ELSEVIER

Journal of Chromatography A, 782 (1997) 33–40

JOURNAL OF CHROMATOGRAPHY A

Ion-exclusion chromatography of benzenecarboxylic acids on an unmodified silica-gel column[1]

Kazutoku Ohta[a,*], Kazuhiko Tanaka[a], Paul R. Haddad[b]

[a]*National Industrial Research Institute of Nagoya, 1-1. Hirate-cho, Kita-hu, Nagoya, Aichi 462, Japan*
[b]*Department of Chemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia*

Received 16 September 1996; received in revised form 30 November 1996; accepted 2 May 1997

Abstract

In order to demonstrate the effectiveness of two unmodified silica-gel (Develosil 30-5) columns (300×7.8 mm I.D.) used in series for the ion-exclusion chromatographic separation of mono-, di-, tri- and tetrabenzenecarboxylic acids, the elution behavior of benzenecarboxylic acids and phenol was investigated. When using water as an eluent, many benzenecarboxylic acids were co-eluted and the separation was not satisfactory. However, when using 5 m*M* sulfuric acid (pH 2.0) as the eluent, excellent separation and high detection sensitivities for many benzenecarboxylic acids and phenol were achieved in 40 min using UV detection at 200 nm. © 1997 Elsevier Science B.V.

Keywords: Benzenecarboxylic acids; Carboxylic acids

1. Introduction

Ion-exclusion chromatography developed by Wheaton et al. [1] is a very useful method for the determination of both inorganic and organic weak acids. In ion-exclusion chromatography, weak acids are separated on a cation-exchange resin depending mainly on their first acid dissociation constant (pK_{a1}) and their hydrophobicity [2]. Separation columns packed with a high-capacity sulfonated polystyrene–divinylbenzene (PS–DVB) resin or a high-capacity carboxylated polyacrylate resin are commonly used. However, hydrophobic weak acids such as higher aliphatic carboxylic acids and benzenecarboxylic acids are strongly retained on the columns due to hydrophobic adsorption. Therefore, ion-exclusion chromatography with PS–DVB resins is applied mainly to the separation of carboxylic acids which have either hydrophilic or weakly hydrophobic characteristics. One approach to the separation of strongly hydrophobic solutes is to use a column packed with a cation-exchange resin having strongly hydrophilic characteristics. Unmodified silica gel is recognized to be a suitable stationary phase for this application because the silica has strong hydrophilic characteristics and the silanol group on the surface of the silica can act as a weakly acidic cation exchanger [3,4]. We have demonstrated that a commercially available unmodified silica gel (Develosil 30-5) acts as a cation exchanger at pH 2 [5] and that the cation-exchange characteristics of this material are due mainly to the presence of some metal impurities in the silica. On the basis of the characteristics of the Develosil 30-5 silica gel, we have established a new

*Corresponding author.

[1] Presented at the International Ion Chromatography Symposium, Reading, 16–19 September 1996.

PII S0021-9673(97)00523-2

ion-exclusion chromatography method for the simultaneous separation of a strong acid (nitric acid) and C_1–C_{10} aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic, caprylic, pelargonic and capric acids) using sulfuric acid–heptanol as eluent [5]. However, in this work, the elution behavior of benzenecarboxylic acids was not investigated in detail.

The purpose of the present study was to demonstrate the effectiveness of the Develosil 30-5 stationary phase for the ion-exclusion chromatographic separation of various kinds of benzenecarboxylic acids and to establish a new ion-exclusion chromatography method for the determination of benzenecarboxylic acids.

2. Experimental

2.1. Instrumentation

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) SC-8010 chromatographic data processor, a Tosoh CCPM delivery pump operated at 1 ml/min, a Tosoh CO-8000 column oven at 35°C, a Tosoh SD-8012 on-line eluent degasser, a Shimadzu (Kyoto, Japan) SPD-10AV UV–Vis spectrophotometric detector operated at 200 nm, an Otsuka Electronics (Osaka, Japan) MCPD-3500 photodiode array UV-Vis spectrophotometric detector operated at 200–400 nm, and a Rheodyne (Cotati, CA, USA) 7125 injector equipped with a 100 μl sample loop.

Two stainless-steel separation columns (300×7.8 mm I.D.) were packed with a Nomura Chemical (Seto, Japan) Develosil 30-5 spherical porous silica gel. The columns were connected in series and equilibrated thoroughly with eluent before each chromatographic run. The series configuration of columns was used for all experiments. A Tosoh TSKgel SCX column and a TSKgel OA-Pak A column (both 300×7.8 mm I.D.) were used as conventional separation columns to compare the elution behavior of benzenecarboxylic acids and phenol with that obtained on the Develosil 30-5 column. The details of all columns used are shown in Table 1.

2.2. Reagents

All chemicals were of analytical-reagent grade and were purchased from Tokyo Kasei (Tokyo, Japan) and Wako (Osaka, Japan). Distilled deionized water was used for the preparation of standard solutions and eluents. The eluents were prepared by diluting 0.5 *M* sulfuric acid solution with distilled deionized water. Sample solutions were prepared by dissolving the required acids in the eluent to be used for their separation. The eluent pH was measured using a Toa Denpa (Tokyo, Japan) IM-40S ion-meter with a glass electrode.

3. Results and discussion

3.1. Elution behavior of benzenecarboxylic acids on Develosil 30-5

In order to demonstrate the effectiveness of the Develosil 30-5 stationary phase for the ion-exclusion chromatographic separation of various kinds of benzenecarboxylic acids, including 1,2,4,5-benzenetetracarboxylic acid (pyromellitic acid), 1,2,3-benzene-

Table 1
Properties of ion-exclusion chromatography columns used in this study

Column	Develosil 30-5	TSKgel SCX	TSKgel OA-Pak A
Matrix	Silica	PS–DVB	PA
Functionality	-SiOH	$-SO_3H$	$-CO_2H$
Capacity (mequiv. g^{-1})	nd	>4.2	uk
Particle size (μm)	5	5	5
Pore size (Å)	ca. 27	uk	uk
Surface area ($m^2 g^{-1}$)	ca. 770	uk	uk
Column size (mm)	300×7.8×2	300×7.8	300×7.8

PS–DVB=styrene–divinylbenzene co-polymer resin; PA=polyacrylate resin; nd=not determined; uk=unknown.

tricarboxylic acid (hemimellitic acid), 1,2,4-benzenetricarboxylic acid (trimellitic acid), 1,2-benzenedicarboxylic acid (*o*-phthalic acid), 1,3-benzenedicarboxylic acid (*m*-phthalic acid), 1,4-benzenedicarboxylic acid (*p*-phthalic acid), benzoic acid, *o*-hydroxybenzoic acid (salicylic acid) and phenol, the elution behavior of these solutes was investigated in detail. As shown in Fig. 1a, when using water as an eluent, almost all of the benzenecarboxylic acids (except for benzoic acid) were co-eluted.

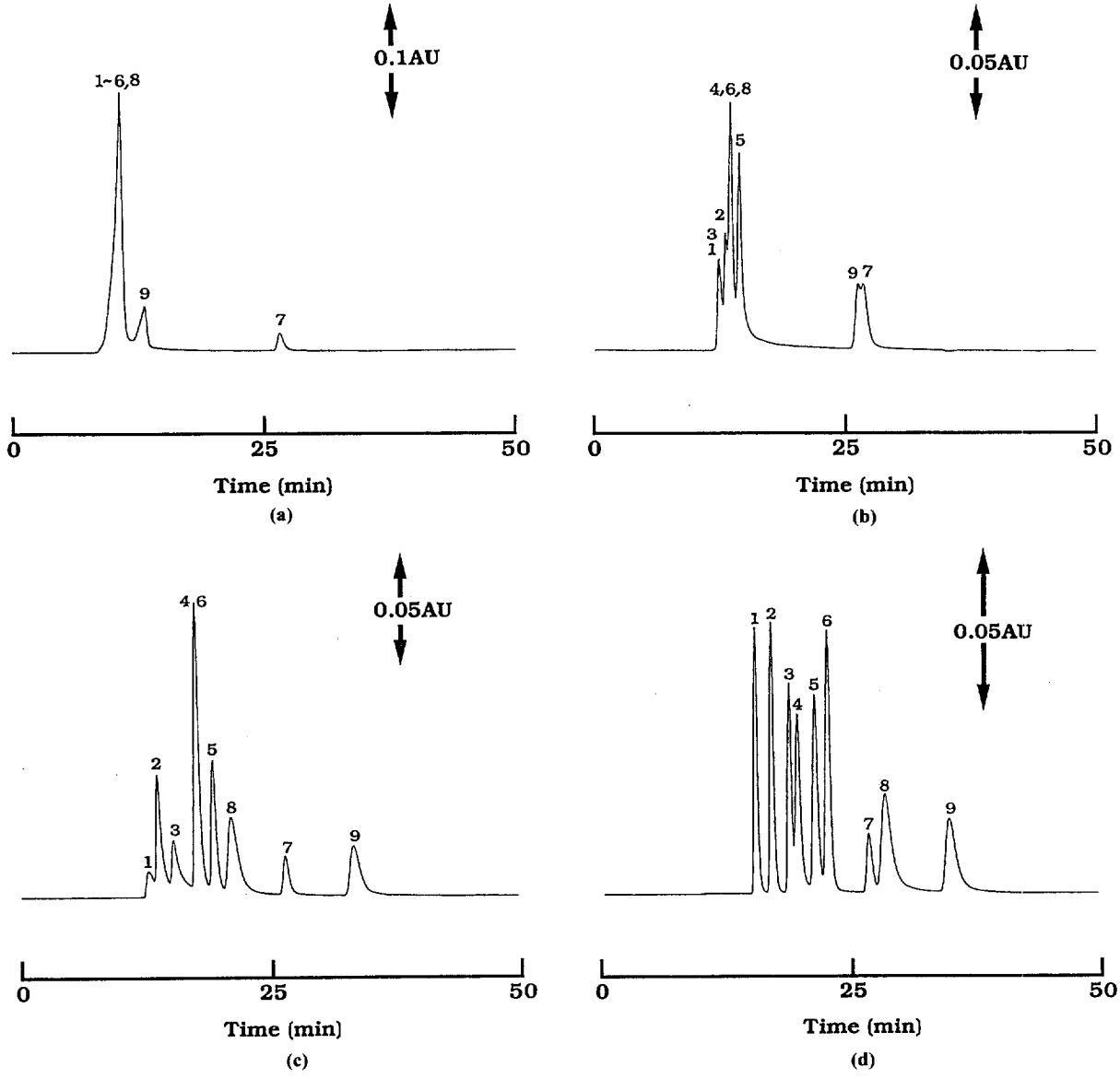

Fig. 1. Ion-exclusion chromatograms of benzenecarboxylic acids and phenol on the Develosil 30-5 column with various concentrations of sulfuric acid in the eluent. Conditions: column: two Develosil 30-5, 300×7.8 mm I.D., in series; column temperature: 35°C; eluent: (a) water, (b) 0.05 m*M* sulfuric acid, (c) 0.5 m*M* sulfuric acid, (d) 5 m*M* sulfuric acid; flow-rate: 1 ml/min; detection: UV at 200 nm; injection volume: 100 μl; sample concentration: 0.01 m*M*. Peaks: 1=pyromellitic acid, 2=trimellitic acid, 3=hemimellitic acid, 4=*p*-phthalic acid, 5=*m*-phthalic acid, 6=*o*-phthalic acid, 7=phenol, 8=salicylic acid, 9=benzoic acid.

In ion-exclusion chromatography it is well known that under conditions where solute retention is determined solely by electrostatic effects, solutes are eluted at retention volumes which fall between V_o (complete ion exclusion) and V_o+V_i (complete ion permeation), where V_o is the column void volume and V_i is the volume of liquid inside the resin in the column [6]. In Fig. 1a, the values of the retention volumes (V_R) of the benzenecarboxylic acids were between V_o (10.4 ml) and V_o+V_i (21.0 ml), indicating that the partly dissociated benzenecarboxylic acids were strongly excluded from the silica-gel surface by electronic repulsion from the dissociated silanol group on the Develosil 30-5 silica-gel surface. The separation of benzoic acid from the remaining benzenecarboxylic acids was due to the difference in the pK_{a1} value and the hydrophobicity of this solute. It was also noteworthy that some benzenepolycarboxylic acids gave tailed peaks, perhaps due to hydrophilic adsorption between these partly dissociated benzenepolycarboxylic acids and the partly dissociated silanol group. The poor peak shape made it difficult to quantitatively determine peak areas for solutes at low concentration (<0.01 m*M*).

Turkelson et al. [7] have shown that the use of an acidic eluent in ion-exclusion chromatography is very effective in the improvement of both the peak shapes of carboxylic acids and the resolution between carboxylic acids. Therefore, the effects of the concentration of sulfuric acid in the eluent were investigated. The relationship between the eluent pH and the V_R values of the benzenecarboxylic acids and phenol is shown in Fig. 2. The V_R values of the benzenecarboxylic acids increased with decreasing the eluent pH, whereas the V_R value of phenol remained almost constant. The retention behavior of the benzenecarboxylic acids was due to an increase in hydrophobic adsorption between the solutes and the stationary phase arising from suppression of the dissociation of both the benzenecarboxylic acids and the silanol group. The degree to which the V_R values varied with the eluent pH was depended on the pK_a values and the hydrophobicity of the individual solutes. Typical chromatograms obtained using 0.05, 0.5 and 5 m*M* sulfuric acid as eluent are shown in Fig. 1b,c and d, respectively. These figures show that the peak shapes of the benzenepolycarboxylic acids were improved by decreasing the eluent pH, perhaps due to a decrease in hydrophilic adsorption resulting from suppression of the dissociation of benzenepolycarboxylic acids and the silanol group. However, the peak shape for salicylic acid remained slightly tailed, probably due to interaction with metal impurities in the Develosil 30-5 silica gel. Considering both the resolution and the peak shape, the optimum eluent conditions were 5 m*M* sulfuric acid (pH 2). As shown in Fig. 1d, an excellent separation of the benzenecarboxylic acids and phenol was achieved in 40 min with the use of this eluent.

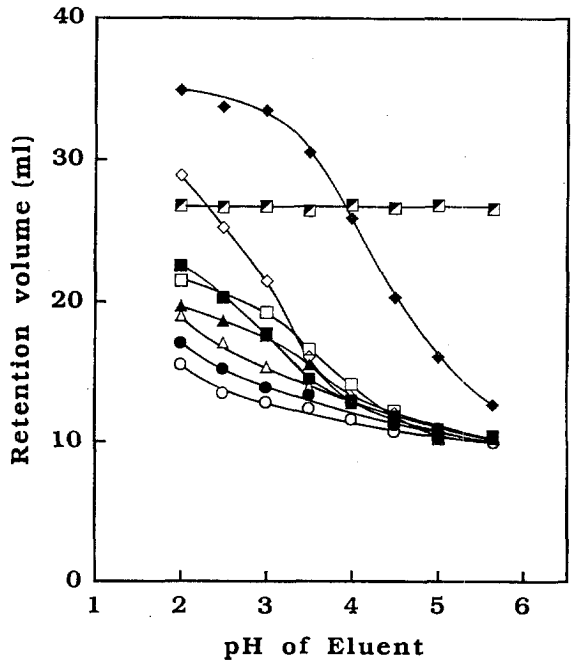

Fig. 2. Effect of eluent pH on the retention volumes (V_R) of the benzenecarboxylic acids and phenol with the Develosil 30-5 stationary phase. Symbols: ○=pyromellitic acid, ●=trimellitic acid, △=hemimellitic acid, ▲=*p*-phthalic acid, □=*m*-phthalic acid, ■=*o*-phthalic acid, ◩=phenol, ◇=salicylic acid, ◆=benzoic acid. Chromatographic conditions are as for Fig. 1.

3.2. Elution behavior of benzenecarboxylic acids on conventional ion-exclusion chromatography columns

In order to compare the effectiveness of the Develosil 30-5 stationary phase for the ion-exclusion

chromatographic separation of benzenecarboxylic acids with conventional ion-exclusion chromatography phases, the elution behavior of these solutes and phenol was studied using the TSKgel SCX and TSKgel OA-Pak A columns. The relationship between the eluent pH and the V_R values on the TSKgel SCX and the TSKgel OA-Pak A columns are shown in Figs. 3 and 4, respectively. The V_R values of most of the solutes on the conventional ion-exclusion chromatography columns were much larger than those on the Develosil 30-5 column. This is due to the strongly hydrophobic characteristics of polymer-based cation-exchange resins, so that the V_R values of the benzenecarboxylic acids increased dramatically by decreasing the eluent pH. On the other hand, the V_R values of some benzenecarboxylic acids on the TSKgel OA-Pak A column were larger than those on the TSKgel SCX column, despite the fact that the hydrophobicity of the TSKgel OA-Pak A resin (polyacrylate) is lower than that of the TSKgel SCX

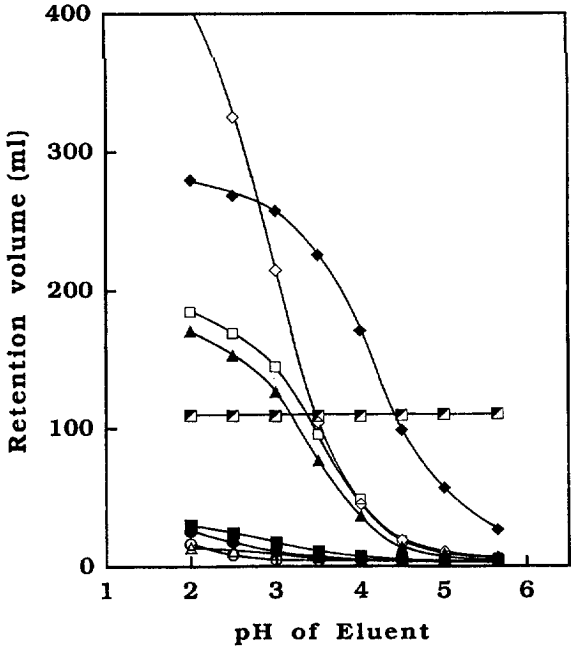

Fig. 3. Effect of the eluent pH on the retention volumes of the benzenecarboxylic acids and phenol on the TSKgel SCX stationary phase. Conditions: column: TSKgel SCX, 300×7.8 mm I.D. The other chromatographic conditions and the symbols are as for Fig. 2.

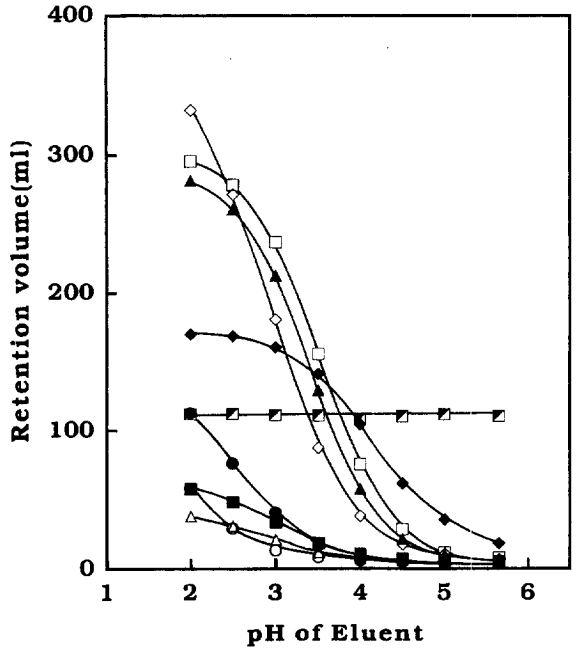

Fig. 4. Effect of the eluent pH on the retention volumes of the benzenecarboxylic acids and phenol on the TSKgel OA-Pak A stationary phase. Conditions: column: TSKgel OA-Pak A, 300× 7.8 mm I.D. The other chromatographic conditions and the symbols are as for Fig. 2.

resin (PS–DVB co-polymer). This is due mainly to decrease in electronic repulsion caused by suppression of the dissociation of the carboxylic group on the TSKgel OA-Pak A resin under acidic conditions. Typical chromatograms obtained on the TSKgel SCX and the TSKgel OA-Pak A columns are shown in Figs. 5 and 6, respectively.

In Figs. 5a and 6a with water as eluent, fronted peak shapes were obtained for many benzenecarboxylic acid and the separation was not satisfactory. However, when using 5 m*M* sulfuric acid as eluent (Figs. 5b and 6b), improved peak shapes and enhanced resolution were achieved although the separation times were long due to strongly hydrophobic adsorption effects. Fig. 6b shows that whilst the peak shapes of benzenecarboxylic acids and phenol on the TSKgel OA-Pak A column were good, the V_R value of salicylic acid was ca. 350 ml. Considering the resolution, the peak shape and the separation time, the Develosil 30-5 column was concluded to be the

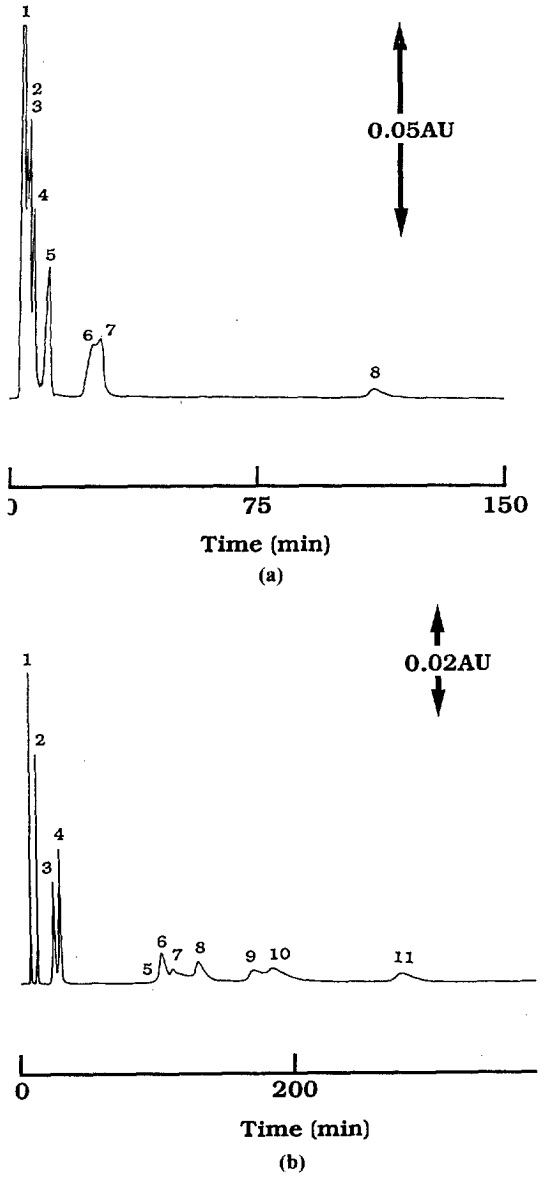

Fig. 5. Ion-exclusion chromatograms of benzenecarboxylic acids and phenol on TSKgel SCX column using (a) water, and (b) 5 m*M* sulfuric acid as an eluent. Conditions: eluent: (a) water, (b) 5 m*M* sulfuric acid; column: TSKgel SCX, 300×7.8 mm I.D. Peaks: (a) 1=pyromellitic, hemimellitic; trimellitic, trimesic, and *o*-phthalic acids, 2=*p*-phthalic acid, 3=*m*-phthalic acid, 4=salicylic acid, 5=*m*-hydroxybenzoic acid, 6=*p*-hydroxybenzoic acid, 7=benzoic acid, 8=phenol; (b) 1=pyromellitic acid, 2=hemimellitic acid, 3=trimellitic acid, 4=*o*-phthalic acid, 5=trimesic acid, 6=*m*-hydroxybenzoic acid, 7=phenol, 8=*p*-hydroxybenzoic acid, 9=*p*-phthalic acid, 10=*m*-phthalic acid, 11=benzoic acid. Other chromatographic conditions are as for Fig. 1.

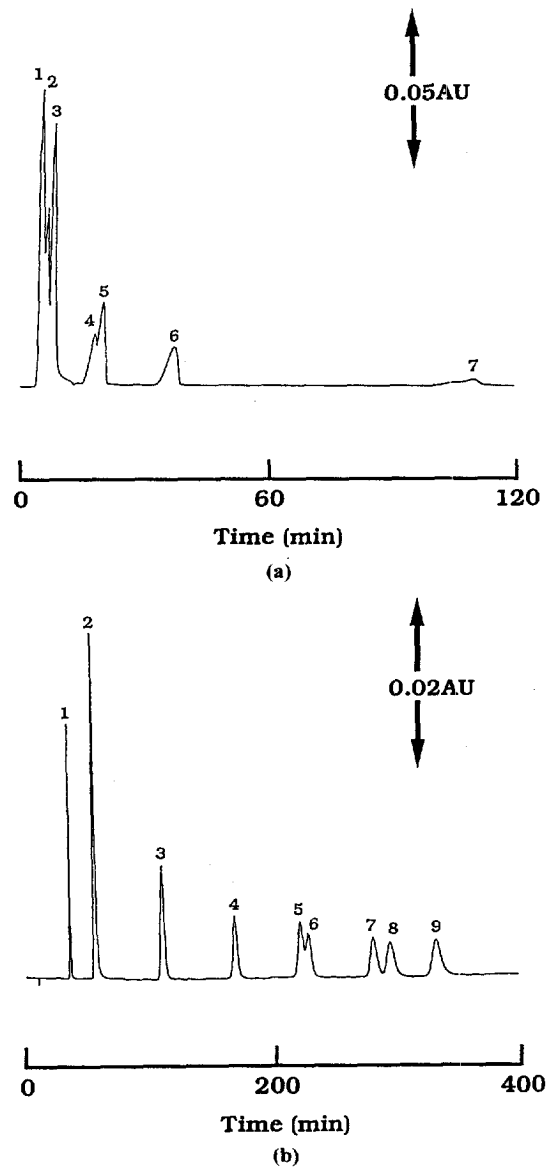

Fig. 6. Ion-exclusion chromatograms of benzenecarboxylic acids and phenol on TSKgel OA-Pak A column using (a) water, and (b) 5 m*M* sulfuric acid as eluent. Conditions: eluent: (a) water, (b) 5 m*M* sulfuric acid; column: TSKgel OA Pak A, 300×7.8 mm I.D. Peaks: (a) 1-3=pyromellitic, hemimellitic, trimellitic, trimesic, *o*-phthalic, *m*-phthalic, *p*-phthalic, and salicylic acids, 4=benzoic acid, 5=*m*-hydroxybenzoic acid, 6=*p*-hydroxybenzoic acid, 7=phenol; (b) 1=hemimellitic acid, 2=pyromellitic and *o*-phthalic acids, 3=trimellitic acid and phenol, 4=benzoic acid, 5=*m*-hydroxybenzoic acid, 6=*p*-hydroxybenzoic acid, 7=*m*-phthalic acid, 8=*p*-phthalic acid, 9=trimesic acid. Other chromatographic conditions are as for Fig. 1.

Table 2
Detection limits of benzenecarboxylic acids and phenol under the optimum chromatographic conditions (injection volume 100 μl)

Benzenecarboxylic acid	Detection limit[a]	
	(μ*M*)	(ppb)
Pyromellitic acid	0.0036	0.90
Hemimellitic acid	0.0047	0.97
Trimellitic acid	0.0036	0.75
o-Phthalic acid	0.0034	0.65
m-Phthalic acid	0.0052	0.87
p-Phthalic acid	0.0047	0.65
Benzoic acid	0.015	1.8
Salicylic acid	0.011	1.5
Phenol	0.019	1.8

[a] Signal-to-noise ratio=3.

most useful for the ion-exclusion chromatographic separation of benzenecarboxylic acids.

3.3. Analytical performance parameters

Calibration graphs obtained by plotting peak height of the benzenecarboxylic acids and phenol against the concentration were linear over the concentration range 0.001–0.075 m*M*. Detection limits (*S*/*N*=3) are listed in Table 2 and show that high detection sensitivities were achieved by the proposed ion-exclusion chromatography method. The reproducibility of the peak heights of the benzenecarboxylic acids and phenol under the optimum chromatographic conditions (Fig. 1D) was less than 1.2 R.S.D. (n=8) and reproducible chromatograms were obtained during repeated chromatographic runs. Fig. 7 shows a chromatogram of the benzenecarboxylic acids and phenol obtained using a photodiode array detector and demonstrates that either selective detection or identification of benzenecarboxylic acids can be achieved with this detector.

4. Conclusion

A simple, convenient and selective ion-exclusion chromatography method has been developed for the separation of benzenecarboxylic acids on a Develosil 30-5 stationary phase using sulfuric acid as eluent. This work expands the utility of this stationary phase which has already been shown to be applicable to the ion-exclusion chromatographic separation of C_1–C_{10} aliphatic carboxylic acids using similar eluents.

Acknowledgments

This work was supported, in part, by the Agency of Industrial Science and Technology of the Ministry

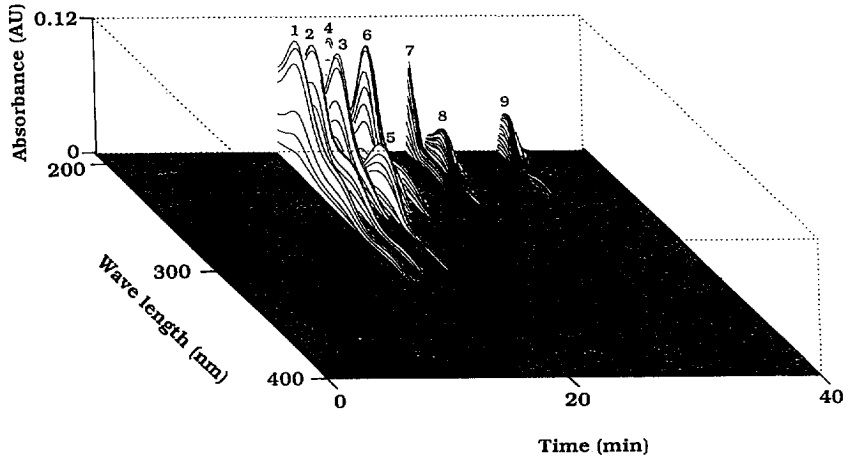

Fig. 7. Ion-exclusion chromatogram of the benzenecarboxylic acids and phenol with photodiode array detector under the optimum eluent conditions. Conditions: eluent: 5 m*M* sulfuric acid; column: two Develosil 30-5, 300×7.8 mm I.D., columns; detection: UV at 200–400 nm. Peaks: 1=pyromellitic acid, 2=trimellitic acid, 3=hemimellitic acid, 4=*p*-phthalic acid, 5=*m*-isophthalic acid, 6=*o*-phthalic acid, 7=phenol, 8=salicylic acid, 9=benzoic acid. Other chromatographic conditions are as for Fig. 1.

of International Trade and Industry in Japan, based on a Cooperative Agreement on Science and Technology for the development of acid rain monitoring system by ion chromatography between the National Industrial Research Institute of Nagoya and the University of Tasmania.

References

[1] R.M. Wheaton, W.C. Bauman, Ind Eng. Chem. 45 (1953) 228.
[2] P.R. Haddad, P.E. Jackson, Ion Chromatography: Principles and Applications, Elsevier, Amsterdam, 1990.
[3] K.K. Unger, Porous Silica, Elsevier, Amsterdam, 1979.
[4] R.P.W. Scott, Silica Gel and Bonded Phases: Their Production, Properties and Use in LC, Wiley, Chichester, 1993.
[5] K. Ohta, K. Tanaka, P.R. Haddad, J. Chromatogr. A 739 (1996) 359.
[6] K. Tanaka, T. Ishizuka, H. Sunahara, J. Chromatogr. 174 (1979) 153.
[7] V.T. Turkelson, M. Richards, Anal. Chem. 50 (1978) 1420.