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Determination of beta-blocker drugs in pharmaceutical preparations by non-suppressed ion chromatography

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Abstract

A rapid and feasible chromatographic method for determining atenolol, metoprolol, alprenolol, oxprenolol, acebutolol and propranolol by non-suppressed ion chromatography is proposed. The mobile phase consisted of 50 mM nitric acid in an aqueous solution of 4% (v/v) acetonitrile. Detection limits are in the range 0.1–2.7 mg l^{-1}. The method was successfully applied to the determination of beta-blockers in several pharmaceutical formulations.

Keywords: Beta-blockers; Ion chromatography; Pharmaceutical preparations

1. Introduction

Beta-adrenoceptor blocking drugs are of therapeutic value in the treatment of various cardiovascular disorders, such as angina pectoris, cardiac arrhythmia and hypertension [1,2]. Sone beta-blockers are also successfully used in the treatment of migraine and glaucoma and as minor tranquillizers.

Several analytical methods have been described to determine the concentration of these compounds in plasma, urine and pharmaceutical preparations. A spectrophotometric method for atenolol [3] and spectrofluorimetric methods for atenolol [4,5], propranolol [6,7] and nadolol [8] have been reported. Gas chromatography has been used for determinations of propranolol [9], oxprenolol [10], and atenolol [11], thin layer chromatography was described for oxprenolol [12], propranolol [13] and atenolol [14], high-performance liquid chromatography (HPLC) with spectrofluorimetric detection was reported for atenolol [15,16], alprenolol [17], metropolol [17,18], propranolol [19,20] and acebutolol [21], and HPLC with spectrophotometric detection was reported for oxprenolol [22,23], acebutolol [24,25] and some other compounds [26]. Likewise, the quantitative analysis of several beta-blockers by reversed-phase ion-pair HPLC [27] has been described. However, these compounds have not been studied much by ion-exchange HPLC [28–30]. Moreover, in the USP NF current edition [31], only three beta-blockers are assayed by

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HPLC: namely, metoprolol tartrate, pindolol and timolol maleate (oxprenolol tablets are assayed by spectrophotometry). In each case, different mobile phases and chromatographic conditions are required for their quantitation, which is an important handicap in quality control.

In this paper a rapid, simple, accurate and reproducible ion chromatographic (IC) method, using spectrophotometric detection, is described for the determination of alprenolol, atenolol, acebutolol, metopropolol, oxprenolol and propranolol in pharmaceutical preparations.

2. Materials and methods

2.1. Chemicals and solutions

Atenolol (4-(2′-hydroxy-3′-isopropylamino-propoxy)phenylacetamide), metoprolol (1-isopropylamino-3-(*p*-(β-methoxyethyl)phenoxy)-2-propanol), alprenolol (1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol), oxprenolol (1-(2-(allyloxy)-phenoxy)-3-isopropylamino-2-propanol) and acebutolol (*N*-3-acetyl-4-(2-hydroxy-3(isopropylamino)-propoxy)-phenylbutanamide) as hydrochlorides (except metoprolol which is available as the tartrate), were obtained from Sigma (St. Louis, MO); propranolol (1-isopropylamino-3-(1-naphthyloxy)-2-propanol) as the hydrochloride was obtained from ICN Biochemicals (Costa Mesa, CA).

Nitric acid 65% (w/v) and acetonitrile, both of analytical grade, were obtained from Merck (Darmstadt, Germany). Milli-Q® (Millipore, Millford, MA) treated water was used throughout.

2.2. Chromatographic system

A Waters Model 501 solvent delivery system was used together with a Waters IC-Pak™ C M/D column packed with silica base coated with a polybutadiene/maleic acid copolymer (5 μm particle size and exchange capacity of 2.0 ± 0.2 μeq/ ml^{-1}). Samples were injected using a Rheodyne (Cotati, CA) injector with a 100 μl sample loop. Detection was done with a Waters 486 Tunable Absorbance Detector operating at 270 nm with an AUFS of 0.11. Peak evaluations were made with a Hewlett Packard (Palo Alto, CA) Model HP3395 Integrator. The mobile phase consisted of 50 mM nitric acid in an aqueous solution of 4% (v/v) acetonitrile, and the flow rate was 1 ml min^{-1}.

2.3. Samples

Beta-blockers were determined in eight pharmaceutical formulations: Trasicor Retard® and Trasitensin Retard® (Ciba-Geigy, S.A.), Sumial 40® (Zeneca Farma, S.A.), Lopresor® (Laboratorio Padró, S.A.), Atenolol Ratiopharm 100® (Ratiopharm España, S.A.), Atenolol Alter® (Alter, S.A.), Sectral 400® (Rhône-Poulenc Farma, S.A./ Italfármaco, S.A.) and Normopresil® (Laboratorios Semar, S.A.). The formulation of each pharmaceutical is given in the Appendix.

2.4. Procedures

2.4.1. Conditioning of solid-phase extraction (SPE) cartridges

SPE cartridges (Waters C18 Sep-Pak™ Plus short body cartridge) were used with the aim of preventing retention of neutral organics via hydrophobic interactions. The cartridges were previously conditioned with 5 ml of acetonitrile and then 5 ml of mobile phase. The conditioned cartridges must not dry out.

2.4.2. Sample treatment

Pharmaceutical samples containing beta-blockers were dissolved in aqueous 50 mM nitric acid, passed through a previously conditioned (see above) Waters C18 Sep-Pak™ Plus short body cartridge and microfiltered (0.22 μm) before injection.

2.4.3. Calibration features

The experimental conditions permit the resolution of atenolol, metoprolol, acebutolol and propranolol mixtures; the calibration of alprenolol and oxprenolol must be performed separately. For the calibration atenolol is used as internal standard (20 mg l^{-1}) for determining acebutolol, alprenolol and oxprenolol, and acebutolol (10 mg

Table 1
Calibration data

Substance	t_R[a]	k'[b]	Regression parameters	r[d]	Calibration range (mg ml^{-1})	LOD[e]	Recovery (%)
Atenolol	4.8	1.8	$y = 0.04(3) + 0.034(9)x$	0.9938	3.0–75.0	2.7	98.3
Metoprolol	7.6	3.5	$y = 0.00(2) + 0.0334(5)x$	0.9998	2.0–75.0	1.8	99.8
Alprenolol	8.4	3.9	$y = 0.02(2) + 0.0669(4)x$	0.9996	1.0–70.0	0.9	100.3
Oxprenolol	8.5	4.0	$y = 0.05(4) + 0.083(1)x$	0.9981	2.0–71.0	1.5	101.2
Acebutolol	10.3	5.1	$y = -0.005(4) + 0.1208(4)x$	0.9999	1.0–20.0	0.1	99.3
Propranolol	23.0	12.5	$y = 0.00(5) + 0.113(2)x$	0.9983	2.0–71.0	1.3	99.1

[a] Retention time (min).
[b] $k' = (t_R - t_0)/t_0$.
[c] Figures in parentheses are the standard errors associated with the last significant figures.
[d] Correlation coefficient.
[e] Detection limit (mg l^{-1}).

ml^{-1}) for determining atenolol, metoprolol and propranolol. Calibration data (linear concentration ranges, correlation coefficients and detection limits) are collected in Table 1.

Sensitivities are adequate, with detection limits in the range 0.1–2.7 mg l^{-1}. Recoveries in the range 98.3–101.2% were found for the analytes studied.

2.4.4. Method validation

For the study of precision, probes containing 10 mg l^{-1} of each beta-blocker were analyzed according to the proposed procedure. The within-day precision or repeatability (as RSD) is within 2.5–3.0%. The between day precision or reproducibility was averaged to 4.2% (five randomized determinations over 1 month using the same materials, apparatus and stock reagent solutions).

In order to assess the absence of systematic errors the proposed method was compared with another independent method [26] applied to the same set of five pharmaceutical preparations (Trasicord Retard, Sumial 40, Lopresor, Atenolol Alter, Sectral 400). The regression method was applied, considering the results obtained by the proposed method as y-values and those obtained by the independent method as x-values [32]. The resulting straight line is $y = (-9 \pm 8) + (1.08 \pm 0.03)x$. The corresponding Student t tests on slope and intercept indicate that at a 5% significance level there are no significant differences in the results obtained from either of the two methods, i.e. the proposed method is suitably validated.

3. Results and discussion

Fig. 1(a) shows the chromatographic separation of a 20 mg l^{-1} atenolol (AT)/20 mg l^{-1} metoprolol (ME)/10 mg l^{-1} acebutolol (AC)/20 mg l^{-1} propranolol (PR) mixture. Figs. 1(b) and 1(c) show chromatograms corresponding to 20 mg l^{-1} alprenolol (AL) and 10 mg l^{-1} oxprenolol (OX) respectively; in both cases, 20 mg l^{-1} atenolol was used as internal standard.

Although only four of the six beta-blockers considered can be analyzed simultaneously, this is not a disadvantage of the proposed method because in beta-blocker-based pharmaceuticals only one active ingredient appears in the formulation.

The method was applied to the determination of beta-blockers in several pharmaceutical preparations according to the above-described procedure. The results obtained are collected in Table 2 where, as can be seen, a good agreement was found with respect to the label claims for the analyzed samples, within the confidence ranges commonly accepted.

The absence of undeserable peaks due to the presence of other ingredients is noticeable; only cations actually present in the treated samples were separated and detected, the remaining components being eliminated in the clean-up step. Fig. 2 shows the chromatogram corresponding to the analysis of a pharmaceutical sample.

These facts show the possible applicability of the proposed method for pharmacological analy-

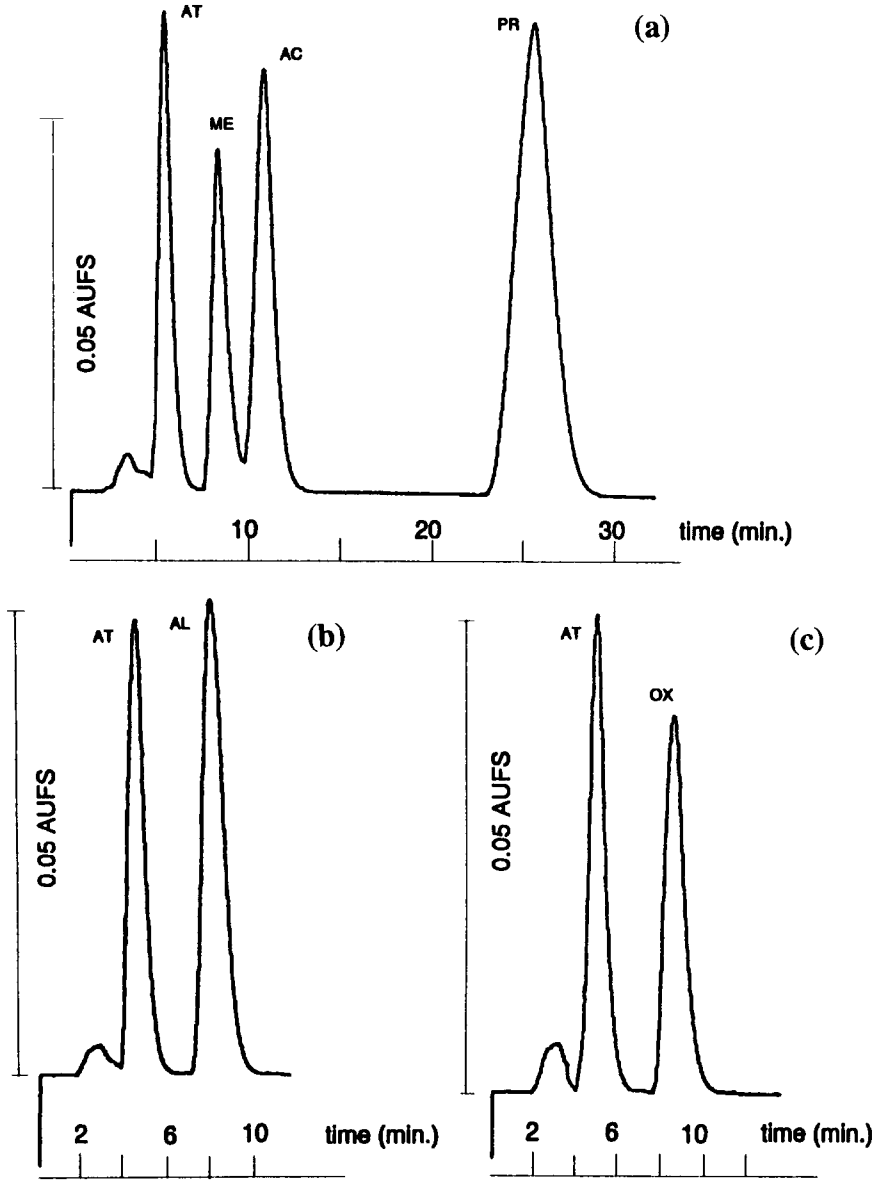

Fig. 1. Chromatograms corresponding to the analyzed compounds: (a) atenolol, metoprolol, propranolol (all 20 mg l^{-1}) and acebutolol (10 mg l^{-1}); (b) atenolol and alprenolol (both 20 mg l^{-1}); (c) atenolol (20 mg l^{-1}) and oxprenolol (10 mg l^{-1}). (Abbreviations are described in the text).

sis, without possible interference problems derived from other substances which frequently appear in the formulations.

4. Conclusions

The proposed method for the determination of beta-blocking drugs is sensitive, rapid and, unlike HPLC methods, practically does not require the use of organic solvents with their environmental and cost problems; only a small amount of acetonitrile is used in the unique mobile phase.

One important advantage regarding the instrumentation is that any conventional HPLC equipment can be used for applying the proposed

Table 2
Results for the pharmaceutical samples analyzed

Pharmaceutical	Claimed[a] (mg)	Found[b] (mg)
Trasicor Retard	160	158 ± 3
Trasitensin Retard	160	157 ± 4
Sumial 40	40	39.7 ± 0.2
Lopresor	100	94 ± 1
Atenolol Ratiopharm 100	100	106 ± 2
Atenolol Alter	100	105 ± 3
Sectral 400	400	426 ± 3
Normopresil	100	104 ± 2

[a] See Appendix for the beta-blocker present.
[b] Average of 10 determinations ± standard deviation.

procedure simply by substituting the column for an IC-Pak M/D column.

Although only four of the six beta-blockers analyzed can be simultaneously separated, this is not a problem for the proposed method because only one beta-blocker is present as the active principle in the pharmaceuticals. The proposed method does, however, permit the determination of all the beta-blockers under the same conditions (same mobile phase).

The methodology was successfully applied to several pharmaceutical formulations, and possible interference problems were eliminated.

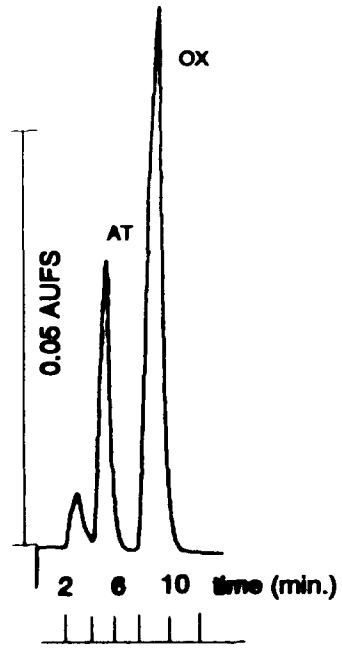

Fig. 2. Chromatogram corresponding to the analysis of Trasitensin Retard (atenolol as internal standard).

Appendix A: Composition of the analyzed samples

Trasicor Retard® (contents of one lacquered tablet)

Oxprenolol, chlorhydrate	160 mg
Lactose and other excipients	

Trasitensin Retard® (contents of one pill)

Oxprenolol, chlorhydrate	160 mg
Chlortalidone	20 mg
Saccharose	101 mg
Lactose and other excipients	

Sumial 40® (contents of one tablet)

Propranolol, chlorhydrate	40 mg
Lactose and other excipients	

Lopresor® (contents of one tablet)

Metoprolol, tartrate	100 mg
Excipient	

Atenolol Ratiopharm 100® (contents of one tablet)

Atenolol	100 mg
Excipient	

Atenolol Alter® (contents of one tablet)

Atenolol	200 mg
Starch	30 mg
Excipient	

Sectral 400® (contents of one tablet)

Acebutolol, chlorhydrate	400 mg
Excipients (lactose, wheat starch and others)	

Normopresil® (contents of one tablet)

Atenolol	100 mg
Chlortalidone	100 mg
Excipient	

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