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Short communication

Stationary phase structures enhancing electroosmotic flow in capillary electrochromatography

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Abstract

Several chemically bonded silicas with C₁₈ groups were examined with respect to electroosmotic flow (EOF) velocities under CEC conditions. Stationary phases with low hydrophobic selectivity generally provided high EOFs. The stationary phases prepared by using octadecyltrichlorosilane showed greater EOF than those from octadecyldimethylchlorosilane. Restricted-access reversed-phase (RARP) packing materials having C₁₈ groups inside the pores and silanols on the external surfaces showed higher EOF than monomeric C₁₈ phases with similarly high hydrophobic selectivity. The RARP-type structure having silanols at the external surface seems to be effective for increasing EOF while maintaining the hydrophobic character of the solute binding sites. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

For successful application of capillary electrochromatography (CEC), it is important to develop stable, high-performance columns. Increased electroosmotic flow (EOF) is necessary for fast separations, and controlled EOF for reproducibility. EOF is known to be a function of pH, the dielectric constant, viscosity and buffer concentration of the mobile phase [1–6]. It has been reported that the EOF is higher at a lower buffer concentration, and at a higher pH. Although surface modification of silica with C₁₈ groups usually suppresses EOF, C₁₈ phases showed an increase in EOF at an acetonitrile content

higher than 80% [1–3]. The EOF in a particle-packed column is dominated by the silanols on the particles, not the tube surface [2,7–9]. The effect of the preparation method of RPLC packing materials on EOF velocity, however, is not easily understood on the basis of the surface coverage with C₁₈ groups, or the surface area and pore size of the packing material [3,10]. Apparently charge density at the surface affects the EOF velocity [9,10]. Recent C₁₈ stationary phases prepared from high-purity silicas or base-deactivated materials generate low EOF [1].

High EOF is desirable for fast and efficient separations. However, studies of the relation between plate height and linear velocity, van Deemter plots have been carried out at the low velocity regime (0.25–3 mm/s). They cannot be carried out at high linear velocities (>3 mm/s) due to physical and electrical limitations and maximum voltage con-

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straints on commercial instruments [11]. This limits the utilization of the full capacity of CEC using small particles. Stationary phases with ion-exchange groups show higher EOF [9,10], although they require complicated preparation procedures. In addition ion-exchange-type stationary phases are expected to cause retention by ion-exchange effects. The selectivity of such stationary phases, especially those from organic polymers, will be different from ordinary C_{18} phases.

Here we report a study on the effect of stationary phase structures on EOF, particularly the structure of C_{18} phases including the effect of surface coverage as well as the location of the C_{18} groups. Restricted-access reversed-phase (RARP)-type materials with C_{18} groups in the pores and external silanol groups [12,13] were examined for their effect on EOF velocity under CEC conditions.

2. Experimental

2.1. Instrument

A Beckman P/ACE system MDQ (Beckman, Fullerton, CA, USA) was used with column length of 30.5 cm (20 cm effective length) unless otherwise stated. A pressure of 50 p.s.i. was applied to the both ends of the capillary to prevent bubble formation.

2.2. Column and measurement

The outlet frit was prepared by forcing a mixture of 40- μm glass beads and potassium silicate solution into a capillary followed by external heating for ~ 3 min and washing with water. After packing the particles in acetone as a slurry medium and acetonitrile–water (60:40) as a driving liquid, the inlet frit was prepared similarly as the outlet frit. The column was then equilibrated with acetonitrile–buffer solution. Mobile phase was prepared from HPLC grade acetonitrile and 50 mM trihydroxymethylamino-methane (Tris)–HCl, pH 8. Detection was carried out at 254 nm. The measurement of EOF was carried out at 24.4 kV applied voltage using thiourea as an EOF marker at 25°C. Sample injection was carried out electrokinetically (5 kV, 2 s).

2.3. Packing materials

Several C_{18} phases were prepared as reported previously [14], and listed in Table 1. Silica particles (particle diameter: 5 μm ; pore size: 11 nm; specific surface area: 330 m^2/g) were used, unless stated otherwise. Monomeric C_{18} phases (C_{18} -M) were prepared by reacting the silica particles with octadecyldimethylchlorosilane at refluxing temperature in toluene in the presence of pyridine at maximum surface coverage (C_{18} -M_{max}) and at ≈ 1 $\mu\text{mol}/\text{m}^2$

Table 1
Properties of C_{18} phases

Stationary phase ^a	C (%)	Surface coverage ($\mu\text{mol}/\text{m}^2$)	$\alpha(\text{C/P})^b$ silanol activity	$\alpha(\text{CH}_2)^c$ hydrophobic selectivity	$k'(\text{AB})^d$
C_{18} -T _{max}	18.8	3.5	0.87	1.46	5.56
C_{18} -T ₂	12.3	2.1	1.39	1.42	3.78
C_{18} -T ₁	7.0	1.1	2.07	1.33	1.44
C_{18} -M _{max}	18.0	3.0	0.76	1.49	7.04
C_{18} -M ₁	8.2	1.0	2.22	1.35	1.82
C_{18} -RARP-I	7.4 (18.0) ^e	–	0.93	1.48	1.84
C_{18} -RARP-II ^f	10.7 (14.5) ^e	–	2.18	1.49	1.77

^a Silica particles: particle diameter: 5 μm ; pore size: 11 nm; surface area: 330 m^2/g ; unless stated otherwise. T: polymeric, M: monomeric C_{18} phase.

^b $\alpha(\text{C/P})$: $k'(\text{caffeine})/k'(\text{phenol})$ in 20% methanol.

^c $\alpha(\text{CH}_2)$: $k'(\text{amylbenzene})/k'(\text{butylbenzene})$ in 80% methanol.

^d $k'(\text{AB})$: $k'(\text{amylbenzene})$ in 80% methanol.

^e Before acid decomposition.

^f C_{18} -RARP-II was prepared from 3- μm silica particles with 6 nm pore size, and surface area of 400 m^2/g .

coverage (C_{18} - M_1). Similarly, polymeric C_{18} phases (C_{18} -T) were prepared by the reaction with octadecyltrichlorosilane at maximum surface coverage (C_{18} - T_{max}), at $\approx 2 \mu\text{mol}/\text{m}^2$ coverage (C_{18} - T_2), and at $\approx 1 \mu\text{mol}/\text{m}^2$ coverage (C_{18} - T_1).

RARP materials were prepared from the monomeric C_{18} silica particles of maximum surface coverage (C_{18} - M_{max}) by refluxing with concentrated hydrochloric acid to remove C_{18} groups mainly from external surfaces, as previously described [12,13]. Octadecanol was added to the mixture to protect the stationary phase inside the pores from acid hydrolysis. From the C_{18} - M_{max} -II phase of 14.5% carbon, C_{18} -RARP-II phase of 10.7% carbon was obtained. C_{18} - M_{max} -II (not used for measurement) and C_{18} -RARP-II phase were prepared from 3- μm silica particles (pore size: 6 nm; surface area: 400 m^2/g).

3. Results and discussion

3.1. Chromatographic properties of stationary phases

In order to study the relation between the stationary phase structure and EOF velocity, we prepared several C_{18} phases, as shown in Table 1. They included C_{18} phases with maximum surface coverages with a polymeric structure prepared from octadecyltrichlorosilane and a monomeric structure prepared from octadecyldimethylchlorosilane, and stationary phases with controlled surface coverages at about 2 or 1 $\mu\text{mol}/\text{m}^2$.

We also prepared a restricted-access type C_{18} phase which had C_{18} groups in the pores and bare silica surface at the external surface. Such a stationary phase was prepared by the acid decomposition of a full-coverage monomeric C_{18} stationary phase [12,13]. The acid decomposition was carried out by suspending the monomeric C_{18} phase with maximum surface coverage in concentrated HCl at a refluxing temperature for 5 h, which removed a part of the bonded phase at the peripheral regions. If the decomposed products were not washed out from the particles with an organic solvent, the decomposition did not proceed beyond certain limit. Because the C_{18} silica is not wettable with concentrated HCl at the beginning, the reaction presumably starts from

the external surfaces. The addition of 1-octadecanol to the starting mixture further protected the stationary phase at the internal surfaces against decomposition. We did not attach diol functionality to cover the silanols, because we wanted to have maximum EOF. The RARP-I was prepared from 5- μm silica particles, and RARP-II from 3- μm particles. The carbon content decreased from 18.0 to 7.4% with RARP-I, and from 14.5 to 10.7% with RARP-II under similar reaction conditions. The difference seems to be caused by the difference in the pore size, 11 nm for RARP-I and 6 nm for RARP-II.

The carbon contents of the RARP materials and k' values for amylbenzene in 80% methanol are similar to those with the C_{18} stationary phases of surface coverages of 1–2 $\mu\text{mol}/\text{m}^2$. Chromatographic tests [14] were carried out to get information on alkyl group density, or hydrophobic selectivity, $\alpha(\text{CH}_2) = k'(\text{amylbenzene})/k'(\text{butylbenzene})$, and the effect of silanols, $\alpha(\text{C/P}) = k'(\text{caffeine})/k'(\text{phenol})$. The stationary phases of low surface coverages showed smaller $\alpha(\text{CH}_2)$ as well as smaller $k'(\text{amylbenzene})$ and larger $\alpha(\text{CIP})$ values than those with maximum coverages.

The RARP materials showed small k' values for amylbenzene, compared to the phases with full coverage. Yet the hydrophobic selectivity shown as a separation factor caused by one methylene group, $\alpha(\text{CH}_2)$, is close to the maximum value found with the material of the maximum surface coverage, indicating that the acid decomposition left the bonded phase as patches inside the pores, and preferentially removed the bonded phase from the external surfaces of the particles. We tested the EOF velocity in a capillary column packed with these stationary phases.

3.2. Electroosmotic flow

The relation between the linear flow velocity and the applied voltage is described as Eq. (1), where ϵ_0 and ϵ_r are relative and vacuum permittivities, respectively, and u is the linear velocity, E is the applied electric field, ξ is the zeta potential and η is the viscosity of the solvent. The linear velocity in a packed capillary can also be expressed as Eq. (2), where σ = charge density and $1/\kappa$ = the thickness of the double layer:

Table 2
Electroosmotic mobilities (μ_{eo}) of the eluent in a capillary packed with various C_{18} phases

Stationary phase	$\mu_{\text{eo}} \times 10^4 \text{ cm}^2/\text{V s}$		
	Acetonitrile content of mobile phase		
	90%	80%	70%
$\text{C}_{18}\text{-T}_{\text{max}}$	2.2	2.2	1.6
$\text{C}_{18}\text{-T}_2$	2.1	1.8	1.6
$\text{C}_{18}\text{-T}_1$	2.0	1.9	1.7
$\text{C}_{18}\text{-M}_{\text{max}}$	1.2	0.9	0.8
$\text{C}_{18}\text{-M}_1$	1.5	1.3	1.0
$\text{C}_{18}\text{-RARP-I}$	1.2	1.2	1.1
$\text{C}_{18}\text{-RARP-II}^{\text{a}}$	2.0	1.7	1.4
CEC Hypersil	–	2.2 ^b	1.8 ^b
HP- C_{18}^{c}	1.9	1.5	1.4
HP- C_{18}^{d}	1.8	1.4	1.2

^a 3- μm particles.

^b Taken from Ref. [1].

^c Commercially obtained column from Hewlett-Packard; 5- μm particles.

^d Commercially obtained column from Hewlett-Packard; 3- μm particles.

$$u = (\epsilon_0 \epsilon_r E \xi) / \eta \quad (1)$$

$$u = \sigma E / \kappa \eta \quad (2)$$

Under otherwise identical conditions, the charge density on the stationary phase surface, or the availability of dissociated silanols, determines the EOF velocity in CEC. Table 2 lists the EOF mobilities of acetonitrile–buffer mixtures obtained with the stationary phases. The comparison between polymeric and monomeric phases indicates that the stationary phase prepared from octadecyltrichlorosilane gave higher EOF velocity than the monomeric stationary phase. The reaction of silanols on the silica surface with octadecyltrichlorosilane leaves one or two chlorine atoms unreacted to regenerate silanols upon contact with water [15]. Thus polymeric stationary phases possess more silanols than the monomeric stationary phase, leading to the greater charge density. Similar-

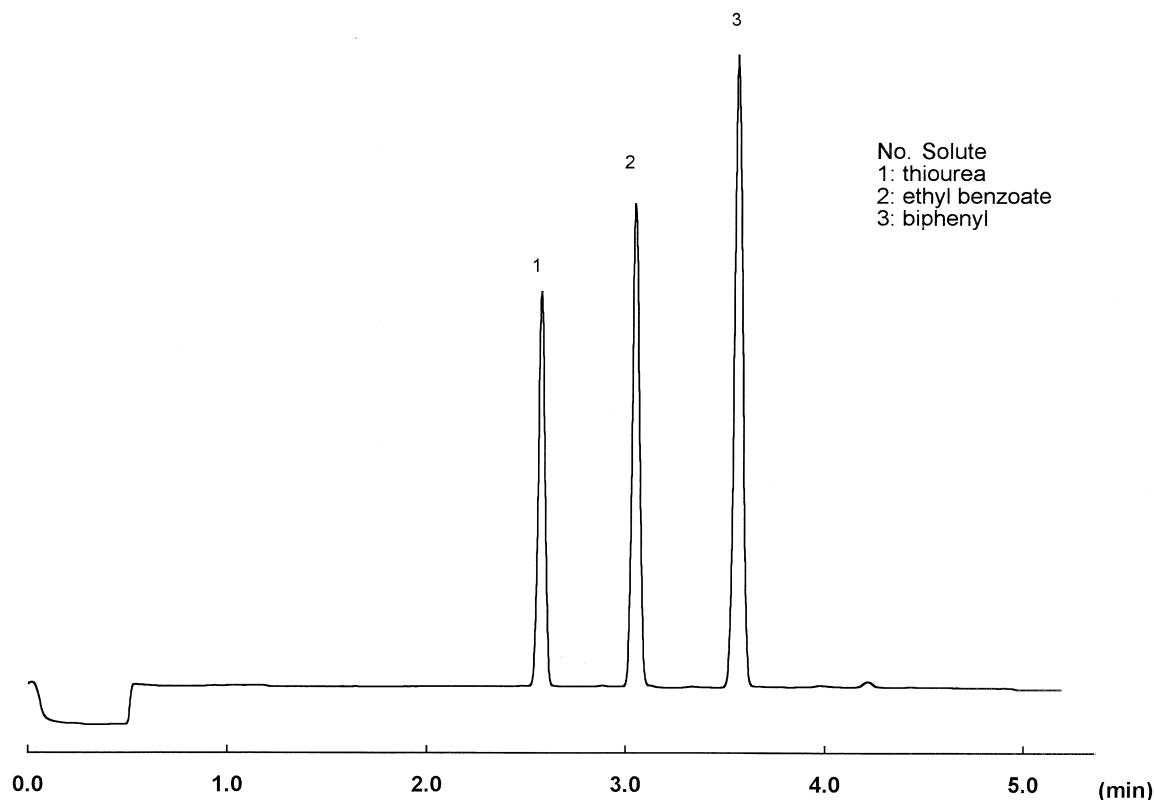


Fig. 1. CEC separation on $\text{C}_{18}\text{-RARP-II}$. Mobile phase: acetonitrile–50 mM Tris–HCl, pH 8 (80:20). Applied voltage: 21.4 kV; detection wavelength: 254 nm; temperature: 25°C; column: 30.5 cm (effective length 20 cm). Solutes: 1, thiourea; 2, ethyl benzoate; 3, biphenyl.

ly the C_{18} - M_{max} phase provided lower EOF velocity than C_{18} -M [1] due to the presence of smaller amount of silanols.

A decrease in EOF velocity was observed with the decrease in acetonitrile content. The results are generally in agreement with those found in the literature [1–3,10], and can be attributed to the differences in accessibility of silanols. Increase in silanophilic interaction was reported in a mobile phase with high organic solvent content [15], where the C_{18} alkyl chains on the silica surface assume the more extended conformation [16]. The EOF mobility obtained with monomeric C_{18} phase having the maximum surface coverage was smaller than on the phases having the lower surface coverage, or polymeric C_{18} phases. The stationary phases which generated high EOF velocity generally showed low hydrophobic selectivity, $\alpha(CH_2)$ values, as shown in Table 1.

Originally RARP materials were developed for the

direct injection of serum into reversed-phase columns [17–19]. These materials commonly possess hydrophilic organic groups at the external surface so as not to denature the serum proteins in buffered acetonitrile. The external surfaces of the RARP materials in this study, however, were not covered by hydrophilic organic groups. The silanols at the external surfaces and hydrophobic groups at the internal surfaces were expected to generate high EOF, while maintaining the retentivity.

C_{18} -RARP-I prepared from the C_{18} - M_{max} phase resulted in slightly higher EOF than C_{18} -M [1] with the lower surface coverage or the original C_{18} - M_{max} phase, while showing the similar $\alpha(CH_2)$ value to the C_{18} - M_{max} phase. The results in Table 1 indicate that the carbon content of RARP-I is similar to that of C_{18} -M or C_{18} -T of low coverage, while much higher $\alpha(CH_2)$ values should reflect the much higher hydrophobic property of the binding sites in the pores. Another batch of RARP material prepared

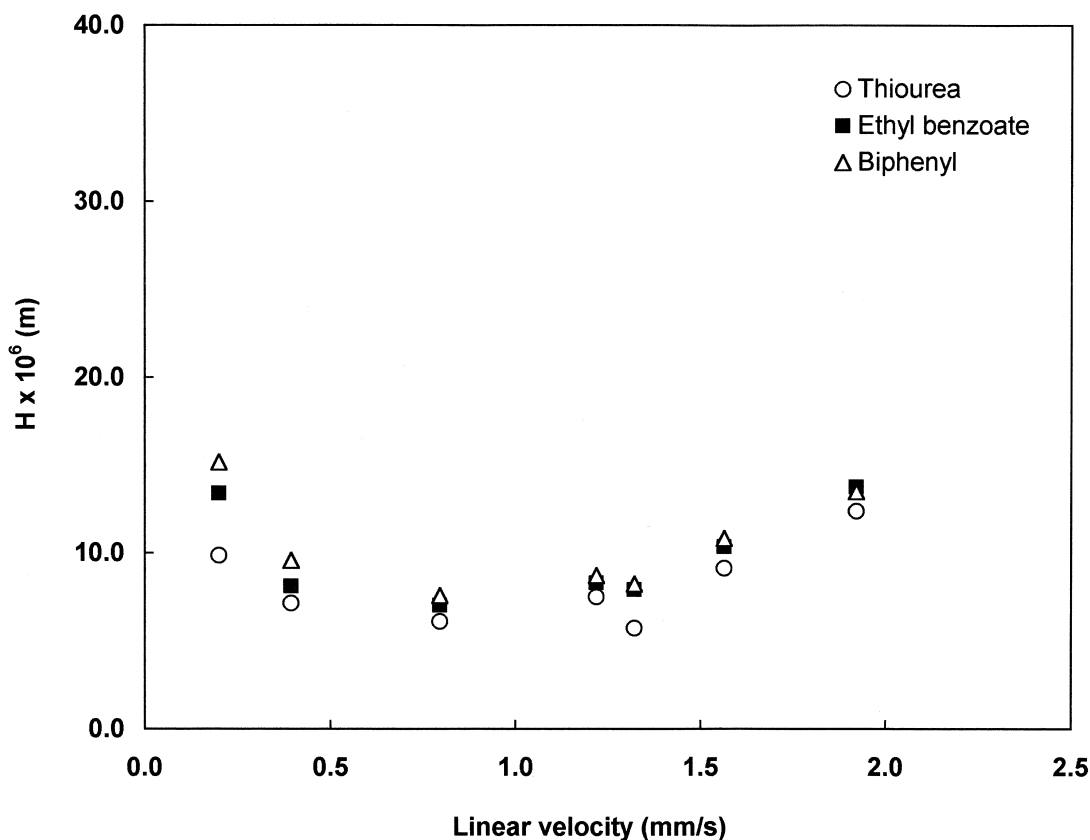


Fig. 2. The van Deemter plot obtained with C_{18} -RARP-II. Mobile phase: acetonitrile–50 mM Tris–HCl, pH 8 (90:10).

from 3- μm particles, C_{18} -RARP-II, showed relatively fast EOF among the monomeric stationary phases — comparable with that found for commercially obtained CEC columns (HP- C_{18}). Thus RARP materials seem to be one possible means to obtain a high EOF while maintaining the hydrophobic selectivity of stationary phase.

The chromatogram in Fig. 1 shows the performance of C_{18} -RARP-II in 80% acetonitrile, and Fig. 2 shows the van Deemter plot obtained with this material in 90% acetonitrile. A minimum plate height of $\sim 6 \mu\text{m}$ was observed at $\sim 1 \text{ mm/s}$. The previous study indicates that among several RARP materials, the C_{18} type showed the greatest retention, and a selectivity relatively similar to those of ordinary C_{18} phases [13]. Other RARP materials, having peptide or amide linkage, showed very different selectivity from ordinary C_{18} phases as well as

lower retentive properties. The C_{18} -RARP phases, however, do have problems. EOF was still not as fast as desired. The most serious problem is the poor performance for basic compounds. As shown in Fig. 3, bases (peaks No. 6 and 8) were eluted with severe tailing from this stationary phase, although good performance was obtained for the neutral solutes. Good performance for basic solutes is reported in other CEC systems [20].

The present study showed that relatively high EOF can be obtained with the low-coverage C_{18} phase, polymeric C_{18} phase, or the RARP type materials that can keep the hydrophobic properties of the binding sites. One problem with the approach of exposing more silanols is that it is exactly opposite to the approach taken in the past to improve the ODS packing materials. How successfully CEC is going to be applied in the future will depend, at least partially,

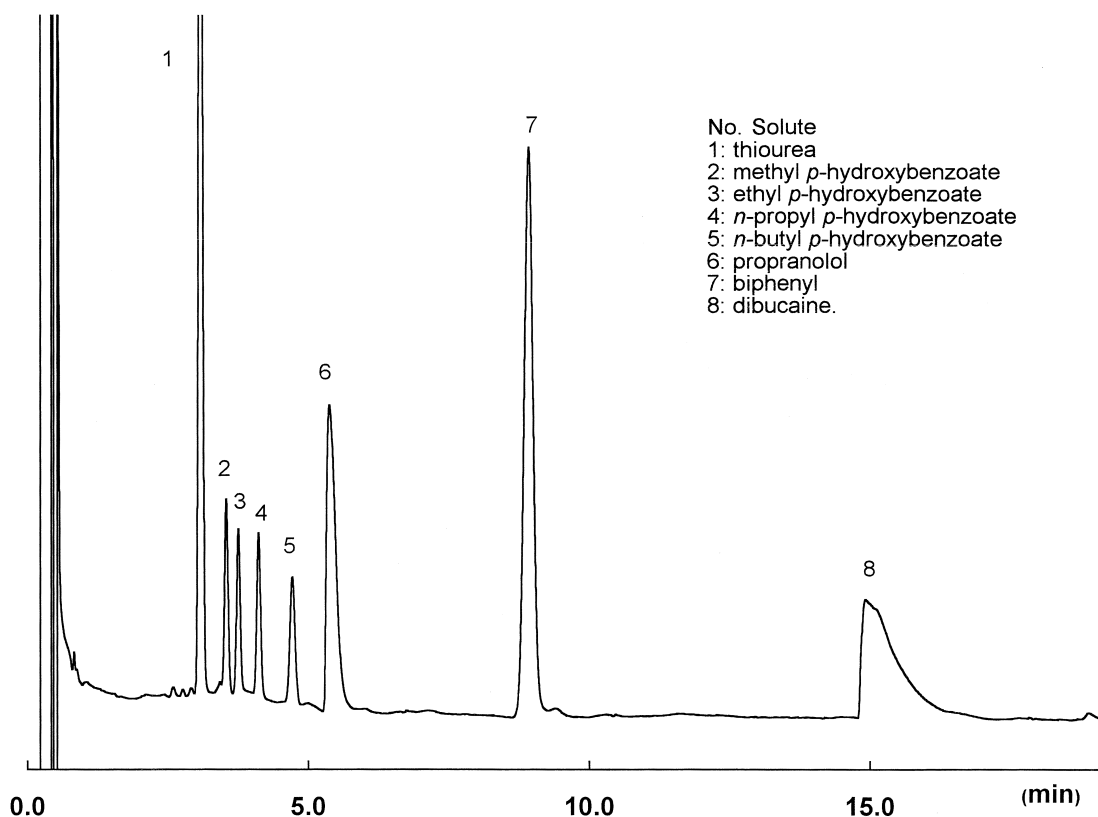


Fig. 3. Performance for basic compounds observed with C_{18} -RARP-II. Mobile phase: acetonitrile–50 mM Tris–HCl, pH 8 (60:40). Applied voltage: 24.4 kV; other conditions as in Fig. 1. Solutes: 1, thiourea; 2, methyl *p*-hydroxybenzoate; 3, ethyl *p*-hydroxybenzoate; 4, *n*-propyl-*p*-hydroxybenzoate; 5, *n*-butyl-*p*-hydroxybenzoate; 6, propranolol; 7, biphenyl; 8, dibucaine.

on how well the problems are going to be solved without affecting the performance [20].

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