0.01 *M* Na_2HPO_4 . Before the addition of propanol, the pH was adjusted to 3 with HCl. The drugs were also eluted with a hydro-organic mobile phase of methanol–0.05 *M* NaH_2PO_4 at pH 3 (40:60). However, atenolol required a mobile phase with a lesser eluent strength: methanol–0.01 *M* NaH_2PO_4 at pH 4.3 (22:78). β-Blocker solutions and mobile phases were prepared in nanopure water (Barnstead, Sybron, Boston, MA, USA).

The mobile phases were filtered through Nylon membranes of 0.45 μm and 47 mm diameter (MSI, Westboro, MA, USA). β-Blocker solutions were also filtered before their injection into the chromatographic column through Teflon membranes of 0.45 μm and 13 mm diameter (MSI), which should be conditioned to avoid adsorption of the drugs. The use of Nylon (MSI) and Durapore (Millipore, Bedford, MA, USA) membranes of 0.45 μm and 13 mm diameter was also considered. The conditioning process of the filters consisted in passing through at least 3 ml of the β-blocker solution. This volume was larger (minimum 5 ml) for propranolol and labetalol.

The chromatograph was from Hewlett-Packard

Table 1
Structures and protonation constants of some β-blockers

Compound (manufacturer)		log K[a]	Compound (manufacturer)		log K[a]
Acebutolol (Italfarmaco, Alcobendas, Madrid)		9.2	Metoprolol (Ciba-Geigy, Barcelona)		9.7
Atenolol (Zeneca Farma, Madrid)		9.6	Nadolol (Squibb, Esplugues de Llobregat, Barcelona)		9.4
Carteolol (Miquel-Otsuka, Barcelona)			Oxprenolol (Ciba-Geigy)		9.5
Celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid)			Propranolol (ICI-Farma, Madrid)		9.5
Labetalol (Glaxo, Tres Cantos, Madrid)		8.7, 7.4	Timolol (Merck Sharp & Dohme, Madrid)		9.2

[a] Ref. [27].

(HP 1050) and was provided with an isocratic pump, autosampler and integrator (HP 3396A) (Palo Alto, CA, USA). A Spherisorb ODS-2 analytical column (5 μm, 120×4.6 mm I.D.) and a guard column of similar characteristics (35×4.6 mm I.D.) from Scharlau were used. The flow-rate was 1 ml/min, the injection volume, 20 μl, and the detection wavelength, 225 nm. The dead volume was determined by the measurement of the first deviation of the baseline following the injection of the sample [28]. Data acquisition was made with the Peak-96 software from Hewlett-Packard (Avondale, PA, USA). Data were treated with MICHROM, a package of programs developed in our laboratory [29].

2.2. Sample preparation

The pharmaceuticals analyzed were tablets, capsules and an ophthalmic solution. Ten tablets were weighed, powdered and homogenized, a portion was taken, weighed and dissolved in 0.05 *M* SDS using an ultrasonic bath. Water was used for dilution. A similar procedure was followed with the contents of the capsules, whose weight was determined by the difference between the weight of the filled and empty capsules. The capsules were carefully cleaned in order to obtain an accurate weight of the capsule contents. On the other hand, an aliquot of the ophthalmic solution was taken, mixed with 0.05 *M* SDS solution and diluted with water. The solutions of the pharmaceuticals were injected into the chromatograph without any other treatment than filtration. The filtration was performed directly on the autosampler vials.

When methanol–water mobile phases were employed, the procedure followed for the preparation of the samples was similar. In this case, the pharmaceuticals were dissolved in the solution used as mobile phase.

3. Results and discussion

3.1. Study of the retention behaviour of β-blockers in micellar mobile phases

The retention in a C_{18} column of β-blockers eluted with pure micellar eluents (without modifier) was excessive. Also, the efficiency was extremely low and the chromatographic peaks were very asymmetrical. An increase in the concentration of SDS in the mobile phase from 0.075 to 0.15 *M* scarcely modified the efficiency and peak asymmetry, but a 60% decrease in the retention was achieved, except for timolol, with a 40% reduction. It should be noted that this drug has a chemical structure differentiated from the other β-blockers (see Table 1). The capacity factors ranged between the values obtained for atenolol, $k'=34$ in 0.075 *M* SDS and $k'=13.9$ in 0.15 *M* SDS, and for propranolol, $k'=280$ in 0.075 *M* SDS and $k'=115$ in 0.15 *M* SDS.

The addition of an alcohol lowered the retention times (Table 2), with an increasing eluent strength in the order pentanol>butanol>propanol. However, it was observed that the addition of 10% propanol largely improved the efficiency and symmetry of the chromatographic peaks, whereas the presence of 5% butanol or 2% pentanol did not produce a significant improvement, and the shape of the peaks was even deteriorated for some β-blockers with respect to the pure micellar eluents. This was the case of metoprolol and oxprenolol with butanol, and of propranolol with pentanol. Also, the eluent strength of butanol and pentanol for these drugs was lower than for the other β-blockers. We considered that the use of propanol as modifier was the better choice for the chromatographic separation of the β-blockers with SDS micellar mobile phases, because of the improved efficiencies. Dorsey et al. [31] were the first to describe the use of propanol to increase the efficiency in MLC.

Two peaks were obtained for nadolol with mobile phases without alcohol or containing a low propanol concentration (below 5%). These peaks corresponded to the two racemates: +−/−+ (*RS/SR*) and ++/−− (*RR/SS*). Commercial nadolol is a mixture of approximately equal proportions of the two racemates.

Table 1 includes the protonation constants of the β-blockers in water. The presence of micelles probably increased these constants in the eluents, owing to the stabilization of the protonated positively charged species of the drug by association to the anionic micelles, this species will thus predominate in the working pH range of the C_{18} column (3–7). It was

Table 2
Chromatographic parameters of the β-blockers eluted with mobile phases containing 0.1 *M* SDS[a]

Compound		Without alcohol	10% Propanol	5% Butanol	2% Pentanol
Acebutolol	k'	47	10.2	10.1	7.4
	N	100	1160	300	310
	B/A	5.0	1.5	2.9	2.4
Atenolol	k'	23	4.4	4.5	3.6
	N	960	1740	470	820
	B/A	2.2	1.3	2.5	1.5
Carteolol	k'	39	6.9	6.3	5.0
	N	430	1550	530	490
	B/A	2.6	1.3	2.3	1.9
Celiprolol	k'	96	13.9	13.0	9.3
	N	70	1070	280	240
	B/A	5.0	1.5	3.0	2.8
Labetalol	k'	110	24	17.2	15.2
	N	150	730	100	130
	B/A	1.7	1.8	2.5	2.6
Metoprolol	k'	100	16.4	22	12.4
	N	180	1220	40	120
	B/A	3.3	1.7	4.4	3.5
Nadolol	k'	50 55	7.8	7.1	6.0
	N	–	1630	1120	710
	B/A	–	1.1	1.5	1.6
Oxprenolol	k'	178	25	32	20
	N	310	1440	110	130
	B/A	2.2	1.7	2.6	3.1
Propranolol	k'	196	29	28	31
	N	190	1520	130	90
	B/A	1.6	1.3	3.0	3.1
Timolol	k'	134	15.9	13.7	11.9
	N	280	1940	310	500
	B/A	3.5	1.4	2.7	2.0

[a] N (efficiency) was calculated according to the equation of Foley and Dorsey for skewed peaks [30]; B and A are the distance between the centre and the tailing and leading edge of the chromatographic peak, respectively, measured at 10% of peak height.

checked indeed that the pH of the mobile phase scarcely changed the elution times of the β-blockers. However, an interesting effect was observed: at a lower pH the efficiency of the peaks increased, and their asymmetry decreased. Thus, when atenolol was eluted in a 0.125 *M* SDS–7.5% propanol mobile phase, $k'=4.7$ ($N=1470$, $B/A=1.3$) at pH 7, $k'=4.4$ ($N=2070$, $B/A=1.3$) at pH 5, and $k'=4.5$ ($N=2130$, $B/A=1.2$) at pH 3. For propranolol, $k'=27$ ($N=900$, $B/A=1.7$) at pH 7, $k'=25$ ($N=980$, $B/A=1.5$) at pH 5, and $k'=25$ ($N=1720$, $B/A=1.2$) at pH 3.

Kiel et al. [32], and Villanueva Camañas et al. [21] also observed an improvement in efficiency and a diminution in the asymmetry of the peaks at decreasing pH when some amines were eluted with hydro-organic mobile phases of acetonitrile–water, and micellar mobile phases of SDS–propanol, respectively. In both cases, alkylated silica columns were used. This effect was explained by the protonation of the free silanol groups on the stationary phase. Owing to the improvement in peak shape, we decided to elute further the β-blockers with mobile phases buffered at pH 3.

Two series of experiments were also performed,

where the concentration of surfactant and propanol in the mobile phase was varied. The usual behaviour was observed: the retention decreased at increasing SDS and alcohol concentration. Table 3 gives the retention parameters for four mobile phases of SDS-propanol. The surfactant showed a high eluent strength, as occured with some catecholamines eluted with SDS–propanol mobile phases [21]. This study indicated that the β-blockers required relatively large concentrations of both SDS and propanol to attain sufficiently low capacity factors.

3.2. Correlation between the retention and hydrophobicity of the β-blockers

The $1/k'$ vs. SDS micellar concentration plots, obtained for different amounts of propanol, always gave straight-lines, indicating that the retention behaviour was described by the equation [33]:

$$k' = \frac{K_{SW}}{1 + K_{AM}[M]} \quad (1)$$

where $K_{SW} = \phi P_{SW}$ is the product of the phase ratio by the partition coefficient between stationary phase and water, and K_{AM} is the solute-micelle association constant. The intercept of the line was statistically zero for almost all the β-blockers, and for different amounts of propanol in the mobile phase, but atenolol, carteolol and the less retained racemate of nadolol gave slightly positive intercepts for some mobile phases. This indicated that the β-blockers had a large affinity for the C_{18} stationary phase. The large value of K_{SW} made the calculation of K_{AM} difficult, however, the values of the slopes of the straight lines (K_{AM}/K_{SW}) suggested that the compounds were also strongly associated to the micelles. When these slopes were plotted against the concentration of modifier, straight-lines were obtained for all the drugs (Fig. 1), even for eluents containing a relatively large amount of propanol (15%). The elution order of the β-blockers in a mobile phase of 0.075 *M* SDS–5% propanol was: atenolol, carteolol, nadolol, acebutolol, celiprolol, metoprolol, timolol, labetalol, oxprenolol, propranolol. This order was usually the same for different mobile phase compositions, with the only exception of timolol.

Fig. 2 represents the logarithm of the octanol–water partition coefficient, log *P*, of the ten β-blockers against the log of the capacity factor, for two SDS micellar mobile phases (with and without propanol) and a methanol–phosphate buffer mobile phase. The log *P* values were taken from the literature [27]. The correlation between log *P* and log k' is similar in the methanol–water and micellar mobile phase without propanol. Vilá et al. [34] also obtained linear correlations between log *P* (corrected at several pH values), and log k' for several β-blockers, eluted with methanol–water mobile phases.

The addition of propanol to the micellar mobile

Table 3
Chromatographic parameters of the β-blockers eluted with SDS–propanol and methanol–water mobile phases

Compound	0.1 *M* SDS						0.15 *M* SDS						Methanol–NaH_2PO_4[a]		
	5% Propanol			15% Propanol			5% Propanol			15% Propanol					
	k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A
Acebutolol	15.6	940	1.9	7.8	1530	1.5	10.5	720	1.9	5.3	1380	1.4	–	–	–
Atenolol	7.3	1640	1.3	3.1	1970	1.3	5.0	1360	1.3	2.3	1620	1.3	4.4	70	5.2
Carteolol	11.1	1260	1.4	5.0	1870	1.3	7.6	780	1.7	3.5	1450	1.3	1.5	980	1.8
Celiprolol	24	510	2.4	10.6	1140	1.8	16.3	420	2.6	7.2	1100	1.7	8.2	80	4.8
Labetalol	33	980	1.2	15.8	1640	1.3	21	900	1.2	10.5	1690	1.2	8.6	1460	1.7
Metoprolol	25	1140	1.8	11.2	1880	1.5	17.0	990	1.6	7.4	1760	1.4	5.1	330	3.1
Nadolol	12.2	670	1.8	5.4	2220	1.2	8.9	850	1.2	3.7	1760	1.2	1.8	1180	1.5
Oxprenolol	38	1320	1.7	16.3	1480	1.9	25	1150	1.6	10.7	1700	1.5	10.6	130	4.0
Propranolol	46	1190	1.2	19.1	2110	1.4	29	1120	1.2	12.3	1840	1.4	22	180	3.8
Timolol	27	1420	1.6	10.5	2330	1.3	18.0	1080	1.6	7.2	2040	1.3	4.1	330	3.2

[a] A 40:60 methanol–0.05 *M* NaH_2PO_4 mobile phase at pH 3 was used, except for atenolol, where a 22:78 methanol–0.01 *M* NaH_2PO_4 mobile phase at pH 4.3 was required.

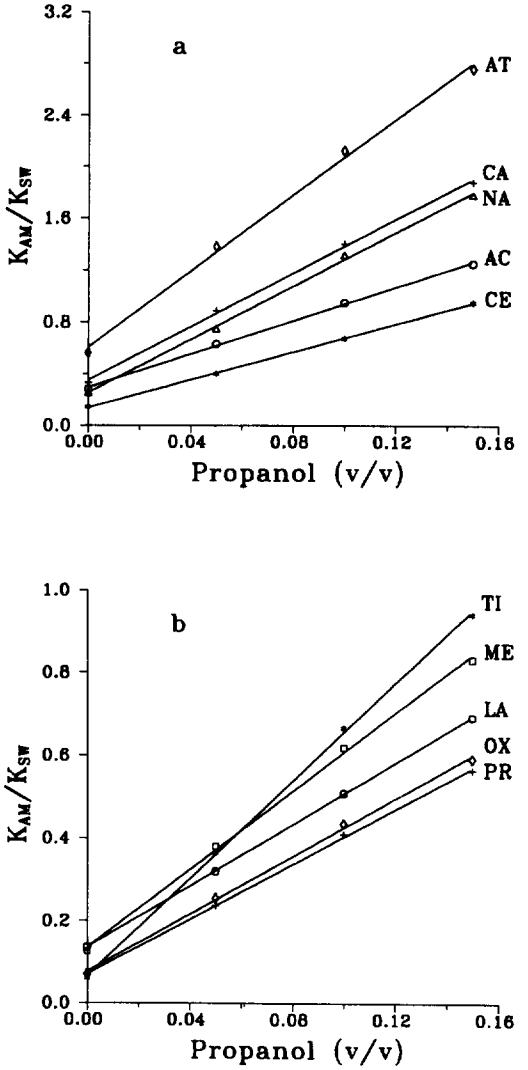


Fig. 1. K_{AM}/K_{SW} graphs vs. propanol concentration in the mobile phase for: (a) the less retained β-blockers: atenolol (AT), carteolol (CA), nadolol (NA), acebutolol (AC), celiprolol (CE), and (b) the most retained β-blockers: timolol (TI), metoprolol (ME), labetalol (LA), oxprenolol (OX), propranolol (PR).

phase improved the linear regression coefficients (Fig. 2). The excellent correlation suggested that the difference in retention among the β-blockers was mainly governed by their hydrophobicity, in spite of the electrostatic interaction between the protonated secondary amine group of the drugs and the anionic surfactant adsorbed on the surface of the modified stationary phase and forming the micelle. The pres-

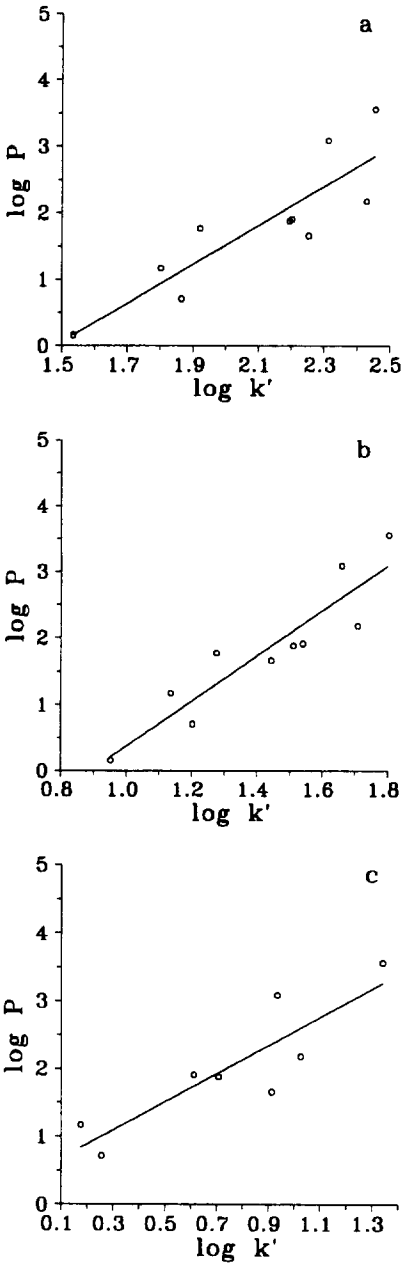


Fig. 2. Correlation between log P and log k' for the β-blockers eluted with mobile phases of: (a) 0.075 M SDS–0% propanol ($r=0.873$), (b) 0.075 M SDS–5% propanol ($r=0.919$), and (c) 40:60 methanol–0.05 M phosphate buffer at pH 3 ($r=0.873$).

ence of alcohol in the micellar mobile phase affected more the retention of the most hydrophobic β-blockers, owing to the decrease in the polarity of bulk water. Consequently, the alcohol enhanced the properties related with the hydrophobicity.

3.3. Analysis of pharmaceuticals containing β-blockers

A mobile phase with a relatively large eluent strength was selected for the determination of the β-blockers, in order to obtain sufficiently low retention times: 0.15 *M* SDS–15% propanol at pH 3 (phosphate buffer). The addition of a high amount of propanol originated well-shaped peaks (see Table 3). The sample preparation was simple and only consisted in the dissolution of the pharmaceutical in SDS medium, dilution with water and filtration. However, low and non-reproducible recoveries were obtained for some β-blockers owing to their adsorption on the filter membrane.

The losses in drug concentration depended on the type of filter and hydrophobicity of the drug. Three types of filter with Teflon, Nylon and Durapore membranes were checked. The larger losses were produced with propranolol and labetalol, the two most hydrophobic β-blockers of those studied (log *P* was 3.56, 3.09 and 2.18 for propranolol, labetalol and oxprenolol, respectively [27]). On the other hand, the adsorption on the filters increased in the order Nylon>Teflon>Durapore. Also, the number of drugs retained in the filter was larger with the Nylon membrane. The retention was decreased by increasing the volume of drug solution used to condition the filter. The use of the Nylon filter was not further considered, and the Teflon filter was finally used.

The results obtained with the micellar mobile phase were compared with others achieved with hydro-organic mobile phases. The chromatographic procedures proposed by the USP-XXII [3], British Pharmacopeia [4], and Analytical Profiles of Drug Substances [16], for the determination of β-blockers in pharmaceuticals make use of hydro-organic mobile phases containing 35–60% acetonitrile or methanol, buffered at pH 3–4 with phosphate. Taking into account these conditions, we selected a mobile phase with an intermediate eluent strength, that permitted the adequate elution of most β-blockers found in several pharmaceutical preparations commercialized in Spain. A 40:60 methanol–0.05 *M* phosphate buffer (pH 3) eluent allowed the determination of

Table 4
Calibration parameters for the β-blockers eluted in SDS–propanol and methanol–water mobile phases

Compound	Mobile phase	Intercept	Slope	*r*
Atenolol	[a]	0.004±0.019	83 700±1900	0.9992
	[b]	0.01±0.02	77 200±500	0.99993
Carteolol	[a]	0.02±0.03	166 000±4000	0.9992
	[c]	−0.01±0.03	164 000±4000	0.9993
Celiprolol	[a]	−0.02±0.03	198 000±2000	0.9998
	[c]	−0.08±0.03	188 000±2000	0.9998
Labetalol	[a]	−0.20±0.07	88 000±3000	0.998
	[c]	−0.030±0.016	84 400±800	0.99990
Metoprolol	[a]	0.00±0.04	163 000±4000	0.9991
	[c]	0.135±0.016	157 600±1500	0.99990
Nadolol	[a]	−0.007±0.010	62 100±600	0.99990
	[c]	0.012±0.008	60 900±400	0.99992
Oxprenolol	[a]	−0.23±0.05	70 100±1800	0.9991
	[c]	−0.02±0.05	60 000±2000	0.998
Propranolol	[a]	−0.5±0.2	280 000±9000	0.998
	[c]	−0.43±0.16	316 000±5000	0.9996
Timolol	[a]	−0.013±0.005	19 230±170	0.99990
	[c]	0.086±0.006	18 300±180	0.99990

[a] 0.15 *M* SDS–15% propanol at pH 3.
[b] 22:78 methanol–water at pH 4.3.
[c] 40:60 methanol–water at pH 3.

eight β-blockers (carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol and timolol). The determination of atenolol required a mobile phase with a lower eluent strength, such as 22:78 methanol–0.01 *M* phosphate buffer (pH 4.3), since with the previous eluent its chromatographic peak was overlapped with the noise at the head of the chromatogram. The chromatographic procedures could not be applied to the evaluation of acebutolol in pharmaceuticals, since they do not exist in the spanish market at the present time.

The chromatographic parameters obtained with the methanol–water mobile phases are shown in Table 3, together with the values achieved in micellar eluents. It may be observed that the elution order is the same in both cases, except for celiprolol, metoprolol and timolol, which eluted at close times with the micellar eluent. On the other hand, the peaks obtained with the SDS–propanol eluents had better characteristics than the peaks in the hydro-organic eluents, with a tenfold increase in efficiency for some drugs. The peaks in the hydro-organic mobile phase were often very asymmetrical.

Calibration curves were made with five solutions of increasing concentration for each drug, with duplicate injections. The areas of the chromatographic peaks were measured. The values of slope and intercept of the calibration straight-lines, and the regression coefficients are given in Table 4, for the chromatographic procedures with the SDS–propanol and methanol–water mobile phases. The sensitivity was similar for both procedures.

The excipients in the tablets and capsules could not be dissolved in the micellar or hydro-organic medium (except for Sumial 10), and therefore, the solutions should be filtered before the injection. It was observed that the required dilution of the sample should be performed before the filtration, to reduce the concentration loss by adsorption on the excipient.

The analyses were made by quintuplicate, and an average value of the measurements was taken to calculate the amount of drug. Fig. 3 shows the chromatograms for three pharmaceuticals containing atenolol, metoprolol and propranolol, obtained with the micellar and hydro-organic eluents. Table 5 gives the values declared by the manufacturers and the values found, together with the repeatibilities. The recoveries were in the 94–107% range for the micellar mobile phase and in the 85–107% range for the hydro-organic eluent. The low recovery obtained for some β-blockers with the hydro-organic mobile phase may be due to an incomplete extraction of the drug when the pharmaceutical was dissolved. The 107% recovery achieved with propranolol in Sumial 10 with the micellar eluent, was checked by repeated analyses of this pharmaceutical made in different days.

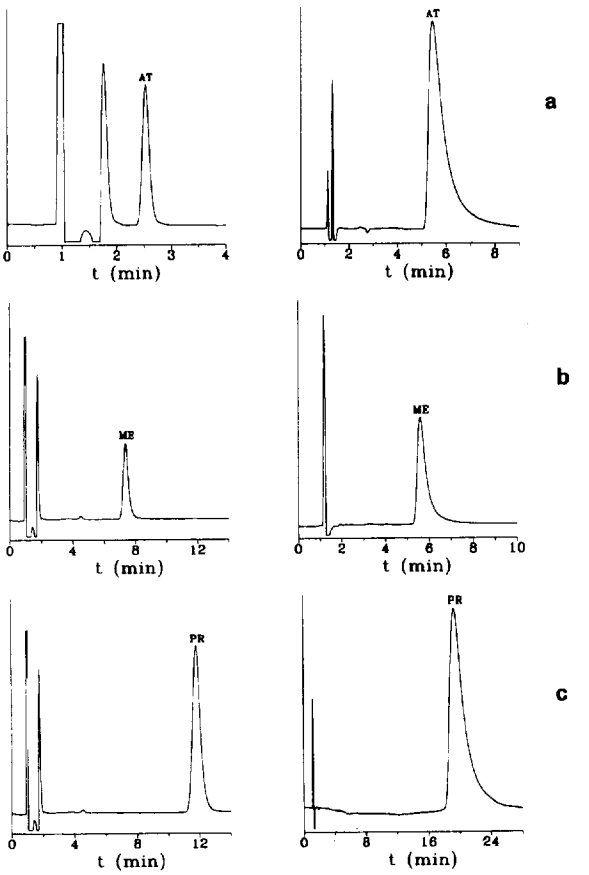


Fig. 3. Chromatograms of the pharmaceuticals: (a) Blokium 50 (atenolol), (b) Lopresor (metoprolol), (c) Sumial Retard (propranolol), obtained with an SDS–propanol mobile phase (left), and a methanol–water mobile phase (right).

The procedure proposed in this work for the determination of β-blockers in pharmaceuticals with a micellar mobile phase of SDS and propanol is rapid, with elution times of less than 15 min. The variation coefficients were below 2.5% and the values found agreed with those declared.

Table 5
Analysis of pharmaceuticals containing several β-blockers

Compound	Formulation (laboratory)	Composition (mg)	Found[a] (mg)	C.V.[a] (%) $n=5$	Found[b] (mg)	C.V.[b] (%) $n=5$
Atenolol	Blokium 50 (Prodes, Sant Just Desvern, Barcelona)	per tablet: atenolol (50), excipient	49.9	1.4	44.5	2.5
Carteolol	Arteolol (Lácer, Barcelona)	per tablet: carteolol chlorhydrate (5), lactose and other excipients	4.80	0.6	4.26	0.9
	Elebloc 1% (Cusí, El Masnou, Barcelona)	per ml: carteolol chlorhydrate (10) in aqueous medium	9.94	0.4	10.6	2.8
	Mikelan 1% (Miquel-Otsuka, Barcelona)	per ml: carteolol chlorhydrate (10), benzalkonium chlorhydrate (0.05)	9.66	0.8	10.66	0.5
Celiprolol	Cardem (Rhone-Poulenc Rorer, Alcorcón, Madrid)	per tablet: celiprolol chlorhydrate (200), excipient	197	2.5	214	0.6
Labetalol	Trandate (Glaxo, Aranda de Duero, Burgos)	per tablet: labetalol chlorhydrate (100), lactose and other excipients	99.0	0.6	97.0	1.0
Metoprolol	Lopresor (Padró, Barcelona)	per tablet: metoprolol tartrate (100), excipient	99.4	1.8	89.4	1.9
Nadolol	Solgol 40 (Uriach, Barcelona)	per tablet: nadolol (40), excipient	40.0	1.8	38.5	0.8
Oxprenolol	Trasicor (Ciba-Geigy, Barcelona)	per tablet: oxprenolol chlorhydrate (80), excipient	84.5	1.1	77.0	0.5
Propranolol	Sumial 10 (Zeneca Farma, Porriño, Pontevedra)	per tablet: propranolol chlorhydrate (10), lactose and other excipients	10.7	1.7	10.3	0.8
	Sumial Retard (Zeneca Farma, Porriño, Pontevedra)	per capsule: propranolol chlorhydrate (160), excipient	165	1.1	151	0.6
Timolol	Blocadrem (Merck Sharp and Dohme España, Alcalá de Henares, Madrid)	per tablet: timolol maleate (10), excipient	9.43	0.7	9.20	2.2
	Cusimolol 0.25% (Cusí, El Masnou, Barcelona)	per ml: timolol (maleate) (2.5) in aqueous medium	2.48	0.4	2.31	0.9

[a] SDS–propanol micellar mobile phase.
[b] Methanol–water mobile phase.

Acknowledgments

This work was supported by the DGICYT Project PB94/967.

References

[1] C.S.P. Sastry, T. Thirupathi Rao and A. Sailaja, Talanta, 38 (1991) 1057.

[2] Y.K. Agrawal, K. Raman, S. Rajput and S.K. Menon, Anal. Lett., 25 (1992) 1503.
[3] U.S. Pharmacopeia, XXII revision, U.S. Pharmacopeial Convention, Rockville, MD, 1990.
[4] British Parmacopoeia, Stationary Office, London, Vols. I and II, 1988.
[5] L. Ersoy and S. Kocaman, Archiv der Pharmazie, 324 (1991) 259.
[6] D. Radulovic, Z. Vujic and M. Vasiljevic, Spectroscopy Lett., 27 (1994) 503.
[7] Z. Vujic, D. Radulovic and L. Zivanovic, Farmaco, 50 (1995) 281.
[8] M. Veronico, G. Ragno and C. Vetuschi, Spectroscopy Lett., 28 (1995) 407.
[9] A.M. de la Peña, F. Salinas and M.S. Durán, Anal. Chim. Acta, 255 (1991) 317.
[10] A. El-Yazigi, J. Pharm. Sci., 73 (1984) 751.
[11] S.C. Olsen and H.S. Scroggins, J. Assoc. Off. Anal. Chem., 71 (1988) 761.
[12] S.I. Sa'-sa, J. Liq. Chromatogr., 11 (1988) 929.
[13] D.J. Mazzo and P.A. Snyder, J. Chromatogr., 438 (1988) 85.
[14] D. Radulovic, L.J. Zivanovic, G. Velimirovic and D. Stevanovic, Anal. Lett., 24 (1991) 1813.
[15] Z. Pawlak and B.J. Clark, J. Pharm. Biomed. Anal., 10 (1992) 329.
[16] Analytical Profile of Drug Substances, Vols. 9, 12, 13, 16, 19 and 20, Academic Press, New York, NY, 1980–90.
[17] T.A. Biemer, J. Chromatogr., 410 (1987) 206.
[18] E. Bonet Domingo, M.J. Medina Hernández, G. Ramis Ramos and M.C. García Alvarez-Coque, Analyst, 117 (1992) 843.
[19] E. Bonet Domingo, M.J. Medina Hernández and M.C. García Alvarez-Coque, J. Pharm. Biomed. Anal., 11 (1993) 711.
[20] S. Torres Cartas, M.C. García Alvarez-Coque and R.M. Villanueva Camañas, Anal. Chim. Acta, 302 (1995) 163.
[21] R.M. Villanueva Camañas, J.M. Sanchis Mallols, J.R. Torres Lapasió and G. Ramis Ramos, Analyst, 120 (1995) 1767.
[22] M.C. García Alvarez-Coque, E. Simó Alfonso, G. Ramis Ramos and J.S. Esteve Romero, J. Pharm. Biomed. Anal., 13 (1995) 237.
[23] M. Catalá Icardo, M.J. Medina Hernández and M.C. García Alvarez-Coque, J. Liq. Chromatogr., 18 (1995) 2827.
[24] L.J. Cline Love and J.J. Fett, J. Pharm. Biomed. Anal., 9 (1991) 323.
[25] J.M. Vadillo, M.E. González, I. Carretero and J.J. Laserna, Mikrochim. Acta, 118 (1995) 273.
[26] D. Haupt, C. Pettersson and D. Westerlund, Chirality, 5 (1993) 224.
[27] C.J. Drayton (Editor), Comprehensive Medicinal Chemistry, Volume 6, Pergamon, Oxford, 1990.
[28] J.R. Torres Lapasió, J.J. Baeza Baeza and M.C. García Alvarez-Coque, J. Liq. Chromatogr., 19 (1996) 1205.
[29] J.R. Torres Lapasió, Ph.D. Thesis, University of Valencia, 1996.
[30] J.P. Foley and J.G. Dorsey, Anal. Chem., 55 (1983) 730.
[31] J.G. Dorsey, M.T. DeEchegaray and J.S. Landy, Anal. Chem., 55 (1983) 924.
[32] J.S Kiel, S.L. Morgan and R.K. Abramson, J. Chromatogr., 320 (1985) 313.
[33] M.C. García Alvarez-Coque, J.R. Torres Lapasió and J.J. Baeza Baeza, Anal. Chim. Acta, 324 (1996) 163.
[34] J.I. Vilá, R. Obach, R. Prieto and J. Moreno, Chromatographia, 22 (1986) 48.