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Optimization of mobile phase in the separation of β-blockers by HPLC

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Abstract

β-blockers are generally determined using high-performance liquid chromatography (HPLC). Previous HPLC separations of β-blockers have often required a mobile phase containing three components; acetonitrile or methanol to control the retention; buffer to control the ionic strength and pH of the mobile phase; ion-pairing reagent to provide adequate retention of β-blockers or organic amines as masking agent to reduce peak tailing. Due to the complexity of the mobile phases employed, development of these assays can be a laborious process. Additionally, alkyl sulphonates and organic amines dramatically reduces the life-time reduction of silica based C_{18} columns. The results of this study demonstrated that the addition of tested alkyl sulphonates and organic amines is not essential for an adequate separation of β-blockers. In this study, we developed a simple HPLC method for the simultaneous separation of model β-blockers, atenolol, practolol, metoprolol, oxprenolol and propranolol. Atenolol, practolol, metoprolol, oxprenolol and propranolol adequately separated with high peak symmetries using a mobile phase consisted of methanol/acetonitrile/phosphate buffer (10 mM, pH 3.0) (15:15:70, v/v/v). By altering only the fraction of methanol with respect to acetonitrile, method development becomes a more efficient separation. Furthermore, atenolol, practolol, metoprolol, oxprenolol and propranolol can be detected up to 0.25, 5, 10, 50 and 10 ng ml^{-1}. In this publication, we present the simultaneous separation of β-blockers having a wide range of polarity. It is proposed that this new mobile phase, consisting only acetonitrile, methanol and phosphate buffer can be used for the analysis of the several β-blockers presently in doping control analysis as well as others.

Keywords: β-blockers; RP-HPLC; Ion-pairing; Amine modifiers; Alkyl sulphonate modifiers

1. Introduction

β-blockers are clinically important drugs and are used in the treatment of disorders such as

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hypertension, angina pectoris and arrhythmia [1]. They are also abused in sports because of their blood pressure regulatory and tremor decreasing effects, banned by International Olympic Committee [2]. Therefore, simultaneous determination of these drugs is meaningful and lower determination limit values are preferred. β-blockers are determined by a variety of HPLC methods [3–5], particularly by RP-HPLC [6–10], and octadecylsilane (C_{18}) bonded silica is the most widely used stationary phase. Silica can easily be adjusted to the chromatographic requirements because of its specific surface area, average pore diameter and pore volume properties but causes peak tailing and low separation performance with basic solutes, e.g. tricyclic antidepressants, β-blockers [11,12]. Peak asymmetry mainly results from the presence of unbonded surface silanol groups on the stationary phase and their interaction with basic solutes.

In HPLC separation of β-blockers, alkyl sulphonates and organic amines are added to the mobile phase to reduce peak tailing [13–18]. They are supposed to serve major roles in separation as the ion-pairing reagent and as a masking agent for remaining silanols [19]. It was also found that methanol is superior as a mobile phase modifier for enhancing separation of some β-blockers even if they are enantiomers [9,13].

In this study, atenolol, practolol, metoprolol, oxprenolol and propranolol having a wide range of polarity (1000 times difference between atenolol and propranolol) are selected as model β-blockers. A preliminary isocratic separation of a mixture of β-blockers is carried out using μBondapak C_{18} column and an aqueous phosphoric acid buffer (pH 3.0)-acetonitrile mobile phase as a control. Using this separation, the chromatographic behavior of these solutes is studied by addition of alkyl sulphonates (pentane-, hexane-, heptane-, and octane-sulphonate) and organic amines (diethy-, triethyl-, tetraethylamines, tetrabuthlyamine, tetramethyammonium and N,N-dimethyloctylamine) to mobile phase. In addition, the effect of the presence of metoprolol in mobile phase on separation is also examined.

2. Materials and methods

2.1. Chemicals and reagents

Methanol and acetonitrile were HPLC-grade (Baker, Phillipsburg, NJ). Diethylamine (DEA), triethylamine (TrEA), tetramethylammonium (TMA), tetraethylammonium (TEA), tetrabuthylamonium (TBA), *N*, *N*-dimethyloctylamine (DMOA), pentane sulphonate (PSA), hexanesulphonate (HSA), heptanesulphonate (HpSA), octane sulphonate (OSA) and other chemicals were analytical grade. Practolol purchased from ICI (UK), atenolol, metoprolol, oxprenolol and propranolol were kindly donated by Turkish drug companies (Doğu Drug Company, Istanbul, Turkey; Eczacibaşi, Istanbul, Turkey; Novartis, Istanbul branch, Turkey).

2.2. Chromatography

The HPLC system consisted of a pump (Waters M 510), a U6K injection valve (Waters), a UV/visible detector (Waters M 481) and a data integrator (Waters M 750). Separation was carried out on a μBondapak C_{18} stainless-steel analytical column (particle size 10 μm, 300 × 3.9 mm i.d.) in conjunction with a pre-column containing μBondapak C_{18} (40 μm). The mobile phase was consisted of acetonitrile and/or methanol at desired concentration with or without alkyl sulphonate (2.5 mM) or organic amine (25 mM) in phosphate buffer (pH 3.0, 10 mM). Flow rate was 1 ml min^{-1} at ambient temperatures. UV/visible detector was set to the wavelength of 254 nm.

The column was equilibrated with a sufficient amount of eluent and stability of the column was tested for the unchanging solute retention via three repetitive injections of standard mixture of β-blockers (100 μg ml^{-1} of each) in methanol. Stability of the column was tested with the methanol–water (60:40, v/v) eluent. The solute used for testing was acenaphthene. The retention time of unresolved peak (t_0) was determined from the peak obtained when sodium nitrate was injected. To measure the peak symmetry ratio (PSR) the front part of width divided by the back part of width of the peak at the 1/10 of peak

height. The threshold value for peak symmetry ratio (PRS) of 80% was accepted. The capacity factor (k') was calculated from the retention time of solute (t_R) and t_0 described as $k' = (t_R - t_0)/t_0$. For an optimum separation, retention should be in the usual range of $0.5 < k' < 10$ for all β-blockers tested.

3. Results

Selected β-blockers were well resolved from the baseline and their retention ranked from polar to apolar at all concentrations of acetonitrile in phosphate buffer (10 mM, pH 3.0) (Fig. 1). Metoprolol, oxprenolol and propranolol are adequately separated ($k' \geq 0.8$) at 30% acetonitrile and their PSR values were 100.0, 66.7 and 70.0%, respectively. Atenolol and practolol were not separated by mobile phase containing acetonitrile (30%) in phosphate buffer.

Pentane-, hexane-, heptane- and octanesulphonate additions to the mobile phase consisted of acetonitrile (30%) and phosphate buffer (10 mM, pH 3.0) decreased the capacity factors of metoprolol, oxprenolol and propranolol (Fig. 2) without any improvement in their peak symmetries. Moreover, atenolol and practolol could no be separated ($k' < 0.5$) by these mobile phase combinations. Metoprolol, oxprenolol and propranolol are strongly retained by the presence of OSA because of the high ion-pairing efficiency of this long chain apolar alkyl sulphonate.

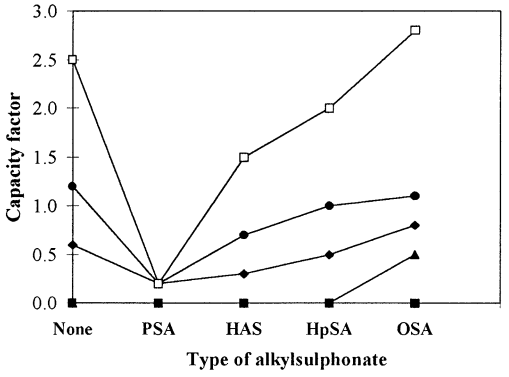

Fig. 2. Effects of alkyl sulphonate type on the separation of β-blockers after addition to the mobile phase containing acetonitrile (30%) and phosphate buffer (10 mM, pH 3.0) (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

The presence of DMOA in the mobile phase (acetonitrile-phosphate buffer, 10 mM, pH 3.0; 30:70, v/v) considerably decreased the capacity factors of all β-blockers. The same trend is obtained by TBA due to the decreased polarity of this long chain amine modifier. Capacity factors of metoprolol, oxprenolol and propranolol are slightly reduced by DEA addition and this reduction is amplified by the presence of TrEA (Fig. 3). Separation of atenolol and practolol was not pos-

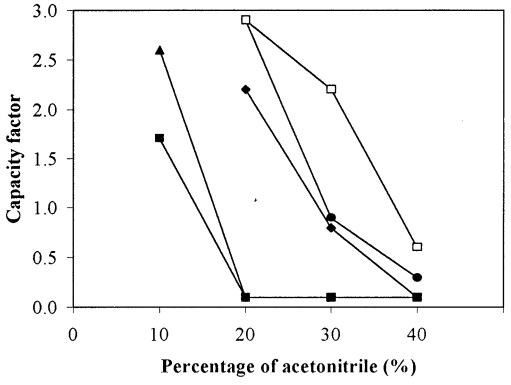

Fig. 1. Effects of acetonitrile concentration in phosphate buffer (10 mM, pH 3.0) on the retention of β-blockers (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

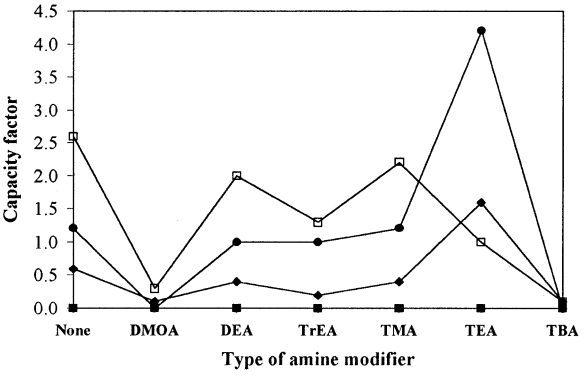

Fig. 3. Separation of β-blockers by acetonitrile (30%)-phosphate buffer (10 mM, pH 3.0) mobile phase containing amine modifier (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

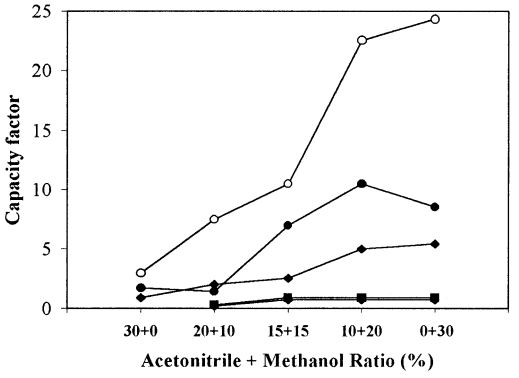

Fig. 4. Retention of β-blockers by mobile phases based on acetonitrile–methanol–buffer (10 mM, pH 3.0) on μBondapak C_{18} (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

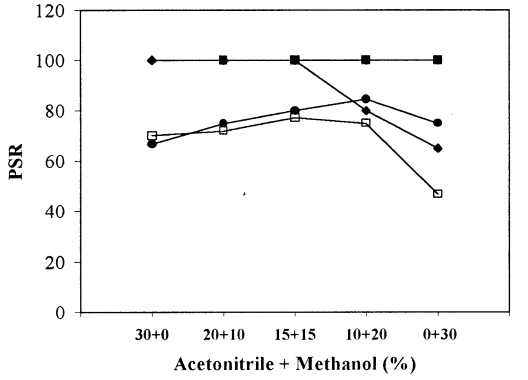

Fig. 5. Effect of methanol addition to the mobile phases based on acetonitrile–methanol–buffer (10 mM, pH 3.0) on peak symmetries of β-blockers on μBondapak C_{18} (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

sible although organic amines in the mixture of acetonitrile (30%) and phosphate buffer were present. PSR values of separated β-blockers, metoprolol, oxprenolol and propranolol were $< 80\%$ in these circumstances.

Retention of β-blockers, including atenolol and practolol, increased after addition of methanol to the mobile phase as a fraction of total organic modifier (30%) component (Fig. 4). Atenolol, practolol, metoprolol, oxprenolol and propranolol simultaneously separated by a mobile phase consisted of acetonitrile–methanol–phosphate buffer (10 mM, pH 3.0) (15:15:70, v/v/v,) in 15 min. Capacity factors of atenolol, practolol, metoprolol, oxprenolol and propranolol were 0.7, 0.9, 2.5, 6.9 and 10.0, respectively. Increased fraction of methanol, in other words decreased fraction of acetonitrile, in organic modifier component increased capacity factors for metoprolol, oxprenolol and propranolol without changing the retardation of atenolol and practolol (Fig. 4). PSR values by this mobile phase combination, for atenolol, practolol and metoprolol were 100.0% and for oxprenolol and propranolol were 80.0 and 77.5%, respectively (Fig. 5).

Retention of metoprolol, oxprenolol and propranolol decreased by DMOA addition to the mobile phase of acetonitrile–methanol–phosphate buffer (10 mM, pH 3.0) (15:15:70, v/v/v). Capacity factors are decreased by increasing carbon number of alkyl amines (TMA, TEA and TBA) and TrEA was much more effective than DEA for reducing retardation of metoprolol, oxprenolol and propranolol (Fig. 6). PSR values of metoprolol, oxprenolol and propranolol with organic amines were not higher than that are found without organic amine (Fig. 7).

4. Discussion

Polarity of the mobile phase has a dominant effect on chromatographic behavior of solutes in HPLC. Therefore, retention of β-blockers at different acetonitrile concentration in phosphate

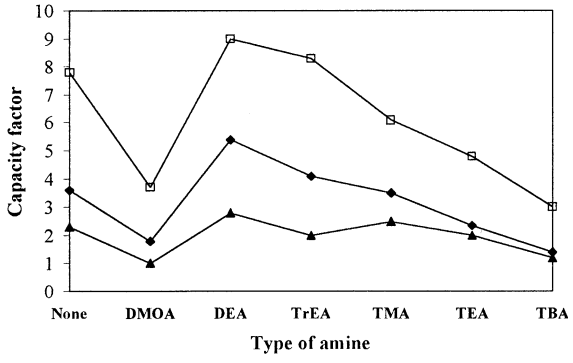

Fig. 6. Separation of β-blockers by a mobile phase containing amine modifier in acetonitrile–methanol–buffer (10 mM, pH 3.0) (15:15:70, v/v/v) (■, Atenolol; ▲, Practolol; ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

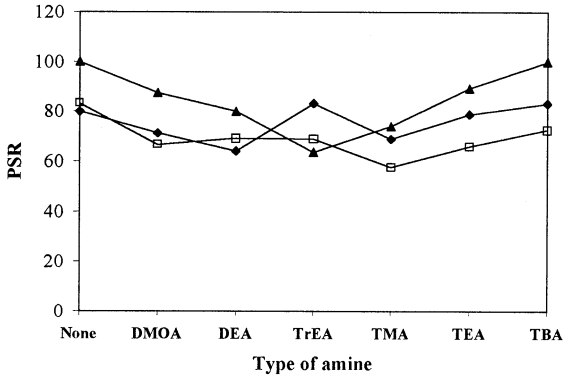

Fig. 7. Amine modifier effect on peak symmetries of β-blockers by a mobile phase of acetonitrile–methanol–buffer (10 mM, pH 3.0) (15:15:70, v/v/v) on μBondapak C_{18} (■, Atenolol; ▲, Practolol, ◆, Metoprolol; ●, Oxprenolol; □, Propranolol).

buffer (10 mM, pH 3.0) is ascertained primarily. Increased concentrations of acetonitrile in phosphate buffer reduced the capacity factors of all β-blockers because of decreased polarity of mobile phase (Fig. 1). It was reported that the influence of remaining silanols on solute retention by polar interactions is most obvious at an eluent composition where the reversed-phase is totally wetted with sufficient water ($< 60\%$) [20]. On the other hand, when high ratios of organic modifier ($\sim 90\%$) are used, silica acts as an ion-exchange medium [21]. Thus, a concentration of 30% for acetonitrile as organic modifier was applied in the other parts of study. PRS values for oxprenolol and propranolol were lower than 80% from the presence of unbonded surface silanols on C_{18} stationary phase and their polar interaction with oxprenolol and propranolol.

Moderately hydrophobic compounds containing amino functions, like β-blockers, create problems in RP-LC in term of asymmetric peaks and poor separation. Alkyl sulphonates are frequently used as ion-pairing agents. As shown in Fig. 2, alkyl sulphonate addition to the mobile phase containing acetonitrile-buffer (30:70, v/v) was not successful for the separation of atenolol and practolol ($k' < 0.5$). Additionally, capacity factors of metoprolol, oxprenolol and propranolol were increased by decreasing polarity of alkyl sulphonates and reached about the values that are found by the mobile phase without alkyl sulphonate (Fig. 2) and PSR values were $< 80\%$, thus longer chained alkyl sulphonates did not examine.

Chromatographic performance of amine solutes can be improved by the presence of positively charged organic amines in mobile phase because of their competition with $(+1)$ charged solutes for residual silanol sites [22]. In this study, common competing agents DEA, TrEA, TMA, TEA, TBA and DMOA are added to the mobile phase based on acetonitrile/phosphate buffer (30:70, v/v). Due to the coverage of free silanols by these organic amines, retention times were changed in some degrees (Fig. 3), but this masking effect was not enough to achieve an adequate separation and to recover the peak asymmetries of tested β-blockers.

The retention pattern of solutes in RPLC is known to be susceptible to the changes in the type and concentration of organic solvent(s) in mobile phase. It was reported that most of silica based columns gave poor peak symmetry with acetonitrile as the only organic modifier in a mobile phase of phosphate buffer pH 3.0 [19]. Additionally, by the mobile phases containing 30% of acetonitrile, atenolol and practolol were not separated because of their higher hydrophillicity and addition of alkyl sulphonates and organic amines were not capable to improve their separation. In this study, methanol is used concurrently with acetonitrile and so atenolol, practolol, metoprolol, oxprenolol and propranolol were adequately separated (Fig. 4) with the highest peak symmetries (Fig. 5), particularly at a concentration of $15+15\%$ (v/v) for methanol and acetonitrile, respectively. It was also demonstrated that, liphophillic alcohols are well adsorbed on silica and performed a homogenous stationary phase [13]. Methanol is a moderately liphophillic alcohol and this can be the mechanism of the improvement in peak symmetries.

Tertiary amine solutes with $pK_a > 9.0$ are much more effected by free silanol groups than primary or secondary amine compounds with $pK_a < 9.0$ [11,23]. β-blockers are secondary amines and their pK_a values are in the range from 8.0 to 9.5. Thus, their peak symmetries may not be effected by the

presence of organic amine or alkyl sulphonate modifiers. Addition of strong silanol masking agents, DMOA, DEA, TrEA, TMA, TEA and TBA, to the mobile phase containing acetonitrile–methanol–phosphate buffer (10 mM, pH 3.0) (15:15:70, v/v/v), reduced capacity factors (Fig. 6) but did not change peak symmetries (Fig. 7). Moreover, it has been reported that peak asymmetry factor of propranolol eluted from μBondapak C_{18} column did not unchanged by addition of DMOA to a mobile phase consisted of methanol/phosphate buffer (1:1, pH 3.0) although retention time is significant decreased [24].

5. Conclusion

In previous HPLC separations of β-blockers, mobile phases generally consisted of acetonitrile or methanol, buffer, ion-pairing reagent or organic amines. The results of this study demonstrated that addition of alkyl amines, PSA, HSA, HpSA and OSA, and organic amines, DMOA, DEA, TrEA, TMA, TEA and TBA, is not essential for an adequate separation. This outcome is established by the data both from the mobile phases containing acetonitrile (30%) and ancetonitrile + methanol (15 + 15%). Atenolol, practolol, metoprolol, oxprenolol and propranolol simultaneously separated with high peak symmetries using a mobile phase consisted of methanol/acetonitrile/phosphate buffer (10 mM) (15:15:70, v/v/v, pH 3.0).

By changing only the fraction of methanol with respect to acetonitrile, method became more efficient for the separation of β-blockers. Furthermore, atenolol, practolol, metoprolol, oxprenolol and propranolol can be detected up to 0.25, 5, 10, 50 and 10 ng ml^{-1}, respectively. It is proposed that this new mobile phase can be used for the analysis of the several β-blockers in doping control analysis as well as others

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