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Review

Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases

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Abstract

The direct chiral separation by chiral stationary phases (CSPs) is one of the most important techniques to analyze enantiomeric purity as well as to get enantiomerically pure material quickly. Among various types of CSPs, polysaccharide type CSPs are well known by their versatility and durability. They are not only effective under normal-phase conditions, but also under reversed-phase conditions. In order to get a good separation under the reversed-phase conditions, it is the key to choose an appropriate mobile phase. For example, a simple mixture of water/acetonitrile or water/methanol are sufficient for a neutral analyte, while it is necessary to use an acidic solution instead of water for an acidic analyte and a solution of a chaotropic salt (or a basic solution) for a basic analyte, respectively. The paper also presents lists of more than 350 separation examples that include 22 validated methods for drug analyses from serum, plasma, or urine samples on polysaccharide type CSPs under reversed-phase conditions. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Enantiomer separations; Chiral stationary phases, LC; Polysaccharides

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1. Introduction

The direct enantioseparation of a chiral compound using a chiral stationary phase (CSP) in high-performance liquid chromatography (HPLC) has had a great impact on the pharmaceutical industry as well as such fields as asymmetric syntheses and biochemistry. Twenty years have passed since the first chiral column was commercialized, and this separation technique has proven to be a powerful, versatile, and convenient procedure for chiral separations [1]. Chiral chromatography can be applied to analytical separations as well as to preparative separations [2,3]. A large number of CSPs have been developed in the last 25 years including brush, ligand-exchange, cyclodextrin, protein, and polysaccharide types [4]. Among these types of CSPs, polysaccharide type CSPs have proven to be one of the most useful CSPs because of their versatility, durability, and in particular, loadability [5].

The application of a carbohydrate to chromatographic chiral separation has a long history. One of the remarkable success was reported by Hesse and Hagel in 1976. They found that microcrystalline cellulose triacetate had fairly good chiral separation ability [6], although the quality of the separation was not good enough for recent analytical requirements. Moreover, it had been believed that the chiral recognition ability would be lost when microcrystalline cellulose triacetate was dissolved in a solvent until proven otherwise by experimentation. Nearly a decade after the report of microcrystalline cellulose triacetate, Namikoshi et al. reexamined the relationship between the morphology of cellulose triacetate and the chiral recognition ability [7]. Based on the results, they and Okamoto et al. [8–10] developed a new type of CSP, which consisted of a derivatized polysaccharide coated on silica-gel. They showed that the chiral selectivity would not be lost by dissolving the polysaccharide in organic solvents, and that the selectivity could be enhanced by introducing various kinds of subsituents on the hydroxyl group of the polysaccharide as well as by coating the polysaccharide on a solid support, like silica-gel. Initially, the polysaccharide type CSPs were mostly used with normal-phase eluents, such as hexane/2-propanol mixtures. The use of alkane/alcohol solvents was possible since there is no ionic functional group in the polysaccharide CSPs, and since interactions such as hydrogen bonding, π–π interaction, and dipole–dipole stacking interaction are considered to be more effective under normal-phase conditions. In contrast to the success of these phases for the separating of enantiomers using normal-phase solvents, there have been significantly fewer reports of the separation of enantiomers using the polysaccharide type CSPs in aqueous mobile phases [11]. The first example of a chiral separation using a buffer was reported by Ikeda et al. in 1989 [12]. Thus, by careful selection of mobile phase conditions, excellent chiral separation can be achieved using reversed-phase conditions with polysaccharide type CSPs [13]. There are a number of advantages to use reversed-phase conditions, for example, good solubility of polar compounds, easier sample preparation from serum or plasma, and use of less costly solvents. Reversed-phase conditions have been especially useful for assay of the enantiomeric ratio of a pharmaceutical in biological matrices.

The aim of this paper is to describe how to develop and optimize a chiral separation for an acidic, neutral, or basic compounds using polysaccharide CSPs with reversed-phase solvents, brief discussion of the separation mechanisms as well as illustrating some applications, which utilize the advantage of the reversed-phase mode.

1.1. Chemical structures and interaction sites of polysaccharide type CSPs

Among various polysaccharides, cellulose and amylose derivatives have proven to be the most

useful CSPs. The structures of the polysaccharide polymers and the functional groups for these CSPs which are commercially available are shown in Fig. 1. From examination of these structures it becomes clear that there are a number of positive interaction sites in each derivative which may interact with functional groups on chiral analytes. For example, there are interaction points for π–π stacking interaction, dipole–dipole stacking interaction and hydrogen bonds. These interactions are relatively weak and are considered to be more effective under normal-phase conditions. One of the major difference between the polysaccharide type CSPs and protein type CSPs is that the protein type CSPs have ionic functional groups in the molecule and hence have been mainly used under reversed-phase conditions to utilize the ionic interactions between the CSPs and the analytes. In contrast, when using the polysaccharide CSPs, it is of primary importance to keep the analyte neutral since there are no functional groups in the polysaccharide that are ionic in nature to interact with charged analytes. It is the same concept to add a counter ion in the mobile phase and form an ion pair with the analyte because the ion pair itself can be considered as neutral. Otherwise, the analyte is charged and is mainly distributed to a polar reversed-phase solvent and it cannot interact sufficiently with the polysaccharide type CSPs. In other words, it is important to control the charge of the analyte to achieve a good separation, especially when the retention of polar analytes which can dissociate in an aqueous solution is attempted. Controlling the ionic state of an analyte will be discussed below.

2. Optimization of conditions in a reversed-phase mode

2.1. Polar organic eluent

For separations using polysaccharide type CSPs, ‘normal-phase eluents’ are typically considered to be

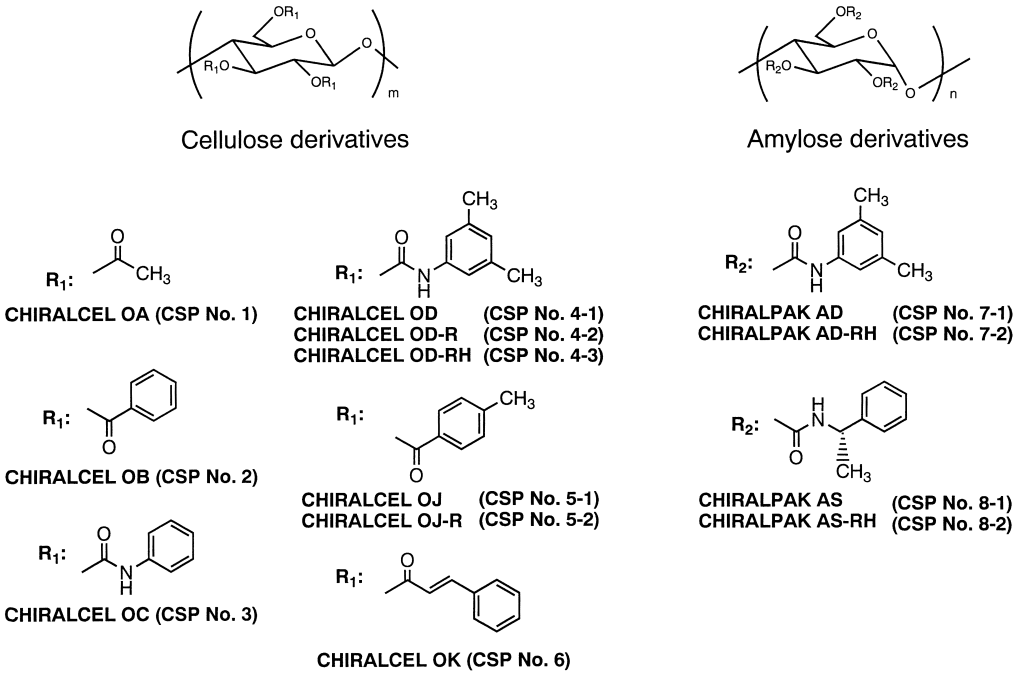


Fig. 1. Structures of polysaccharide type CSPs.

alkane/alcohol mixtures and 'reversed-phase eluents' are considered to be an aqueous/alcohol or aqueous/acetonitrile eluents. In addition, there is another mode in which only pure polar organic solvents are used. Polar solvents referred here as 'polar organic eluents' that can be used with some of polysaccharide type CSPs are: pure acetonitrile, methanol, ethanol, propanol and their mixtures. Although these 'organic eluents' are not reversed-phase solvents, they can be considered as such. As expected, the behavior of analytes with these eluents is somewhat different from that with alkane/alcohol mixtures mainly because of the polarity differences. The polar organic eluents are extremely useful for a preparative scale separations because of the high solubility of polar analyte in these eluents as well as the relative simplicity of their evaporation [14]. Some separations using polar organic eluents are listed in Table 1.

2.2. Organic modifier

It is common to use either acetonitrile or methanol as an organic modifier under reversed-phase conditions because of their low viscosity and low background UV absorption. The retention time of an analyte can decrease with increasing the amount of the organic modifier, or vice versa. In addition, the same amount of acetonitrile usually gives a shorter retention time than an equivalent amount of an alcohol, i.e., methanol. For example, 60/40 of water/acetonitrile results in similar retention time to 40/60 or 30/70 of water/methanol [13]. However, there are examples in which an opposite tendency or even an U-shaped curve have been observed [25,26]. There is no rule as to the choice of a polar organic modifier, either acetonitrile or alcohol, for the optimization of peak shape. In most cases, peak shape is primarily a characteristic of an analyte. In a particular case, the best separation was only achieved by combining three components, that is, acetonitrile/methanol/aqueous $NaClO_4$ [27]. Takakuwa et al. have reported that it is also possible to apply a linear gradient effectively to reduce the analysis time [28].

2.3. Temperature

Normally, the ultimate objective is to achieve sufficient resolution. Resolution factor (*Rs*) is expressed:

$$Rs = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\sqrt{N}\,\frac{k_2'}{1+\overline{k'}} \tag{1}$$

where α is separation factor, N is effective theoretical plate number, k_2' is the capacity factor of the second elected compound and $\overline{k'}$ is the average capacity factor of the first and second elected compounds. As shown in this equation, resolution factor is influenced by two factors, namely, separation factor and efficiency of the column. Resolution factor is increased when separation factor and/or column efficiency is increased. Capacity factor and separation factor may be related to temperature as:

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln\phi \tag{2}$$

$$\ln \alpha = \ln\left(\frac{k_2'}{k_1'}\right) = -\frac{\delta\Delta H^0}{RT} + \frac{\delta\Delta S^0}{R} \tag{3}$$

where R is the ideal gas constant, T is absolute temperature, and ϕ is the phase ratio [29]. The ΔH^0 and ΔS^0 represent the enthalpy and entropy terms for each enantiomer and $\delta\Delta H^0$ and $\delta\Delta S^0$ represent their differences respectively. According to these equations, both capacity factor and separation factor are controlled by an enthalpic contribution which decreases with the elevation of temperature and an entropic contribution which is independent of the temperature. The selectivity is a compromise between differences in enantiomeric binding enthalpy and disruptive entropic effects [30]. The enthalpy term is a function of overall interactions between each enantiomer and the CSP. It is believed that there are multiple types of interactions occurring between the analyte and the polysaccharide type CSP, which is advantageous to separate various compounds. However, that makes it very difficult to predict the sum of each interaction. It is also difficult to conceptualize a chiral character in entropy, though solvation differences and statistical concepts of molecular flexibility are commonly invoked when entropic terms are discussed [30]. Moreover, these parameters are need to be determined for a given analyte, CSP, and mobile phase. Unfortunately, there are too little reports [31,32] to rationalize these

Table 1
Separation samples with polar organic phases[a]

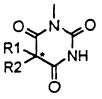
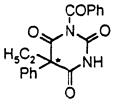
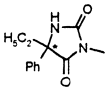
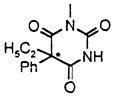
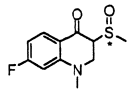
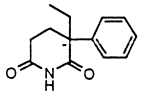
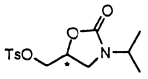
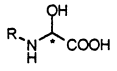
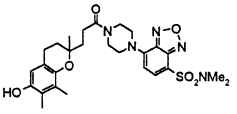
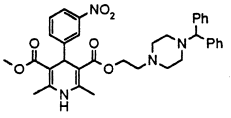
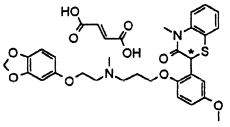
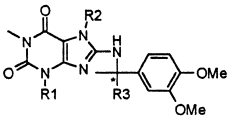
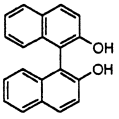
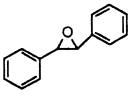
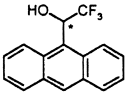
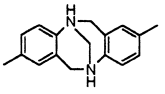
Compounds	Conditions & data
Pharmaceuticals	
 Barbiturates	CSP : 5-1 [15] MeOH (*: EtOH) 1.0 ml/min (*: 0.8 ml/min) 235 nm (*: 228 nm)
R1 / R2 a) $-C_6H_5$ / $-CH_3$ b) $-C_6H_{11}$ / $-C_2H_5$ c) $-C_5H_7$ / $-C_2H_5$ d) $-C_6H_5$ / $-C_3H_7$ e) $-C_2H_5$ / $CH_3(CH_2)_2CHCH_3$*	a) $k_1' = 1.19$, $\alpha = 1.48$ b) $k_1' = 0.49$, $\alpha = 1.22$ c) $k_1' = 0.69$, $\alpha = 1.48$ d) $k_1' = 1.02$, $\alpha = 1.70$ e) $k_1' = 0.18$, $\alpha = 1.56$
 Benzonal	CSP : 5-1 [16] EtOH / MeOH = 90 / 10 0.8 ml/min, 23 °C, 254 nm $k_1' = 3.75$, $\alpha = 1.21$
 Mephenytoin	CSP : 5-1 [16] MeOH 0.5 ml/min, 23 °C, 254 nm $k_1' = 0.86$, $\alpha = 1.46$
 Mephobarbital	CSP : 5-1 [16] MeOH 0.5 ml/min, 23 °C, 254 nm $k_1' = 1.28$, $\alpha = 3.03$
 Flosequinan	CSP : 3-1 [17] EtOH / MeOH = 50 / 50 0.7 ml/min, 320 nm $t_1 = 6.2$, $t_2 = 11.6$
 Glutethimide	CSP : 5-1 [18] MeOH 0.5 ml/min, 210 nm $t_1 = 9.3$, $t_2 = 16.0$
 3-Isopropyl-5-p-toluenesulphonyl-oxymethyloxazolidin-2-one	CSP : 2 [19] MeOH 1.0 ml/min, r.t. 254 nm $t_1 = 4$, $t_2 = 5$
 α-Hydroxyglycine deriv. a) N-Benzoyl b) Cinnamoyl c) 3-Phenylpropionil d) 1-Naphthylacetyl e) Phenylacetyl f) 4'-Methoxyphenylacetyl g) 4'-Nitrophenylacetyl	CSP : 7-1 [20] 1.0 ml/min, 210 nm (*:225 nm, ‡:278 nm) CH_3CN / IPA / TFA = 95 / 5 / 0.1 (a*, f, g) 90 / 10 / 0.1 (b‡, c) 98 / 2 / 0.1 (d, e) a) $t_1 = 5.3$, $t_2 = 6.2$ b) $t_1 = 8.4$, $t_2 = 12.0$ c) $t_1 = 5.1$, $t_2 = 5.7$ d) $t_1 = 5.3$, $t_2 = 6.6$ e) $t_1 = 5.0$, $t_2 = 5.7$ f) $t_1 = 4.7$, $t_2 = 5.1$ g) $t_1 = 5.1$, $t_2 = 6.3$

Table 1. Continued

Compounds	Conditions & data
 DBD-PZ-LLU-α	CSP : 7-1 [21] CH_3CN / MeOH = 5 / 95 $t_1 = 24.7$, $t_2 = 27.4$
 Manidipine	CSP : 5-2 [22] MeOH / DEA 25 °C, 254 nm $t_1 = 8.80$, $t_2 = 9.23$ $k_1' = 2.14$, $\alpha = 1.07$
 Semotiadil fumarate	CSP : 8-1 [23] EtOH 0.6 ml/min $t_1 = 8.4$, $t_2 = 17.1$
 Theophylline deriv.	CSP : 3 [24] MeOH, 0.5 ml/min 20 °C, 210 nm a) $k_1' = 3.65$, $\alpha = 1.33$ b) $k_1' = 3.94$, $\alpha = 1.54$ c) $k_1' = 7.07$, $\alpha = 1.20$ d) $k_1' = 3.07$, $\alpha = 1.22$ e) $k_1' = 4.39$, $\alpha = 1.09$ f) $k_1' = 2.64$, $\alpha = 1.49$
R1 / R2 / R3 a) $-CH_3$ / $-C_2H_4OCH_3$ / $-CH_2OH$ b) $-CH_3$ / $-CH_3$ / $-CH_2OH$ c) $-CH_3$ / $-C_2H_4OCH_3$ / $-CH_2OCONMe_2$ d) $-CH_2$-cyclopropyl / $-C_2H_4OCH_3$ / $-COOC_2H_5$ e) $-CH_3$ / $-C_2H_4OCH_3$ / $-CH_2OCH_3$ f) $-CH_3$ / $-C_2H_4OH$ / $-CH_2OCH_3$	
Chemicals	
 Binaphthol	CSP : 7-1 [22] MeOH 1.0 ml/min, 25 °C, 254 nm $t_1 = 3.90$, $t_2 = 4.19$ $k_1' = 0.30$, $\alpha = 1.32$
 *trans*-Stilbene-oxide	CSP : 5-2 [22] MeOH 0.5 ml/min, 25 °C, 254 nm $t_1 = 8.53$, $t_2 = 12.18$ $k_1' = 1.25$, $\alpha = 1.77$
 2,2,2-Trifluoro-8-anthryl-1-ethanol	CSP : 5-2 [22] MeOH 0.5 ml/min, 25 °C, 254 nm $t_1 = 4.68$, $t_2 = 5.28$ $k_1' = 0.23$, $\alpha = 1.67$
 Tröger's base	CSP : 5-2 [22] MeOH 0.5 ml/min, 25 °C, 254 nm $t_1 = 8.48$, $t_2 = 31.63$ $k_1' = 1.23$, $\alpha = 5.94$

[a] CSP numbers correspond to Fig. 1. References cited in Table [15–24].

parameters on the polysaccharide type CSPs under reversed-phase conditions. However, these references as well as author's experience suggest that most of reversed-phase separations on the polysaccharide type CSPs are rather enthalpy driven in the temperature range between 0°C and 40°C which is commonly used for such separations. In other words, both capacity factor and separation factor decrease with increasing temperature, though their van't Hoff plots may not be linear. One of the example which shows relation between the temperature and chromatographic parameters is illustrated in Fig. 2. On the other hand, the column efficiency is increased with temperature as mass transfer improves [30]. Therefore, the resolution factor may be increased with decreasing temperature when α is close to 1.0 and loss of the column efficiency is moderate. When α is large enough, it is usually effective to increase temperature for a better resolution.

In conclusion, it is very difficult to predict the influence of temperature, but it may be beneficial to investigate it systematically to optimize a difficult separation especially when there are many isomers.

2.4. Eluent

The choice of an appropriate eluent for each type of analytes is the most important part of the method development and optimization. Several comprehensive reports have been published regarding to the choice of an eluent with cellulose tris(3,5-dimethylphenylcarmabate) as CSP [13,33,34]. It is important to control the ionic behavior of the analyte in order to obtain a good separation, as was mentioned earlier, because the polysaccharide phases are not designed for ionic interaction. An analyte bearing an electric charge considerably reduces the interaction with the CSP presumably because solvation of an ionized analytes inhibits the direct interaction between the analyte and the CSP, that eventually results in a poor separation. To avoid the problem, two approaches can be employed, one involves

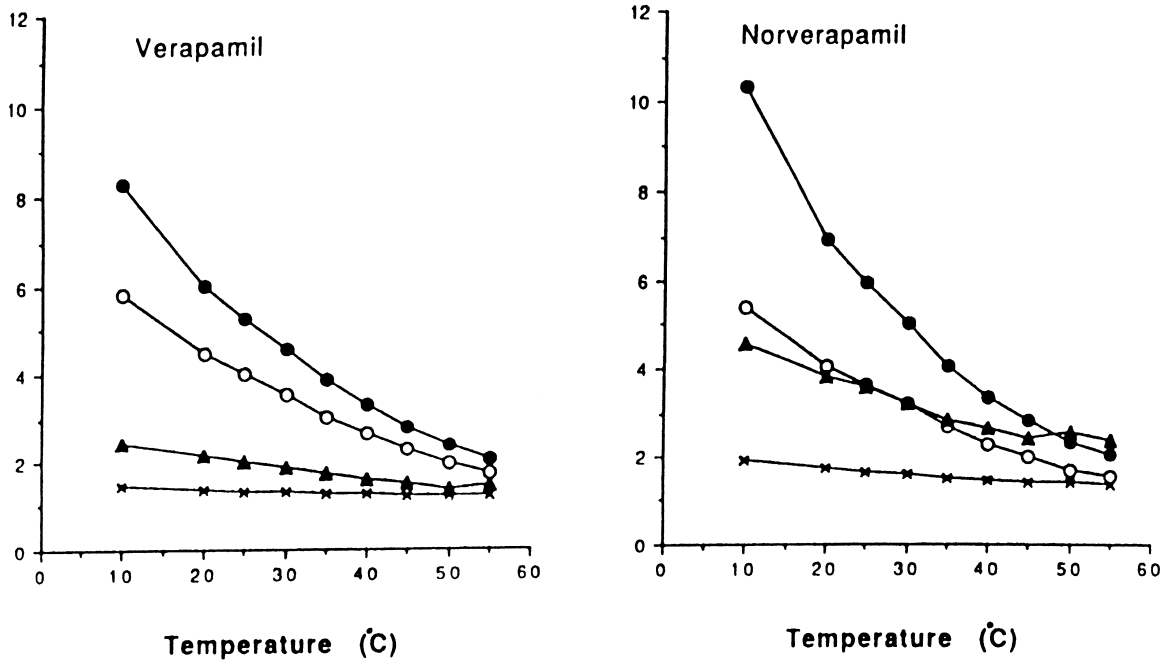


Fig. 2. Effect of temperature on the separation with a CHIRALCEL OD-R (0.46×25 cm) column. Mobile phase: 50 m*M* $NaClO_4$–$HClO_4$ (pH 3)/CH_3CN=67/33, flow-rate: 1.0 ml/min, detection: UV 205 nm, sample injection: 15 μl (1 mg/ml). ○=k' for first eluting enantiomer, ●=k' for second eluting enantiomer, ▲=resolution (Rs), ×=alpha (α). (Reproduced from [31] with permission).

suppression of dissociation of the analyte and the other involves ion pair formation of the analyte with a suitably chosen counter ion (chaotropic reagent). The conditions adaptable to a particular type of analytes will be discussed later on.

2.4.1. Neutral analytes

For a neutral analyte, a simple mixture of water and an organic modifier is sufficient to obtain a satisfactory separation because there is no competitive ionic interaction to deal with. Although it is possible to use eluents with a buffer, acid, or basic modifier, there is essentially no effect on a given separation as illustrated in Fig. 3.

2.4.2. Acidic analytes (Optimization of pH)

For an acidic analyte, it is essential to use an acidic mobile phase to suppress the dissociation of the analyte and minimize ionic interactions as shown below.

$$COO^- + H^+ \rightleftharpoons COOH$$

It is sufficient to use a pH 2 aqueous solution, along with an organic modifier (alcohol or acetonitrile) to obtain the separation of a analyte containing a carboxyl function because most organic acids are in a neutral state (non-ionized) between pH 2 and pH 4. It addition, the use of a mobile phase in the pH range of 2–7 is considered a suitable range for the silica-gel support used in the preparation of polysaccharide CSPs. Typical dependence of the capacity factors on the pH of the mobile phase is shown in Fig. 4.

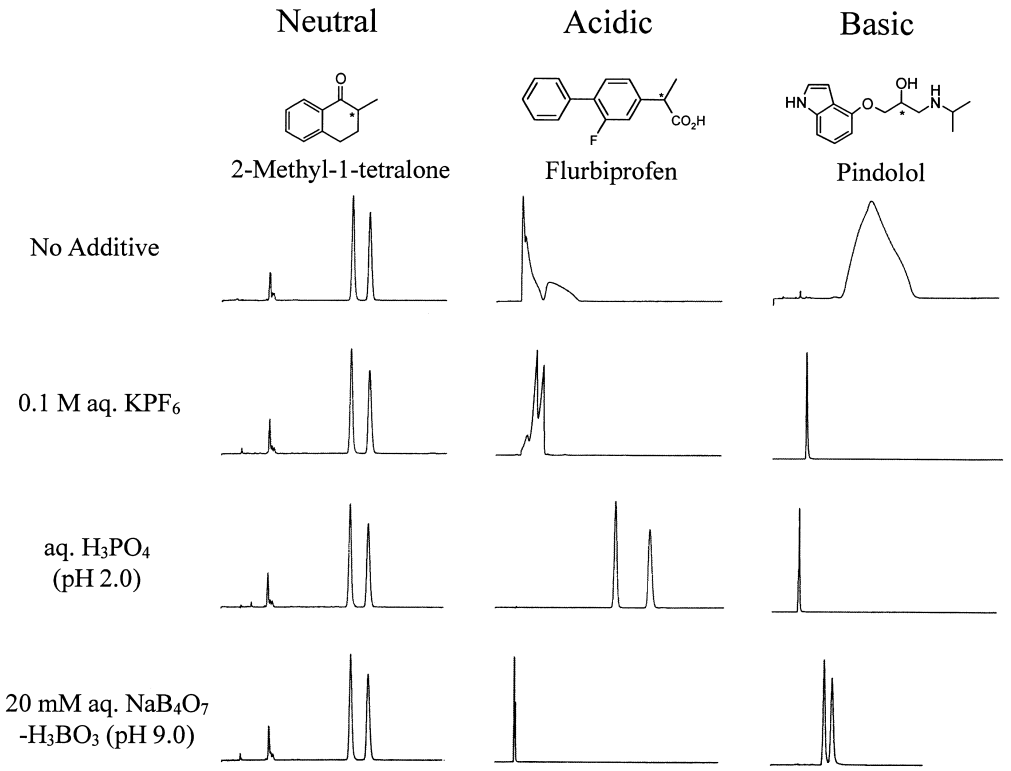


Fig. 3. Effect of various kinds of eluents on the separation of neutral, acidic and basic racemates. Column: CHIRALCEL AD–RH (0.46×15 cm), mobile phase: aq. (shown on the figure)/CH_3CN=60/40, flow-rate: 0.5 ml/min, temp.: 25°C, detection: UV 254 nm. (Reproduced from [36]).

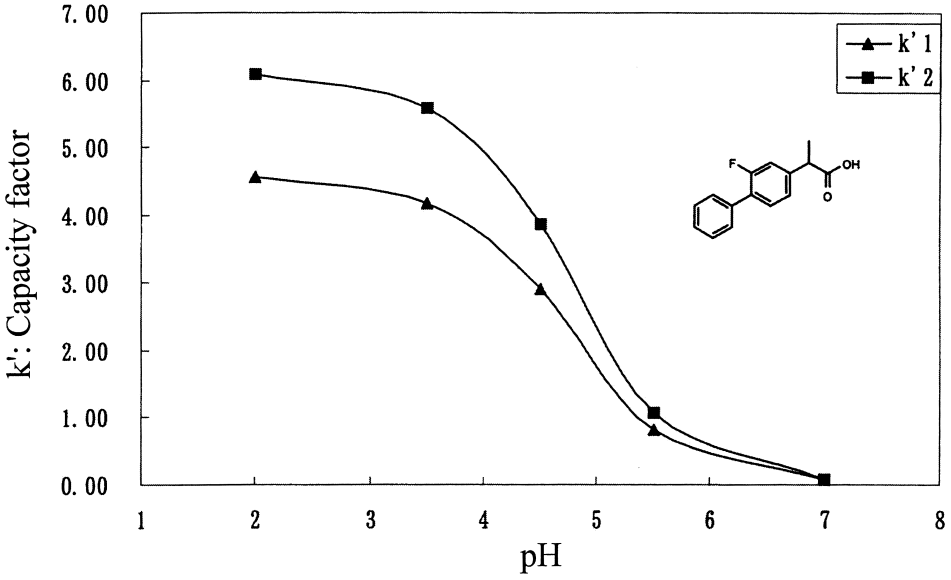


Fig. 4. Effect of pH on the capacity factors of an acidic analyte. Mobile phase: 50 m*M* H_3PO_4 buffer/CH_3CN=60/40. See Fig. 3 for other conditions. (Reproduced from [36]).

2.4.3. Basic analytes (Optimization of buffer and buffer pH)

For an basic analyte, there are basically two parameters which have to be considered in order to optimize a chiral separation: the choice of a buffer system and a suitable pH. It would be relatively straight forward to choose separation conditions except for the fact that silica supports are unstable at pH>7. Literatures reports indicate that the practical limit of pH for a silica-based column is around 9–10, which is barely sufficient to suppress the dissociation of organic amines. Moreover, the expected column life time at pH 10 is considerably shorter than that of at pH 2. Therefore, it is preferable to develop separation conditions at pH<7 whenever possible.

Under neutral and acidic mobile phase conditions, basic analytes are positively charged. That is, the following equilibrium shifts to the right under neutral and acidic mobile phase conditions.

$$RNH_2 + H^+ \rightleftharpoons RNH_3^+$$

The positively charged analyte cannot interact with the CSP efficiently, and the analyte is eluted rapidly and usually without separation. To make the positively charged analyte accessible to the CSP, it is necessary to add a considerable amount of anions in the mobile phase and form an ion pair, which can be considered to be electrically neutral (see equation below).

$$RNH_3^+ + PF_6^- \rightleftharpoons [RNH_3^+PF_6^-]$$

The retentions and the resolution of basic analytes are influenced considerably by the type of anions. Ishikawa et al. reported the order of retention of basic analytes as a function of the counter ion [13]. They mentioned [13] that the order is in good agreement with that of chaotropicity [34]. Miao et al. proposed [35] the following retentive order, with some additional anions compared to Ishikawa et al. Part of the comparison data is summarized in Table 2.

$$PF_6^- > BF_4^- > ClO_4^- > SCN^- > I^- > NO_3^- > H_2PO_4^- > Br^- > Cl^- > AcO^-$$

There is a good correlation between the retention time and the separation factor of amino group-containing analytes on CHIRALCEL OD-R column using these anions. Interestingly, a lipophilic ion pairing reagent such as sodium undecan-1-sulfonate, which is commonly used with an ODS type column,

Table 2
Effect of ion-pair reagent on the separation of basic compounds [35][a]

Mobile phase conditions	t_1	t_2	k'_1	k'_2	α
0.10 *M* $NaPF_6/CH_3CN=60/40$	24.70	32.72	3.84	5.41	1.41
0.10 *M* $NaPF_4/CH_3CN=60/40$	10.54	12.26	1.07	1.40	1.32
0.10 *M* $NaPF_3SO_3$ [b]$//CH_3CN=50/50$	24.30	25.31	3.76	3.96	1.05
0.02 *M* $NaCF_3SO_3+H_2SO_4(Ph2)/CH_3CN=60/40$	8.75	9.89	0.72	0.94	1.31
0.10 *M* $NaC10_4/CH_3CN=60/40$	34.82	38.97	5.83	6.64	1.14
0.10 *M* $NaNO_6/CH_3CN=70/30$	17.13	20.83	2.36	3.08	1.31
0.10 *M* $NaH_2PO_4/CH_3CN=70/30$	11.29	12.85	1.21	1.52	1.25

[a] Sample: Verapamil hydrochloride, Column: CHIRALCEL OD-R, Flow rate: 0.5 ml/min.
[b] pH of the solution was about 10.

was not very effective in the improvement of a given separation despite a longer retention time when this ion pairing reagent is used [13]. It only increases non-stereoselective interactions between the analyte and the CSP. The phenomenon may arise from that the ion pair between the cationic analyte and undecan-1-sulfonate is too large to fit into the chiral recognition site, though its hydrophobicity is increased and it has higher affinity to the CSP.

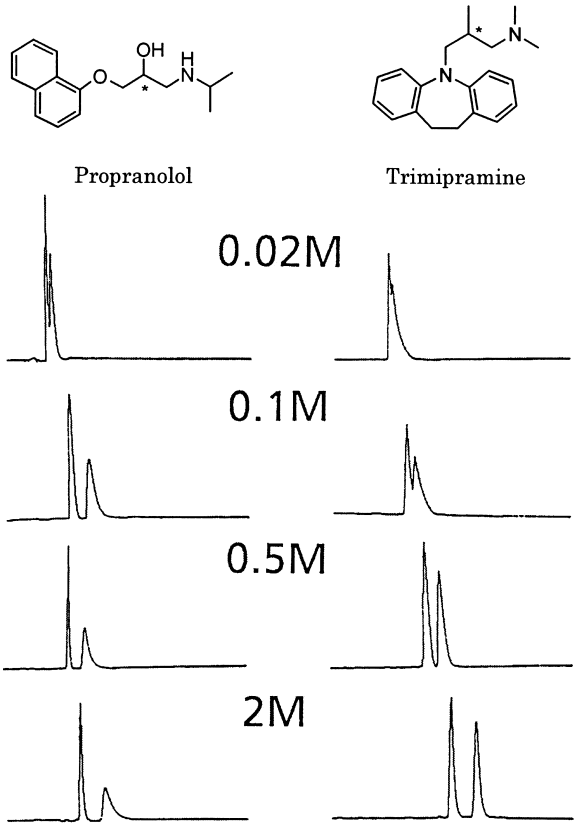


Fig. 5. Effect of concentration of $NaClO_4$ on the separation of propranolol and trimipramine. Column: CHIRALCEL OD-R (0.46×25 cm), mobile phase: aq. $NaClO_4/CH_3CN=60/40$. See Fig. 3 for other conditions. (Reproduced from [13] with permission).

The concentration of an ion pair reagent is also an important factor (see Fig. 5). The higher the concentration of an anion present, the greater the separation obtained. On the other hand, the more concentrated of the ion pair reagent, the greater the chance for precipitation of salts to occur when mixed with organic modifiers. It is suggested to use buffer concentrations at less than 50% of the saturation level of the salt to minimize potential precipitation.

The influence of different counter cations of the chaotropic anions was not so significant as the effect of the anions itself but there was some difference in peak shape and it might worth to try a different combination in the case of a critical separation [13].

Among polysaccharide type CSPs, amylose tris(3,5-dimethylphenylcarmabate) type CSPs (CHIRALPAK AD-RH) showed different tendency against basic analytes (see Table 3). The use of ion pair reagents was not effective (except for a few cases), and the use of basic mobile phase conditions was necessary to achieve a good chiral separation [36]. Fig. 6 demonstrates the dependency of the capacity factor of alprenolol, which is a basic analyte, vs. pH on a CHIRALPAK AD-RH column. At lower pH, the analyte is mainly dissociated and does not interact sufficiently with the CSP. As a result, it is not essentially retained. Once the pH reached 8, the retention time significantly increased and some separation could be observed. This phe-

Table 3
Separation of basic compounds on CHIRALPAK AD-R [36][a]

	Acidic eluent with ion-pair reagent			Basic eluent with buffer		
	Eluents	k'_1	α	Eluents	k'_1	α
Alprenolol*	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=65/35	0.79	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=60/40**	1.97	1.30
Pindolol	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=90/10	11.5	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=60/40**	0.82	1.34
Perisoxal	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=65/35	1.63	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=30/70	3.11	1.95
Tolpeisone	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=65/35	1.53	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=50/50	1.75	1.25
Nefopam	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=65/35	0.99	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=40/60	0.66	1.40
Mextixene	50 m*M* KPF_6 aq. (pH 2)/ CH_3CN=50/50	0.55	1.00	20 m*M* $Na_2B_4O_7$-H_3BO_3 aq. (pH 9)/CH_3CN=30/70	1.82	3.80

[a] Detection: UV 254 nm (*, 220 nm), Flow rate 1.0 ml/min (**, 0.5 ml/min), Temp.: 25°C.

nomenon of the increased retention and selectivity is directly related to the dissociation of the charged amine. At this high pH the ionization of the amine is suppressed and it can interact favorably with the CSP.

$$RNH_3^+ + OH^- \rightleftharpoons RNH_2 + H_2O$$

As a consequence of an newly developed surface treatment of the silica gel, which was applied to CHIRALCEL OD-RH, CHIRALCEL OJ-R, and CHIRALPAK AD-RH, these columns are reasonably stable at pH 9 [36]. Use of a borate buffer around pH 9 is a good starting point. There are reports that the use of silica saturation column, which is placed

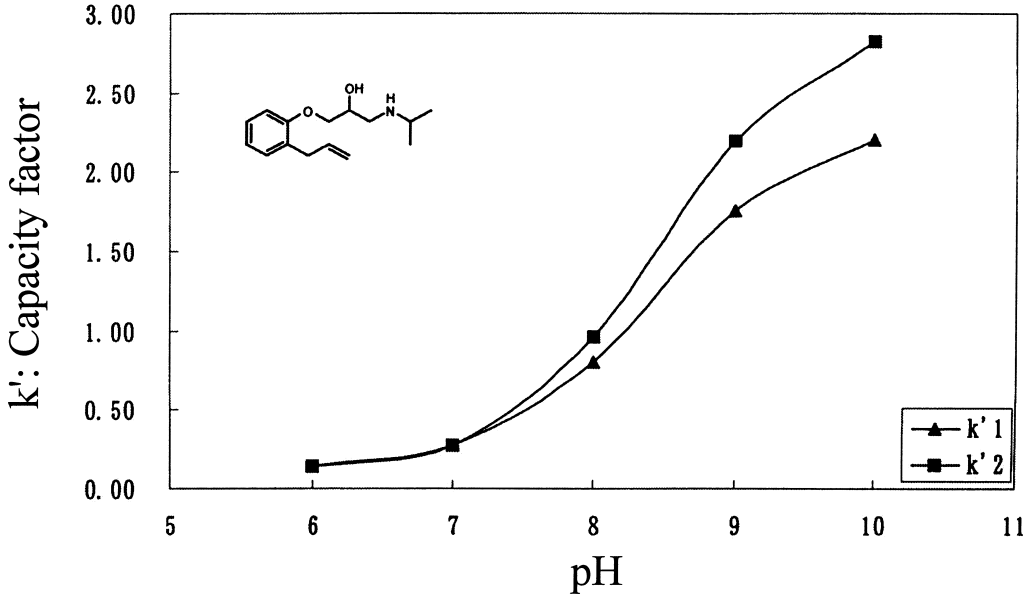


Fig. 6. Effect of pH on the capacity factors of a basic analyte. Mobile phase: 20 m*M* H_3PO_4 buffer/CH_3CN=60/40. See Fig. 3 for other conditions. (Reproduced from [36]).

between a pump and an injector in the HPLC system, is very effective for extending the lifetime of achiral octadecylsilane (ODS) stationary phases when used at higher pH [37]. Use of a basic buffer like borate or the use of diethylamine (DEA) type basic modifiers (0.1%) are believed to be more compatible with silica-based stationary phases than many other available types of bases [37]. These approaches may also be effectively applied to polysaccharide type CSPs.

2.4.4. Bifunctional analytes and onium ion analytes

For bifunctional analytes, such as amino acids, it may be effective to use a mobile phase of lower pH containing an ion-pair reagent. The idea is based on the consideration that the dissociation of the acidic part would be suppressed and an ion-pair would be formed at the basic part as shown below.

$$^{-}OOCRNH_3^{+} + H^{+} + PF_6^{-} \rightleftharpoons [HOOCRNH_3^{+}\,PF_6^{-}]$$

It may be helpful to add a very small amount of an organic amine, that is, 0.1–0.2% of DEA, in order to improve the peak shape [38]. It is maybe suppressing the ionic interaction between the residual silanol on the support and the analyte.

For onium ion analytes, such as quaternary amines, it is essential to use an ion-pair reagent because the other approach, using a basic mobile phase and suppressing the dissociation, cannot be applied to this type of compounds.

$$R_1R_2R_3R_4N^{+} + PF_6^{-} \rightleftharpoons [R_1R_2R_3R_4N^{+}\,PF_6^{-}]$$

Hampe et al. reported results on a comprehensive investigation regarding to analysis conditions for series of quaternary tropane alkaloids [39].

3. Separation examples

Separation examples under reversed-phase conditions are summarized in the following tables. Samples which can be separated in both normal and reversed-phase, are listed in Table 4, samples which can be separated in only reversed-phase mode are listed in Table 5 and other examples are listed in Table 6. The separation under both normal-phases

Table 4
Separation examples of the analytes which can be separated under normal-phase conditions[a]

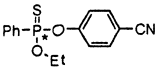
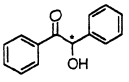
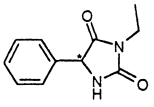
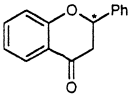
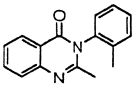
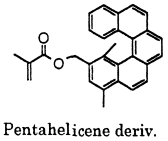
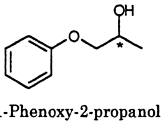
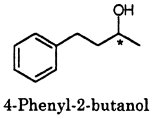
Compounds	Conditions & data
a) Neutral compounds	
 Arylthiophosphate	CSP : 1† [8] H_2O / EtOH = 50 / 50 0.5 ml/min, 25 °C $k_1' = 2.75$, $\alpha = 1.20$
 Benzoin	CSP : 4-3 [40] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm $t_1 = 16.2$, $t_2 = 19.3$ $k_1' = 2.86$, $\alpha = 1.26$
 Ethotoin	CSP : 5-2 [22] H_2O / CH_3CN = 80 / 20 25 °C, 254 nm $k_1' = 4.54$, $\alpha = 1.14$
 Flavanone	CSP : 7-2 [36] H_2O / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm $t_1 = 16.68$, $t_2 = 32.92$ $k_1' = 3.39$, $\alpha = 2.24$
Hexahelicene	CSP : 1† [8] H_2O / EtOH = 50 / 50 0.5 ml/min, 25 °C $k_1' = 5.93$, $\alpha = 1.34$
 Methaqualone	CSP : 5-2 [41] H_2O / CH_3CN = 20 / 80 0.5 ml/min, 25 °C, 254 nm $t_1 = 7.3$, $t_2 = 10.6$ $k_1' = 0.92$, $\alpha = 1.95$
1-(1-Naphthyl)ethylalcohol	CSP : 5-2 [41] H_2O / CH_3CN = 60 / 40 0.3 ml/min, 25 °C, 254 nm $t_1 = 26.78$, $t_2 = 36.37$ $k_1' = 3.23$, $\alpha = 1.47$
 Pentahelicene deriv.	CSP : 1† [8] H_2O / EtOH = 50 / 50 0.5 ml/min 25 °C $k_1' = 3.70$, $\alpha = 1.61$
 1-Phenoxy-2-propanol	CSP : 4-2 [13] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm $t_1 = 13.4$, $t_2 = 17.9$ $k_1' = 1.68$, $\alpha = 1.54$
 4-Phenyl-2-butanol	CSP : 4-2 [13] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm $t_1 = 17.1$, $t_2 = 18.8$ $k_1' = 2.42$, $\alpha = 1.14$

Table 4. Continued

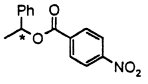
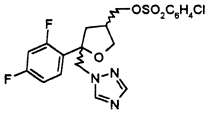
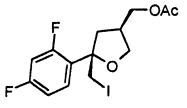
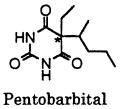
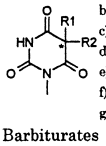
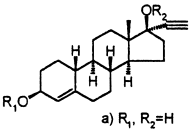
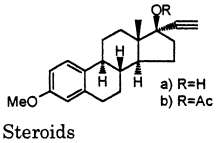
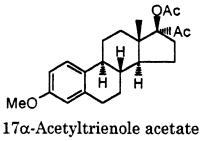
Compounds	Conditions & data
1-Phenylethylalcohol	CSP : 5-2 [41] H_2O / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 19.8, t_2 = 22.0 k_1' = 4.21, α = 1.14
 1-Phenyl-ethylparanitrobenzoate	CSP : 2† [8] H_2O / EtOH = 20 / 80 0.5 ml/min, 25 °C k_1' = 6.35, α = 1.33
 Tetrahydrofuran deriv.	CSP : 7-1 [42] H_2O / MeOH = 10 / 90 1.0 ml/min, 40 °C, 233 nm *trans* : t_1 = 8.0, t_2 = 10.4 *cis* : t_1 = 12.5, t_2 = 19.3
 Tetrahydrofuran deriv.	CSP : 7-1 [42] H_2O / MeOH = 20 / 80 1.0 ml/min, 40 °C, 215 nm t_1 = 6.6, t_2 = 7.5
 Pentobarbital	CSP : 5-2 [43] H_2O / CH_3CN = 80 / 20 0.5 ml/min, r.t., 235 nm k_1' = 6.5, α = 1.15
 a) R_1=Et, R_2=nPr b) R_1=nPr, R_2=iPr c) R_1=Ph, R_2=Me d) R_1=C_6H_{11}, R_2=Et e) R_1=C_6H_7, R_2=Et f) R_1=Ph, R_2=nPr g) R_1=Ph, R_2=Et Barbiturates	CSP : 5-2 [43] H_2O / CH_3CN = 80 / 20 (a), 70 / 30 (b) 65 / 35 (c, d, e, f) 50 / 50 (g) 0.5 ml/min, r.t., 235 nm a) k_1' = 7.5, α = 1.10 b) k_1' = 4.7, α = 1.10 c) k_1' = 2.5, α = 1.45 d) k_1' = 4.0, α = 1.20 e) k_1' = 2.6, α = 1.20 f) k_1' = 4.8, α = 1.46 g) k_1' = 4.0, α = 2.18
 a) R_1, R_2=H b) R_1=H, R_2=Ac c) R_1, R_2=Ac Steroids	CSP : 7-1 [44] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C 215 nm & 240 nm a) t_1 = 4.00, t_2 = 5.50 k_1' = 0.60, α = 2.00 b) t_1 = 4.20, t_2 = 5.43 k_1' = 0.68, α = 1.71 c) t_1 = 3.05, t_2 = 18.9 k_1' = 0.22, α = 30.0
 a) R=H b) R=Ac Steroids	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax a) k_1' = 4.09, α = 1.05 b) k_1' = 0.19, α = 1.63
 17α-Acetyltrienole acetate	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax k_1' = 0.46, α = 1.34

Table 4. Continued

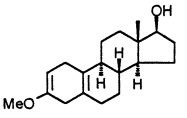
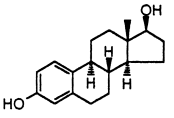
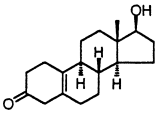
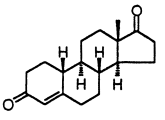
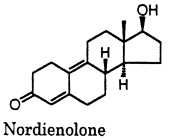
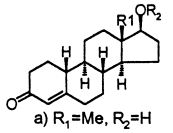
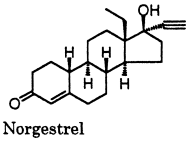
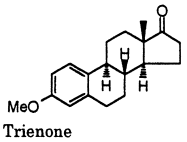
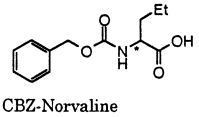
Compounds	Conditions & data
 Dienole	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax k_1' = 1.21, α = 1.18
 Estradiol	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 0.21, α = 1.61
 Isonandrolone	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 1.18, α = 1.04
 Norandrostenedione	CSP : 7-1 [44] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C 215 nm & 240 nm t_1 = 6.25, t_2 = 10.1 k_1' = 1.50, α = 2.02
 Nordienolone	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax k_1' = 0.69, α = 2.46
 a) R_1=Me, R_2=H b) R_1=Me, R_2=Ac c) R_1=Et, R_2=H Steroids	CSP : 7-1 [44] (a, c), [26] (b) H_2O / CH_3CN = 5 / 95, 1.4 ml/min (a, c) H_2O / MeOH = 5 / 95, 1.0 ml/min (b) 35 °C, 215 nm & 240 nm a) t_1 = 5.33, t_2 = 9.90 k_1' = 1.13, α = 2.63 b) k_1' = 3.36, α = 1.21 c) t_1 = 5.53, t_2 = 13.4 k_1' = 1.21, α = 3.59
 Norgestrel	CSP : 7-1 [44] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C 215 nm & 240 nm t_1 = 3.73, t_2 = 3.98 k_1' = 0.49, α = 1.22
 Trienone	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax k_1' = 0.43, α = 2.08
b) Acidic compounds	
 CBZ-Norvaline	CSP : 7-2 [36] H_3PO_4 aq. (pH 2) / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 19.76, t_2 = 22.61 k_1' = 4.20, α = 1.18

Table 4. Continued

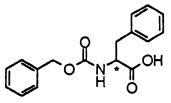
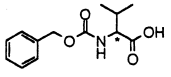
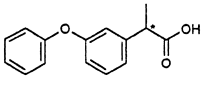
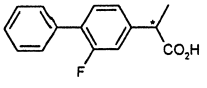
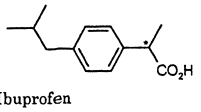
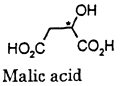
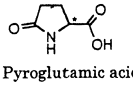
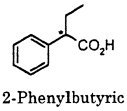
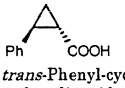
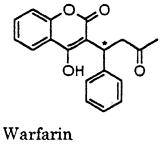
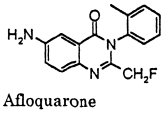
Compounds	Conditions & data
 CBZ-Phenylalanine	CSP : 4-3 [22] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 220 nm t_1 = 22.9, t_2 = 23.6 k_1' = 4.73, α = 1.04
 CBZ-Val	CSP : 4-2 [13] 0.5M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 12.8, t_2 = 14.0 k_1' = 1.56, α = 1.15
 Fenoprofen	CSP : 5-2 [22] Phosphate buffer (pH 2) / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 25.78, t_2 = 27.95 k_1' = 5.78, α = 1.10
 Flurbiprofen	CSP : 7-2 [36] Phosphate buffer (pH 2) / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 22.71, t_2 = 29.48 k_1' = 4.98, α = 1.35
 Ibuprofen	CSP : 7-2 [36] Phosphate buffer (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 20.60, t_2 = 22.32 k_1' = 4.42, α = 1.10
 Malic acid	CSP : 8-2 [36] 0.1M KPF_6-H_3PO_4 aq. (pH2) 1.0 ml/min, 25 °C, 200 nm t_1 = 2.25, t_2 = 4.74 k_1' = 0.11, α = 11.7
 Pyroglutamic acid	CSP : 8-2 [36] 0.1M KPF_6 . H_3PO_4 aq. (pH 2) / CH_3CN = 95 / 5 1.0 ml/min, 25 °C, 200 nm t_1 = 2.47, t_2 = 2.64 k_1' = 0.31, α = 1.29
 2-Phenylbutyric acid	CSP : 4-2 [45] 0.5M $HClO_4$-$NaClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 15.3, t_2 = 16.4 k_1' = 1.55, α = 1.12
 *trans*-Phenyl-cyclopropane-carboxylic acid	CSP : 4-3 [40] H_3PO_4 aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 8.8, t_2 = 9.7 k_1' = 1.10, α = 1.20
 Warfarin	CSP : 4-3 [40] H_3PO_4 aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 23.9, t_2 = 35.7 k_1' = 5.29, α = 1.59
c) Basic compounds	
 Afloquarone	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm t_1 = 18.55, t_2 = 19.28 k_1' = 8.08, α = 1.04

Table 4. Continued

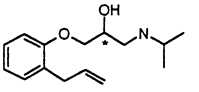
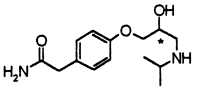
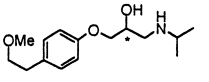
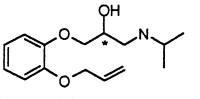
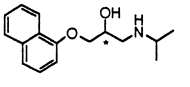
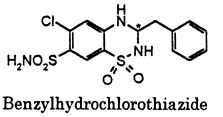
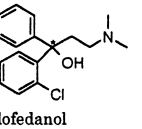
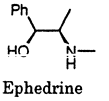
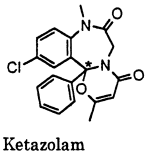
Compounds	Conditions & data
 Alprenolol	CSP : 4-2 [35] 0.1M NaH_2PO_4 + CF_3SO_3H aq. (pH 2) / CH_3CN = 75 / 25 0.5 ml/min, r.t. t_1 = 18.48, t_2 = 19.76 k_1' = 2.62, α = 1.10
 Atenolol	CSP : 4-2 [35] 0.1M $NaPF_6$ aq. / CH_3CN = 80 / 20 0.5 ml/min, r.t. t_1 = 8.90, t_2 = 9.48 k_1' = 0.74, α = 1.16
 Metoprolol	CSP : 4-2 [35] 0.1M NaH_2PO_4 + CF_3SO_3H aq. (pH 2) / CH_3CN = 80 / 20 0.5 ml/min, r.t. t_1 = 11.73, t_2 = 12.74 k_1' = 1.30, α = 1.15
 Oxprenolol	CSP : 4-2 [35] 0.1M NaH_2PO_4 + CF_3SO_3H aq. (pH 2) / CH_3CN = 75 / 25 0.5 ml/min, r.t. t_1 = 13.26, t_2 = 14.81 k_1' = 1.60, α = 1.19
 Propranolol	CSP : 4-2 [13] 0.1M $NaPF_6$ aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 16.7, t_2 = 20.5 k_1' = 2.34, α = 1.32
 Benzylhydrochlorothiazide	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm t_1 = 25.18, t_2 = 28.22 k_1' = 11.34, α = 1.13
 Clofedanol	CSP : 5-2 [41] 50mM KPF_6 aq. / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 60.8, t_2 = 67.2 k_1' = 14.2, α = 1.13
 Ephedrine	CSP : 4-2 [35] 0.1M $NaBF_4$ aq. / CH_3CN = 85 / 15 0.5 ml/min, r.t. k_1' = 0.47, α = 1.12
 Ketazolam	CSP : 4-1† [46] 0.25M $NaClO_4$ aq. / CH_3CN = 60 / 40 44 bar (capillary column) 214 nm k_1' = 3.13, α = 1.21
Lorazepam	CSP : 4-1† [46] 0.25M $NaClO_4$ aq. / CH_3CN = 60 / 40 44 bar (capillary column) 214 nm k_1' = 0.90, α = 2.65

Table 4. Continued

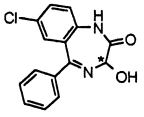
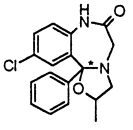
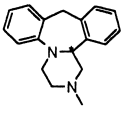
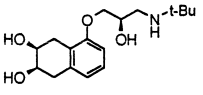
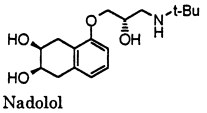
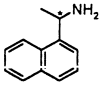
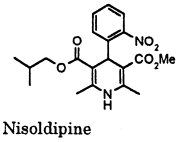
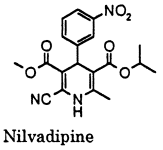
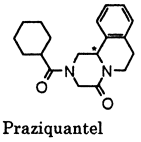
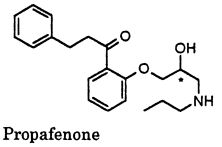
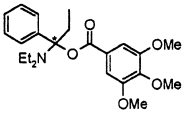
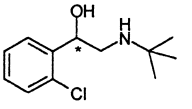
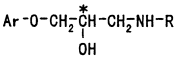
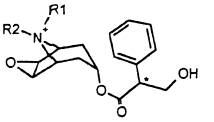
Compounds	Conditions & data
 Oxazepam	CSP : 4-2 [47] 0.3M $NaClO_4$ aq. / CH_3CN = 57 / 43 0.5 ml/min, 210 nm t_1 = 18.44, t_2 = 35.90 k_1' = 2.34, α = 2.34
 Oxazolam	CSP : 5-2 [22] 50mM KPF_6 aq. / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 55.30, t_2 = 67.84 k_1' = 14.36, α = 1.24
 Mianserine	CSP : 5-2 [41] 50mM KPF_6 aq. / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 22.08, t_2 = 24.05 k_1' = 5.68, α = 1.11
 Nadolol	CSP : 7-1 [48] H_2O / EtOH / diethylamine = 25 / 75 / 0.5 0.2 ml/min, 0 °C, 270 nm t_1 = 20.4, t_2 = 39.4
 Nadolol	CSP : 7-1 [48] H_2O / EtOH / diethylamine = 25 / 75 / 0.5 0.2 ml/min, 0 °C, 270 nm t_1 = 24.6, t_2 = 27.3
 1-(1-Naphthyl)ethylamine	CSP : 5-2 [22] 50mM KPF_6 aq. / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 15.85, t_2 = 17.87 k_1' = 3.17, α = 1.17
 Nisoldipine	CSP : 5-2 [22] 100mM KPF_6 aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 15.77, t_2 = 16.01 k_1' = 3.15, α = 1.02
 Nilvadipine	CSP : 8-2 [36] 0.1M $KPF_6 \cdot H_3PO_4$ aq. (pH 2) / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm t_1 = 11.42, t_2 = 11.99 k_1' = 5.04, α = 1.06
 Praziquantel	CSP : 4-2 [49] 0.25M $NaClO_4$ aq. (pH 4) / CH_3CN = 60 / 40 0.7 ml/min, 25 °C, 300 nm t_1 = 24.5, t_2 = 26.0
 Propafenone	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm t_1 = 38.06, t_2 = 48.36 k_1' = 14.0, α = 1.29

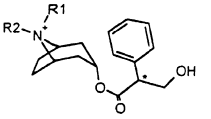
Table 4. Continued

Compounds	Conditions & data
 Trimebutine	CSP : 5-2 [22] 50mM KPF_6 aq. / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 56.30, t_2 = 58.90 k_1' = 14.64, α = 1.05
 Tulobuterol	CSP : 7-2 [36] 0.1M KPF_6 aq. (pH 2) / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 210 nm t_1 = 7.1, t_2 = 8.8 k_1' = 0.87, α = 1.51
 Propranolol analog	CSP : 4-2 [50] 0.1M KPF_6 aq. (pH 6) / CH_3CN = 60 / 40 {*: 0.1M KPF_6 aq. (pH 2.5) / CH_3CN = 50 / 50} 0.7 ml/min, 23 °C 287 nm

Ar	R	
a) 1-naphthyl	$-(CH_2)_3CH_3$	a) α = 1.42
b) 1-naphthyl	$-(CH_2)_2CH_3$	b) α = 1.43
c) 1-naphthyl	$-CH_2CH_3$	c) α = 1.31
d) 1-naphthyl	$-C(CH_3)_3$	d) α = 1.21
e) 1-naphthyl	$-CH_2(CF_2)_2CF_3$	e) α = 1.10*
f) 1-naphthyl	$-CH_2CF_2CF_3$	f) α = 1.04*
g) 1-naphthyl	$-CH_2CF_3$	g) α = 1.10*
h) 2-naphthyl	$-CH_2CH_3$	h) α = 1.12
i) 2-naphthyl	$-(CH_2)_2CH_3$	i) α = 1.10
j) 2-naphthyl	$-(CH_2)_3CH_3$	j) α = 1.13
k) 2-naphthyl	$-C(CH_3)_3$	k) α = 1.17
l) Phenyl	$-CH_2CH_3$	l) α = 1.45
m) Phenyl	$-(CH_2)_2CH_3$	m) α = 1.80
n) Phenyl	$-(CH_2)_3CH_3$	n) α = 1.98
o) Phenyl	$-C(CH_3)_3$	o) α = 1.43

 N,N-Dialkylated noratroscine	CSP : 4-2 [39] 0.05M $NaClO_4$ aq. (pH 2) / CH_3CN = 75 / 25 0.5 ml/min 230 nm

	R1	R2	
a)	Me	Me	a) k_1' = 0.65, α = 1.33
b)	Et	Me	b) k_1' = 0.73, α = 1.89
c)	C_3H_7	Me	c) k_1' = 1.64, α = 1.71
d)	C_4H_9	Me	d) k_1' = 3.23, α = 1.69
e)	C_5H_{11}	Me	e) k_1' = 6.39, α = 1.72
f)	C_6H_{13}	Me	f) k_1' = 13.44, α = 1.86
g)	Me	C_4H_9	g) k_1' = 3.13, α = 1.34
h)	Et	Et	h) k_1' = 1.64, α = 1.47

 N,N-Dialkylated noratropine	CSP : 4-2 [39] 0.05M $NaClO_4$ aq. (pH 2) / CH_3CN = 75 / 25 0.5 ml/min 230 nm

	R1	R2	
a)	Me	Me	a) k_1' = 0.74, α = 1.21
b)	Me	Et	b) k_1' = 1.14, α = 1.59
c)	Me	C_4H_9	c) k_1' = 3.54, α = 2.02
d)	Me	C_5H_{11}	d) k_1' = 5.88, α = 1.27
e)	Me	C_6H_{13}	e) k_1' = 16.16, α = 1.32
f)	Me	C_7H_{13}	f) k_1' = 35.85, α = 1.34
g)	Et	Me	g) k_1' = 1.12, α = 1.19
h)	C_3H_7	Me	h) k_1' = 1.90, α = 1.99
i)	C_4H_9	Me	i) k_1' = 3.57, α = 1.27
j)	C_5H_{11}	Me	j) k_1' = 5.82, α = 1.27
k)	Et	Et	k) k_1' = 1.63, α = 1.54
l)	C_4H_9	C_4H_9	l) k_1' = 10.96, α = 2.32
m)	iPr	Me	m) k_1' = 2.13, α = 2.53
n)	Me	iPr	n) k_1' = 1.87, α = 1.19

[a] †, Homemade CSP; CSP numbers correspond to Fig. 1; References cited in Table [8,13,22,26,35,36,39–50].

Table 5.
Separation examples of the analytes which can be separated under normal-phase conditions[a]

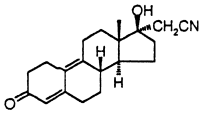
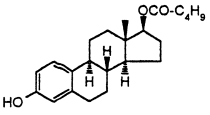
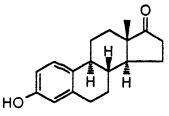
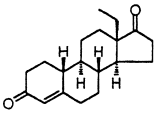
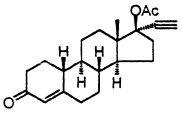
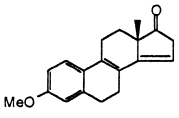
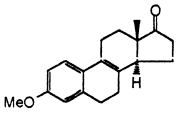
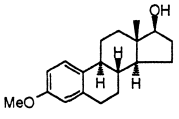
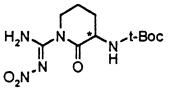
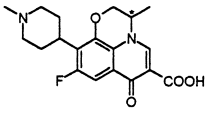
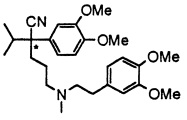
Compounds	Conditions & data
Steroids	
 Dienogest	CSP : 7-1 [26] H_2O / CH_3CN = 60 / 40 0.6 ml/min, 35 °C UV: λmax k_1' = 2.80, α =1.34
 Estradiol-17-valerate	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 1.37, α = 4.11
 Estrone	CSP : 7-1 [26] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C UV: λmax k_1' = 0.22, α = 2.30
 Ethylgonenedione	CSP : 7-1 [44] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C 215 nm & 240 nm t_1 = 6.75, t_2 = 14.6 k_1' = 1.70, α = 2.85
 Norethisterone acetate	CSP : 7-1 [44] H_2O / CH_3CN = 5 / 95 1.4 ml/min, 35 °C 215 nm & 240 nm t_1 = 3.95, t_2 = 4.98 k_1' = 0.58, α = 1.72
 8(9),14-Pentaenone	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 3.39, α = 1.06
 8(9)-Tetraenone	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 1.41, α = 1.10
 Trienol	CSP : 7-1 [26] H_2O / MeOH = 5 / 95 1.0 ml/min, 35 °C UV: λmax k_1' = 0.64, α = 1.63
Alimemazine	CSP : 4-1 [13] 0.1M $NaClO_4$ aq. / CH_3CN = 60 / 40 0.5ml/min, 25 °C, 254 nm t_1 = 22.2, t_2 = 27.3 k_1' = 2.70, α = 1.31
 Boc-N-Arg(NO_2)-Lactam	CSP : 7-1 [42] H_2O / MeOH = 20 / 80 1.0 ml/min, 40 °C 275 nm t_1 = 6.4, t_2 = 8.4

Table 5. Continued

Compounds	Conditions & data
 Ofloxacin	CSP : 8-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 80 / 20 1.0 ml/min, 25 °C, 254 nm t_1 = 16.91, t_2 = 19.81 k_1' = 7.29, α = 1.20
 Verapamil	CSP : 4-3 [40] 0.1M $NaPF_6$ aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 15.6, t_2 = 19.7 k_1' = 2.71, α = 1.36
Epoxides	CSP : 5-2 [51] 1) H_2O / MeOH = 15 / 85 2) H_2O / MeOH = 10 / 90 0.5 ml/min, 215 nm
a) R=nC_6H_{11} b) R=nC_7H_{13} c) R=nC_8H_{15} d) R=nC_9H_{17} e) R=n$C_{10}H_{19}$ f) R=n$C_{11}H_{21}$ g) R=n$C_{12}H_{23}$	a) k_1' = 4.24, α = 1.09 [1)] b) k_1' = 5.60, α = 1.09 [1)] c) k_1' = 7.06, α = 1.10 [1)] d) k_1' = 3.59, α = 1.08 [2)] e) k_1' = 4.38, α = 1.07 [2)] f) k_1' = 5.18, α = 1.07 [2)] g) k_1' = 6.02, α = 1.07 [2)]
a) R=nC_6H_{11} b) R=nC_7H_{13} c) R=nC_8H_{15} d) R=nC_9H_{17} e) R=n$C_{10}H_{19}$ f) R=n$C_{11}H_{21}$ g) R=n$C_{12}H_{23}$	a) k_1' = 4.07, α = 1.18 [1)] b) k_1' = 5.38, α = 1.24 [1)] c) k_1' = 7.00, α = 1.27 [1)] d) k_1' = 3.30, α = 1.23 [2)] e) k_1' = 4.08, α = 1.20 [2)] f) k_1' = 4.84, α = 1.19 [2)] g) k_1' = 5.66, α = 1.17 [2)]
a) R=nC_6H_{11} b) R=nC_7H_{13} c) R=nC_8H_{15} d) R=nC_9H_{17}	a) k_1' = 4.68, α = 1.08 [1)] b) k_1' = 5.98, α = 1.07 [1)] c) k_1' = 7.60, α = 1.04 [1)] d) k_1' = 3.68, α = 1.03 [2)]

[a] CSP numbers correspond to Fig. 1. References cited in Table [13,26,36,40,42,44,51].

and reversed-phase conditions were compared for series of propranolol analogs [91] and steroids [26]. Judging from these results as well as practical experience, roughly 70% of the samples can be separated under both normal and reversed-phase conditions, which is quite surprising. Among these less than 10% of enantiomers showed reversal of elution order when changing from normal to reversed-phase conditions. These results suggests that the separation mechanisms seem to be quite similar under both normal and reversed-phase conditions despite the reduced contribution of hydrogen bonding at the carbamate and ester moieties under the reversed-phase conditions. On the other hand, there

Table 6
Other separation examples[a]

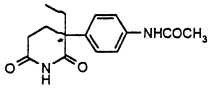
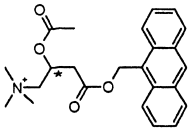
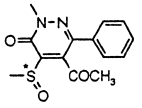
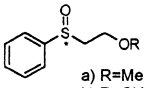
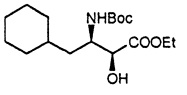
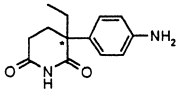
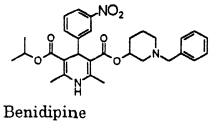
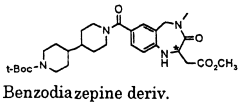
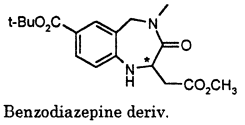
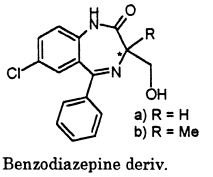
Compounds	Conditions & data
 Acetylaminoglutethimide	CSP : 5-2 [52] H_2O/ CH_3CN = 75 / 25 0.7 ml/min, 287 nm k_1' = 1.13, α = 1.44
 Acetylcarnitine ADAM deriv	CSP : 4-2 [53] 0.5M $NaClO_4$ / CH_3CN = 30 / 20 0.8 ml/min, 25 °C, 254 nm t_1 = 23.8, t_2 = 28.3
 5-Acetyl-2-methyl-4-methylthio-6-phenyl-3(2H)-pyridazinone	CSP : 4-2 [54] H_2O / MeOH = 10 / 90 0.5 ml/min, 244 nm t_1 = 11.86, t_2 = 21.23
 a) R=Me b) R=CH_2OMe c) R=CH_2OBu d) R=Ac 2-Alkoxyethylphenyl sulfide	CSP : 2 [57] H_2O / MeOH = 60 / 40 (*: H_2O / MeOH = 40 / 60) 0.5 ml/min a) t_1 = 45, t_2 = 62 b) t_1 = 42, t_2 = 57 c) t_1 = 57, t_2 = 85 d) t_1 = 25, t_2 = 33*
 3-Amino-4-cyclohexyl-2-hydroxybutanoic acid deriv.	CSP : 4-2 [56] 0.5M $NaClO_4$ / MeOH = 70 / 30 0.5 ml/min t_1 = 22.6, t_2 = 27.7
 Aminoglutethimide	CSP : 5-2 [52] H_2O/ CH_3CN = 70 / 30 0.7 ml/min, 287 nm k_1' = 1.3, α = 1.9
 Benidipine	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 60/40 0.5 ml/min, 25 °C, 254 nm t_1 = 80.75, t_2 = 90.85 k_1' = 24.72, α = 1.13
 Benzodiazepine deriv.	CSP : 4-2 [57] MeOH 0.6 ml/min, 254 nm t_1 = 16.3, t_2 = 19.1
 Benzodiazepine deriv.	CSP : 4-2 [57] H_2O / MeOH = 25 / 75 0.6 ml/min, 254 nm t_1 = 18.2, t_2 = 20.6
 a) R = H b) R = Me Benzodiazepine deriv.	CSP : 4-2 [58] NaClO4 - HClO4 (pH 2) / CH3CN = 70 / 30 a) k_1' = 2.13, α = 1.41 b) k_1' = 3.98, α = 1.08

Table 6. Continued

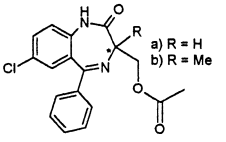
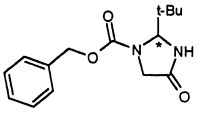
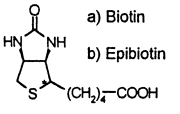
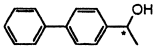
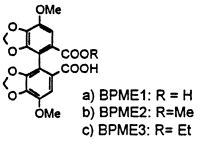
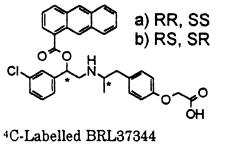
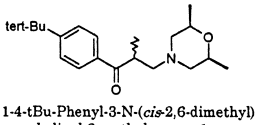
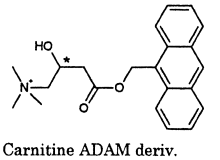
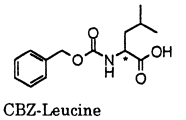
Compounds	Conditions & data
 a) R = H b) R = Me Benzodiazepine deriv.	CSP : 4-2 [58] a) NaClO4 - HClO4 (pH 2) / CH3CN = 50 / 50 k_1' = 1.71, α = 2.15 b) NaClO4 - HClO4 (pH 2) / CH3CN = 30 / 70 k_1' = 1.02, α = 1.23
 Benzyl 2-tert-butyl-4-oxo-imidazolidine-1-carboxylate	CSP : 4-2 [59] 0.5M $HClO_4$ + NaOHaq. (pH 2) / MeOH = 80 / 20 0.5 ml/min, r.t., 205 nm t_1 = 13.54, t_2 = 18.33 k_1' = 1.22, α = 1.64
 a) Biotin b) Epibiotin	CSP : 4-2 [60] 0.05M KH_2PO_4 (pH 2.5) / CH3CN = 90 / 10 0.4 ml/min, 220 nm a) t_1 = 35, t_2 = 40 b) t_1 = 44, t_2 = 47
 1-(p-Biphenyl)ethanol	CSP : 7-2 [36] H_2O / CH_3CN = 50 / 50 1.0 ml/min, 25 °C, 254 nm t_1 = 7.68, t_2 = 9.56 k_1' = 3.04, α = 1.33
 a) BPME1: R = H b) BPME2: R=Me c) BPME3: R= Et	CSP : 4-1[f] [61] 0.25M $NaHPO_4$ aq. / CH_3CN = 70 / 30 0.5 ml/min, 254 nm a) k_1' = 2.33, α = 1.56 b) k_1' = 6.21, α = 1.36 c) k_1' = 8.47, α = 1.17
 a) RR, SS b) RS, SR ^{4}C-Labelled BRL37344 1-anthroyl derivative	CSP : 4-2 [62] 100mM Phosphate buffer (pH 2) / CH3CN = 65 / 35 0.5 ml/min, 30 °C, 260 nm a) t_1 = 58, t_2 = 73 b) t_1 = 51, t_2 = 86
 1-4-tBu-Phenyl-3-N-(*cis*-2,6-dimethyl) morpholinyl-2-methylpropan-1-one	CSP : 4-2 [63] H_2O / MeOH = 15 / 85 0.5 ml/min t_1 = 15.1, t_2 = 16.2 k_1' = 2.02, α = 1.11
 Carnitine ADAM deriv.	CSP : 4-2 [53] 0.5M $NaClO_4$ / CH_3CN = 30 / 20 0.8 ml/min, 25 °C, 254 nm t_1 = 16.80, t_2 = 21.20
 CBZ-Leucine	CSP : 4-2 [13] 0.5M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 17.6, t_2 = 19.2 k_1' = 2.52, α = 1.13 CSP : 7-2 [37] H_3PO_4 aq. (pH 2) / CH_3CN = 70 / 30 1.0 ml/min, 25 °C, 254 nm t_1 = 12.51, t_2 = 14.32 k_1' = 5.58, α = 1.17

Table 6. Continued

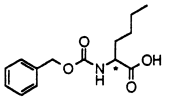
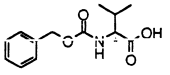
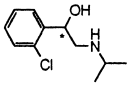
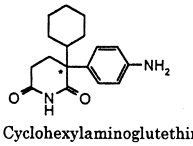
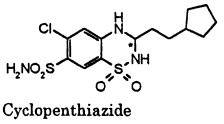
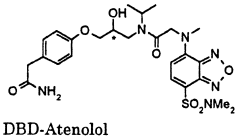
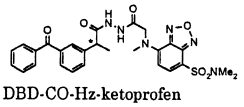
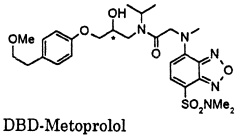
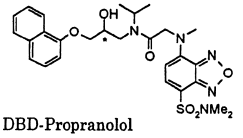
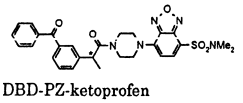
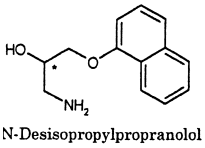
Compounds	Conditions & data
 CBZ-Norleucine	CSP : 7-2 [36] H_3PO_4 aq. (pH 2) / CH_3CN 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 35.62, t_2 = 40.45 k_1' = 8.37, α = 1.15
 CBZ-Valine	CSP : 4-2 [13] 0.5M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 12.8, t_2 = 14.0 k_1' = 1.56, α = 1.15
 Chlorprenaline	CSP : 7-2 [36] 20mM Borate buffer (pH 9 CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 10.22, t_2 = 11.25 k_1' = 1.69, α = 1.16
 Cyclohexylaminoglutethimide	CSP : 5-2 [52] H_2O/ CH_3CN = 73 / 27 0.7 ml/min, 287 nm k_1' = 11.82, α = 1.12
 Cyclopenthiazide	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm t_1 = 7.16, t_2 = 8.48 k_1' = 2.51, α = 1.26
 DBD-Atenolol	CSP : 5-2 [64] MeOH 0.5 ml/min 450 nm (Ex), 560 (Em) k_1' = 1.44, α = 1.53
 DBD-CO-Hz-ketoprofen	CSP : 4-2 [65] H_2O / CH_3CN = 35 / 65 0.5 ml/min t_1 = 10.5, t_2 = 14.8
 DBD-Metoprolol	CSP : 5-2 [64] MeOH 0.5 ml/min 450 nm (Ex), 560 (Em) k_1' = 4.62, α = 1.55
 DBD-Propranolol	CSP : 4-2 [64] H_2O / CH_3CN = 20 / 80 0.5 ml/min 450 nm (Ex), 560 (Em) k_1' = 1.75, α = 1.60
 DBD-PZ-ketoprofen	CSP : 4-2 [65] H_2O / CH_3CN = 35 / 65 0.5 ml/min t_1 = 27.3, t_2 = 28.6 Ref. : 24 c)
 N-Desisopropylpropranolol	CSP : 4-2 [66] 0.5N $HClO_4$ buffer (pH 2) / CH_3CN / MeOH = 65 / 30 / 5 0.5 ml/min 310 nm (Ex), 340 (Em) t_1 = 11, t_2 = 13

Table 6. Continued

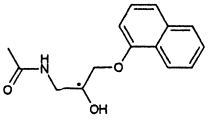
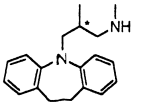
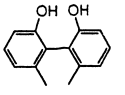
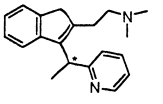
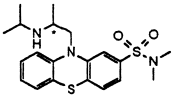
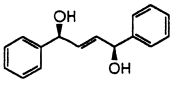
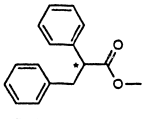
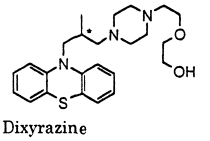
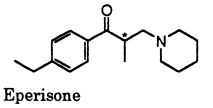
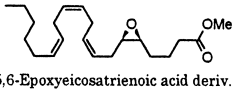
Compounds	Conditions & data
 N-Desisopropylpropranolol deriv.	CSP : 4-2 [66] 0.5N $HClO_4$ buffer (pH 2) / CH_3CN / MeOH = 65 / 30 / 5 0.5 ml/min 310 nm (Ex), 340 (Em) t_1 = 34.5, t_2 = 38
 Desmethyl trimipramine	CSP : 4-2 [67] 0.3M $NaClO_4$ aq. / CH_3CN = 58 / 42 0.50 ml/min, 210 nm t_1 = 16.2, t_2 = 18.0
 2, 2'-Dihydroxy-6, 6'-dimethyl-1, 1'-biphenyl	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 9.86, t_2 = 10.78 k_1' = 1.59, α = 1.15
 Dimethindene	CSP : 5-2 [36] 100mM KPF6aq. / CH_3CN = 70/30 0.5 ml/min., 25 °C, 254 nm t_1 = 23.58, t_2 = 24.42 k_1' = 5.21, α = 1.04
 Dimethothiazine	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 70 / 30 1.0 ml/min, 25 °C, 254 nm t_1 = 26.07, t_2 = 29.34 k_1' = 12.79, α = 1.14
 1,4-Diphenylbut-2-ene-1,4-diol	CSP : 4-2 [68] H_2O / MeOH = 90 / 10 0.9 ml/min t_1 = 8.10, t_2 = 9.21
 2,3-Diphenylpropionic acid methyl ester	CSP : 5-2 [69] H_2O / CH_3CN = 45 / 55 0.5 ml/min t_1 = 16.30, t_2 = 20.50 k_1' = 6.76, α = 1.30
 Dixyrazine	CSP : 4-2 [27] 1M $NaClO_4$ aq. / CH_3CN / MeOH = 40 / 15 / 45 0.5 ml/min k_1' = 9.33, α = 1.36
 Eperisone	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm t_1 = 15.03, t_2 = 17.17 k_1' = 2.96, α = 1.19
 5,6-Epoxyeicosatrienoic acid deriv.	CSP : 2 [70] H_2O / EtOH = 30 / 70 1.0 ml/min t_1 = 47.6, t_2 = 58.4

Table 6. Continued

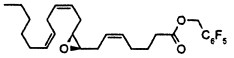
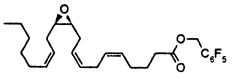
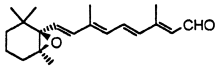
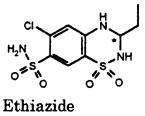
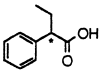
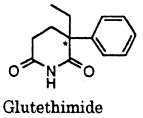
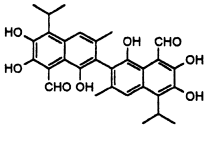
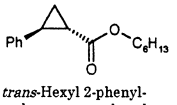
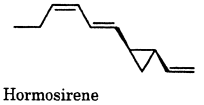
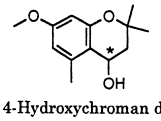
Compounds	Conditions & data
 8,9-Epoxyeicosatrienoic acid deriv.	CSP : 2 [70] H_2O / EtOH = 30 / 70 1.0 ml/min t_1 = 59.5, t_2 = 77.2
 11,12-Epoxyeicosatrienoic acid deriv.	CSP : 2 [70] H_2O / EtOH = 23 / 77 1.1 ml/min t_1 = 19.0, t_2 = 29.4
 5,6-Epoxyetinal	CSP : 6 [71] H_2O / CH_3CN = 55 / 45 1.5 ml/min, 360 nm t_1 = 39, t_2 = 48
 Ethiazide	CSP : 5-2 [62] 100mM KPF6aq. / CH_3CN = 80/20 0.5 ml/min, 25 °C, 254 nm t_1 = 12.67, t_2 = 14.42 k_1' = 2.33, α = 1.20
 1-Ethyl-phenylacetic acid	CSP : 4-2 [13] 0.5M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 15.3, t_2 = 16.4 k_1' = 2.06, α = 1.11
 Glutethimide	CSP : 4-1 [18] H_2O/ CH_3CN = 65 / 35 0.5 ml/min, 210 nm k_1' = 1.33, α = 1.21 CSP : 5-2 [53] H_2O/ CH_3CN = 65 / 35 0.5 ml/min, 287 nm k_1' = 3.00, α = 2.14
 Gossypol	CSP : 4-1† [72] 10mM KH_2PO_4 buffer (pH 3) / CH_3CN = 60 / 40 1.0 ml/min, 287 nm k_1' = 11.30, α = 1.43
 *trans*-Hexyl 2-phenyl-cyclopropane carboxylate	CSP : 4-2 [73] H_2O (pH 4 $HClO_4$) / MeOH = 20 / 80 0.4 ml/min, 280 nm t_1 = 54.03, t_2 = 65.76
 Hormosirene	CSP : 2 [74] H_2O / MeOH = 20 / 80 1.2 ml/min, 247 nm t_1 = 17.3, t_2 = 22.5 k_1' = 3.02, α = 1.40
 4-Hydroxychroman derivative	CSP : 4-2 [75] H_2O / MeOH = 40 / 60 0.5 ml/min, 40 °C t_1 = 22.4, t_2 = 23.9

Table 6. Continued

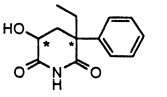
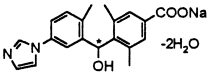
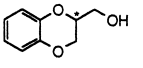
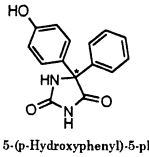
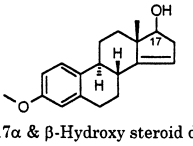
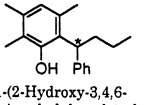
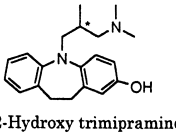
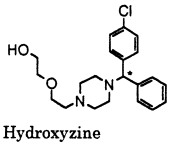
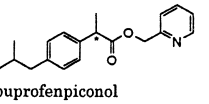
Compounds	Conditions & data
 5-Hydroxy-glutethimide	CSP : 4-2 [18] H_2O/ CH_3CN = 65 / 35 0.5 ml/min, 210 nm k_1' = 1.0, α = 1.14
 4-[α-Hydroxy-5-(1-imidazoyl)-2-methylbenzyl]-3,5-dimethyl-benzoate dihydrate	CSP : 4-1 [12] $NaClO_4$-HClO4 (pH 3.5) / CH_3CN = 80 / 20 1.0 ml/min, 25 °C k_1' = 4.64, α = 1.57
 2-Hydroxymethyl-1, 4-benzodioxane	CSP : 7-2 [37] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 7.74, t_2 = 8.57 k_1' = 1.04, α = 1.20
 5-(p-Hydroxyphenyl)-5-phenylhydantoin	CSP : 5-1 [76] EtOH / H_2O = 70 / 30 0.5 ml/min t_1 = 10.0, t_2 = 15.0
 17α & β-Hydroxy steroid deriv.	CSP : 5-1 [77] H_2O / CH_3CN = 40 / 60 1.0 ml/min t_1 = 9.3 (β), t_2 = 12.6 (α)
 1-(2-Hydroxy-3,4,6-trimethylphenyl-1-phenylbutane	CSP : 4-2 [78] H_2O / CH_3CN = 25 / 75 0.5 ml/min, r.t., 235 nm t_1 = 14.31, t_2 = 15.62
 2-Hydroxy trimipramine	CSP : 4-2 [67] 0.3M $NaClO_4$ aq. / CH_3CN = 58 / 42 0.50 ml/min, 210 nm t_1 = 10.7, t_2 = 11.2
 Hydroxyzine	CSP : 4-2 [13] 0.1M $NaPF_6$ aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 20.6, t_2 = 23.3 k_1' = 3.12, α = 1.17
 Ibuprofenpiconol	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm t_1 = 13.98, t_2 = 15.33 k_1' = 2.68, α = 1.13

Table 6. Continued

Table 6. Continued

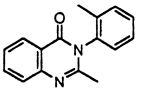
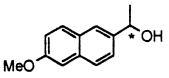
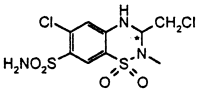
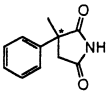
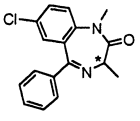
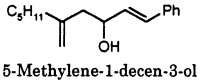
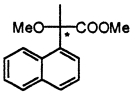
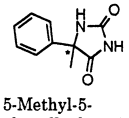
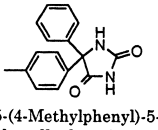
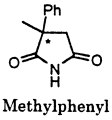
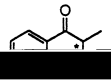
Compounds	Conditions & data
 Methaqualone	CSP : 4-2 [13] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 40 °C, 254 nm t_1 = 40.9, t_2 = 78.6 k_1' = 7.18, α = 2.05
 1-(6'-Methoxynaphthyl)ethanol	CSP : 4-1† [61] 1.0M $NaClO_4$ aq. (pH 2/ CH_3CN = 60 / 40 0.5 ml/min, 254 nm k_1' = 12.23, α = 1.15
 Methychlothiazide	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 80 / 20 1.0 ml/min, 25 °C, 254 nm t_1 = 27.76, t_2 = 29.79 k_1' = 12.61, α = 1.08
 α-Methyl-α-phenylsuccinimide	CSP : 7-2 [36] H_2O / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm t_1 = 10.78, t_2 = 29.12 k_1' = 1.84, α = 3.55
 3-Methyldiazepam	CSP : 3† [46] 0.25M $NaClO_4$ aq. / CH_3CN = 60 / 40 44 bar (capillary column) 214 nm k_1' = 3.02, α = 1.53
 5-Methylene-1-decen-3-ol	CSP : 4-2 [81] H_2O / MeOH = 10 / 90 0.5 ml/min 250 nm t_1 = 17.7, t_2 = 20.4
 Methyl 2-methoxy-2-(1-naphthyl)propanoate	CSP : 4-3 [82] H_2O / CH_3CN = 48 / 52 t_1 = 15.8, t_2 = 16.6
 5-Methyl-5-phenylhydantoin	CSP : 5-2 [62] H_2O / CH_3CN = 70 / 30 1.0 ml/min, 25 °C, 254 nm t_1 = 3.75, t_2 = 5.81 k_1' = 0.79, α = 2.24
 5-(4-Methylphenyl)-5-phenylhydantoin	CSP : 5-2 [62] H_2O / CH_3CN = 70 / 30 1.0 ml/min, 25 °C, 254 nm t_1 = 16.27, t_2 = 19.88 k_1' = 6.74, α = 1.26
 Methylphenyl succimide	CSP : 4-2 [13] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 40 °C, 254 nm t_1 = 10.3, t_2 = 15.2 k_1' = 1.06, α = 1.92
	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 1.0 ml/min, 25 °C, 254 nm

Table 6. Continued

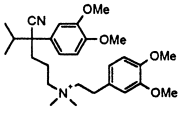
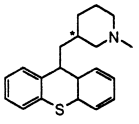
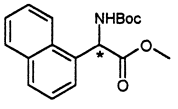
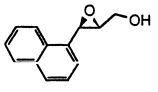
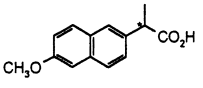
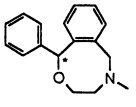
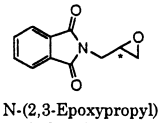
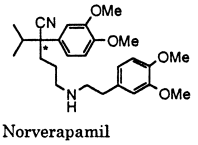
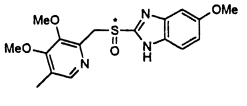
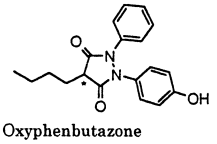
Compounds	Conditions & data
 N-Methyl verapamil	CSP : 4-2 [13] 0.5M $NaClO_4$ aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 15.0, t_2 = 18.2 k_1' = 2.00, α = 1.32
 Metixene	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 30 / 70 1.0 ml/min, 25 °C, 254 nm t_1 = 5.35, t_2 = 15.01 k_1' = 1.82, α = 3.79
 Naphthylglycine deriv.	CSP : 4-2 [83] 0.5M $NaClO_4$ / MeOH = 25 / 75 0.5 ml/min t_1 = 23.34, t_2 = 26.26
 3-(1-Naphthyl)-2-propen-1-ol	CSP : 4-2 [83] 0.5M $NaClO_4$ / MeOH = 10 / 90 0.5 ml/min t_1 = 13.1, t_2 = 19.0
 Naproxen	CSP : 7-2 [36] H_3PO_4 aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 14.22, t_2 = 15.96 k_1' = 2.74, α = 1.16
 Nefopam	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 40 / 60 1.0 ml/min, 25 °C, 254 nm t_1 = 3.16, t_2 = 3.66 k_1' = 0.66, α = 1.40
 N-(2,3-Epoxypropyl) phthalimide	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 14.14, t_2 = 15.26 k_1' = 2.72, α = 1.11
 Norverapamil	CSP : 4-1 [30] 0.05M $NaClO_4$-$HClO_4$ aq. (pH 3) / CH_3CN = 67 / 33 1.0 ml/min k_1' = 4.41, α = 1.60
 Omeprazole	CSP : 5-2 [80] 50mM $NaClO_4$ / CH_3CN = 80 / 20 0.5 ml/min, 40 °C 286 nm k_1' = 2.64, α = 1.13
 Oxyphenbutazone	CSP : 4-2 [49] 0.25M $NaClO_4$ aq. (pH 4) / CH_3CN = 60 / 40 0.7 ml/min, 25 °C, 300 nm t_1 = 13.0, t_2 = 15.1

Table 6. Continued

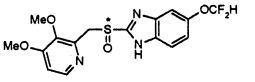
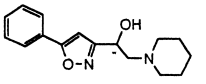
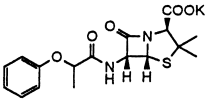
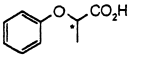
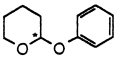
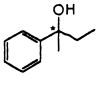
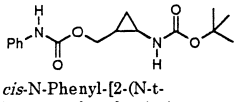
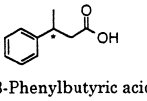
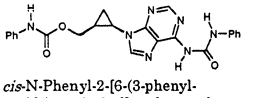
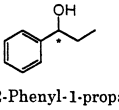
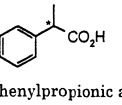
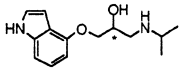
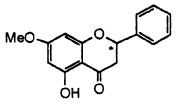
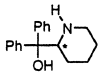
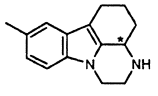
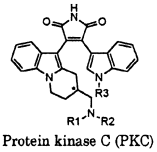
Compounds	Conditions & data
 Pantoprazole	CSP : 5-2 [80] 50mM $NaClO_4$ / CH_3CN = 75 / 25 0.5 ml/min, 40 °C 286 nm k_1' = 2.11, α = 1.26
 Perisoxal	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 25.22, t_2 = 45.42 k_1' = 5.64, α = 1.93
 Phenethicillin potassium	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 100 / 2 1.0 ml/min, 25 °C, 254 nm t_1 = 26.93, t_2 = 36.16 k_1' = 12.2, α = 1.37
 Phenoxypropionic acid	CSP : 5-2 [41] Phosphate buffer (pH 2) / CH_3CN = 80 / 20 0.5 ml/min, 25 °C, 254 nm t_1 = 26.1, t_2 = 27.6 k_1' = 5.87, α = 1.07
 2-Phenoxytetrahydropyran	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 25.54, t_2 = 28.11 k_1' = 5.72, α = 1.12
 2-Phenyl-2-butanol	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 12.82, t_2 = 14.14 k_1' = 2.37, α = 1.15
 *cis*-N-Phenyl-[2-(N-t-butoxycarbonylamino)-cyclopropyl]-methyl carbamate	CSP : 4-2 [84] H_2O (pH 4 $HClO_4$) / MeOH = 15 / 85 0.4 ml/min, 280 nm t_1 = 16.52, t_2 = 21.79
 3-Phenylbutyric acid	CSP : 4-2 [13] 0.5M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 13.0, t_2 = 14.2 k_1' = 1.60, α = 1.15
 *cis*-N-Phenyl-2-[6-(3-phenyl-ureido)-purin-9-yl]-cyclopropyl methyl carbamate	CSP : 4-2 [84] H_2O (pH 4 $HClO_4$) / CH_3CN = 30 / 70 0.4 ml/min, 280 nm t_1 = 18.9, t_2 = 26.3
 2-Phenyl-1-propanol	CSP : 7-2 [36] H_2O / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 7.84, t_2 = 8.60 k_1' = 1.06, α = 1.18
 Phenylpropionic acid	CSP : 5-2 [41] Phosphate buffer (pH 2) / CH_3CN = 70 / 30 0.5 ml/min, 25 °C, 254 nm t_1 = 18.0, t_2 = 19.5 k_1' = 3.74, α = 1.10

Table 6. Continued

Compounds	Conditions & data
2-Phenyltetrahydropyran-4-one	CSP : 5-2 [85] 0.05M NaH_2PO_4 (pH 7) / CH3CN = 80 / 20 1.0 ml/min t_1 = 57, t_2 = 68
 Pindolol	CSP : 4-2 [13] 0.1M $NaPF_6$ aq. / CH_3CN = 60 / 40 0.5 ml/min, 25 °C, 254 nm t_1 = 11.3, t_2 = 19.2 k_1' = 1.26, α = 2.25
 Pinocembrin-7-methylether	CSP : 7-2 [36] H_2O / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm t_1 = 30.68, t_2 = 53.62 k_1' = 7.07, α = 1.85
 Pipradolol	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 80 / 20 1.0 ml/min, 25 °C, 254 nm t_1 = 24.16, t_2 = 29.20 k_1' = 11.78, α = 1.23
 Pirlindole	CSP : 4-2 [86] 50mM Phosphate buffer - 500mM $NaClO_4$ (pH 5) / CH_3CN = 55 / 45 0.5 ml/min, 220 nm k_1' = 0.59, α = 2.02
 Protein kinase C (PKC) inhibitor	CSP : 5-2 [87] 0.05M Et_3N-H_3PO_4 aq. (pH 2.5) / CH_3CN = 75 / 25 0.5 ml/min 215 nm & 254 nm

	R1	R2	R3	
a)	H	H	Me	a) k_1' = 3.64, α = 1.15
b)	H	Me	Me	b) k_1' = 4.78, α = 1.20
c)	Me	Me	Me	c) k_1' = 4.34, α = 1.13
d)	iPr	iPr	Me	d) k_1' = 12.28, α = 1.28
e)	Me	CH_2Ph	Me	e) k_1' = 26.62, α = 1.12
f)	Me	Me	n-C_5H_{11}	f) k_1' = 31.54, α = 1.07
g)	Me	iPr	Me	g) k_1' = 7.54, α = 1.18
h)	nPr	nPr	Me	h) k_1' = 21.40, α = 1.18
i)	H	iPr	Me	i) k_1' = 6.40, α = 1.27
j)	Et	Et	Me	j) k_1' = 7.16, α = 1.15
k)	H	tBu	Me	k) k_1' = 7.80, α = 1.35
l)	Me	Me	H	l) k_1' = 2.94, α = 1.06
m)	H	nPr	Me	m) k_1' = 7.36, α = 1.30
n)	H	H	n-C_5H_{11}	n) k_1' = 27.02, α = 1.05

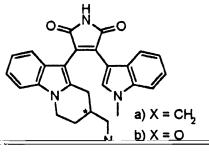
 a) X = CH_2 b) X = O	CSP : 5-2 [87] 0.05M Et_3N-H_3PO_4 aq. (pH 2.5) / CH_3CN = 75 / 25 0.5 ml/min 215 nm & 254 nm

Table 6. Continued

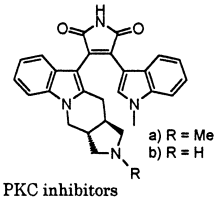
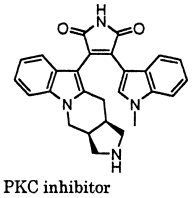
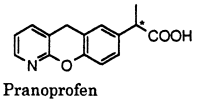
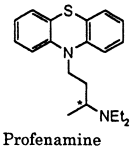
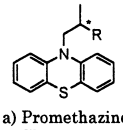
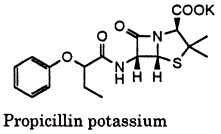
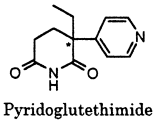
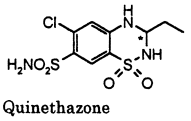
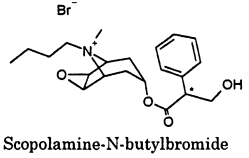
Compounds	Conditions & data
 a) R = Me b) R = H PKC inhibitors	CSP : 5-2 [87] 0.05M Et_3N-H_3PO_4 aq. (pH 2.5) / CH_3CN = 75 / 25 0.5 ml/min 215 nm & 254 nm a) k_1' = 4.56, α = 1.28 b) k_1' = 3.58, α = 1.28
 PKC inhibitor	CSP : 5-2 [87] 0.05M Et_3N-H_3PO_4 aq. (pH 2.5) / CH_3CN = 75 / 25 0.5 ml/min 215 nm & 254 nm k_1' = 4.42, α = 1.04
 Pranoprofen	CSP : 4-1 [12] H_2O / CH_3CN / CH_3COOH = 75 / 25 /0.05 1.0 ml/min, 25 °C t_1 = 31.7, t_2 = 34.5 k_1' = 7.6, α = 1.10
 Profenamine	CSP : 7-2 [36] 20mM Borate buffer (pH 9) / CH_3CN = 40 / 60 0.5 ml/min, 25 °C, 254 nm t_1 = 16.34, t_2 = 17.66 k_1' = 3.30, α = 1.10
 a) Promethazine: R=NMe_2 b) Thiazonamium: R=N^+Me_3 c) Ethopropazine: R=NEt_2 d) Trimeprazine: R=CH_2NMe_2	CSP : 5-2 [27] 1M $NaClO_4$ aq. / CH_3CN / MeOH = 40 / 15 / 45 0.5 ml/min a) k_1' = 4.49, α = 2.20 b) k_1' = 5.49, α = 1.98 c) k_1' = 2.90, α = 1.38 d) k_1' = 4.68, α = 1.34
 Propicillin potassium	CSP : 8-2 [36] 0.1M KPF_6 - H_3PO_4 aq. (pH 2) / CH_3CN = 100 / 2 1.0 ml/min, 25 °C, 254 nm t_1 = 10.51, t_2 = 11.43 k_1' = 4.15, α = 1.11
 Pyridoglutethimide	CSP : 5-2 [52] H_2O/ CH_3CN = 80 / 20 0.7 ml/min, 287 nm k_1' = 0.93, α = 1.73
 Quinethazone	CSP : 8-2 [36] 0.1M KPF_6 aq. / CH_3CN = 80 / 20 1.0 ml/min, 25 °C, 254 nm t_1 = 4.64, t_2 = 5.08 k_1' = 1.27, α = 1.17
 Scopolamine-N-butylbromide	CSP : 4-2 [88] 1 M $NaClO_4$ (pH 2) / CH_3CN = 60 / 40 1 ml/min t_1 = 6.01, t_2 = 7.45 k_1' = 1.49, α = 1.40

Table 7
Validated separation examples[a]

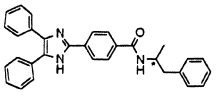
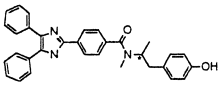
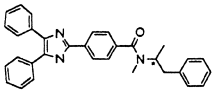
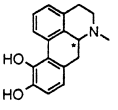
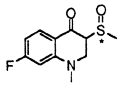
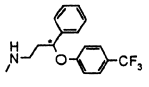
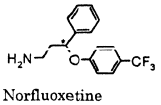
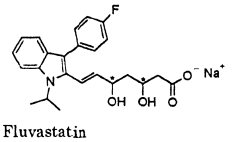
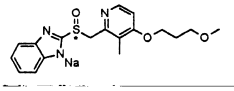
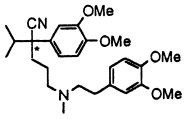
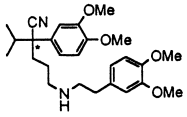
Compounds	Conditions	Linear Range	Sensitivity LOQ/LOD	Accuracy (error, %)	Precision (C.V., %)	Sample
 Amphetamine Deriv.	CSP : 4-2 [92] 2-PrOH / CH_3CN / Na_2HPO_4-citric acid (pH=4.5) = 1 / 59 / 40 35 °C 330 nm (Ex), 440 nm (Em) t_1 = 35, t_2 = 37.5	0.05 - 100 µmol/l	LOD 3.1-3.2 µmol/l (S/N=3)		intra-day 4.4 - 4.5 inter-day 5.9 - 6.0	human urin
 Hydroxymethamphetamine Deriv.	CSP : 4-2 [92] 2-PrOH / CH_3CN / Na_2HPO_4-citric acid (pH=4.5) = 1 / 59 / 40 35 °C 330 nm (Ex), 440 nm (Em) t_1 = 18, t_2 = 19	0.05 - 100 µmol/l	LOD 2.8 µmol/l (S/N=3)		intra-day 6.1 - 7.4 inter-day 6.0 - 7.2	human urin
 Methamphetamine deriv.	CSP : 4-2 [92] 2-PrOH / CH_3CN / Na_2HPO_4-citric acid (pH=4.5) = 1 / 59 / 40 35 °C 330 nm (Ex), 440 nm (Em) t_1 = 40.4, t_2 = 41.8	0.05 - 100 µmol/l	LOD 8.0-8.8 µmol/l (S/N=3)		intra-day 6.1 - 6.7 inter-day 4.3 - 4.9	human urin
 Apomorphine	CSP : 4-2 [93] 0.05M $NaClO_4$-$HClO_4$ aq. (pH 2) / CH_3CN = 65 / 35 0.5 ml/min, 273 nm t_1 = 9.0, t_2 = 10.8	10 - 1000 ng/ml	LOQ 10 ng/ml	0.3 - 6.9	0.2 - 4.7	human serum
 Flosequinan	CSP : 4-1 [94] EtOH / MeOH = 22 / 78 0.7 ml/min, 320 nm t_1 = 7.9, t_2 = 12.1	5 - 10000 ng/ml		0.4 - 11.4	0.3 - 13.6	human plasma
 Fluoxetine	CSP : 4-2 [95] 0.3M $NaClO_4$-$HClO_4$ (pH 2.5) / CH_3CN / Et3N = 34 / 66 / 0.5 0.6 ml/min, 220 nm (Two columns in series were used and the second column was heated at 40 °C.) t_1 = 41.6, t_2 = 44.6	10 - 2000 ng/ml	LOD 10 ng/ml (S/N=3)		intra-day 3.6 - 3.9 inter-day 3.8 - 4.0	human plasma
 Norfluoxetine	CSP : 4-2 [95] 0.3M $NaClO_4$-$HClO_4$ (pH 2.5) / CH_3CN / Et3N = 34 / 66 / 0.5 0.6 ml/min, 220 nm (Two columns in series were used and the second column was heated at 40 °C.) t_1 = 37.0, t_2 = 40.0	10 - 2000 ng/ml	LOD 10 ng/ml (S/N=3)		intra-day 2.5 - 2.9 inter-day 2.9 - 3.1	human plasma
 Fluvastatin	CSP : 4-2 [96] Phosphate buffer (pH 2.5) / CH_3CN = 60 / 40, 0.5 ml/min, 15 °C 305 nm (Ex), 390 nm (Em) t_1 = 28.3, t_2 = 30.3 k_1' = 2.86, α = 1.10	1 - 5000 nmol/l	LOQ 5 nmol/l		3.8	human plasma
	CSP : 4-2 [28] 0.5M NH_4ClO_4 (pH 7.8) / CH_3CN = 80 / 20 - 20 / 80 (linear gradient)	0.03 - 20 µg/ml or 0.1 - 100	LOQ 30 ng/ml	intra-day -3 - 6.7 inter-day -3 - 4	intra-day 3.1 - 12.5 inter-day 0.9 - 4.1	dog & rat plasma

Table 7. Continued

Compounds	Conditions	Linear Range	Sensitivity LOQ/LOD	Accuracy (error, %)	Precision (C.V., %)	Sample
 Verapamil	CSP : 4-2 [104] 0.2M $NaClO_4$ aq. / CH_3CN = 60 / 40 0.8 ml/min 230 nm (Em), 312 nm (Ex) t_1 = 21.8, t_2 = 24.2	2.5 - 300 ng/ml	LOQ 3 ng/ml		intra-day 5.2 - 12.9 inter-day 8.5 - 13.1	human urine
 Norverapamil	CSP : 4-2 [104] 0.2M $NaClO_4$ aq. / CH_3CN = 60 / 40 0.8 ml/min 230 nm (Em), 312 nm (Ex) t_1 = 14.5, t_2 = 18.7	2.5 - 300 ng/ml	LOQ 3 ng/ml		intra-day 3.1 - 11.3 inter-day 4.8 - 14.2	human urine

[a] LOQ, limits of quantitation; LOD, limits of detection; CSP numbers correspond to Fig. 1; References cited in Table [28,49,86,92–104].

4.2. Preparative separation

Polysaccharide type CSPs have been widely used for preparative purposes using normal-phase conditions. However, only a few preparative separations have been reported with the use of reversed-phase conditions. Not only polar organic phases which were mentioned in the earlier section but also simple water/methanol mixtures [107] or volatile acid (aq.)/acetonitrile mixtures [32] are attractive mobile phases for sample preparation, although the aqueous mixtures require more energy to be evaporated and the use of acid may lead to hydrolysis and racemization. In addition, it is even dangerous if a perchlorate buffer is used as a mobile phase for a preparative purpose [31] because perchlorate may explode when it is heated and evaporated with an organic solvent.

5. Conclusion

It is evident that polysaccharide type CSPs have a great separation ability under reversed-phase conditions by choosing an appropriate mobile phase with high durability and good reproducibility. An acidic buffer system with a chaotropic salt can be used for most of analytes, though only the acidic conditions are required for acidic analytes and none is required for neutral analytes. If the separation of a basic analyte is not sufficient, especially on amylose tris(3,5-dimethylphenylcarbamate) type CSP (CHIRALPAK AD-RH), a better separation may be obtained by switching to a basic buffer system. The reversed-phase conditions are particularly useful for bioanalytical applications, and the analysis of drugs in blood serum or plasma can be automated by utilizing the column switching technique. It is anticipated that the reversed-phase applications on polysaccharide type CSPs will continue to grow and facilitate drug development.

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