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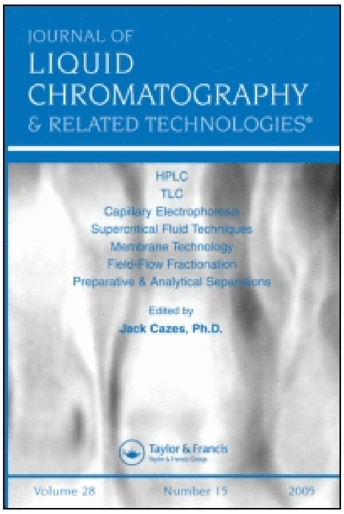

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Reversed-Phase Liquid Chromatography of Alkylphenols Depending on Their Dissociation Constants

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REVERSED-PHASE LIQUID CHROMATOGRAPHY OF ALKYLPHENOLS DEPENDING ON THEIR DISSOCIATION CONSTANTS

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ABSTRACT

The reversed-phase liquid chromatography of structural isomers of alkylphenols has been studied. Octyl-bonded silica gel was useful as stationary phase and addition of a trace of triethylamine to the eluent was effective for this purpose. The separation of nine structural isomers of alkylphenols, ethylphenols and xylenols, was accomplished by such liquid chromatographic system. Thus the good reproducibility of the capacity ratios was obtained.

The increase of retention of the solute was not observed by addition of the ion-pair reagent to the eluent.

INTRODUCTION

The alkylphenols present in nature as the chemical ingredients, coffee [1] and cocoa flavors [2] and the fragrant of tobacco [3]. These are important as the row materials of chemical industry and antioxidants in many fields, artificially.

There were some reports on the chromatography of alkylphenols, on paper partition chromatography [4.5], thin layer chromatography [6~13], and classic liquid chromatography [14~15]. And many studies on

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TABLE 1 The *p Ka* values of alkylphenols

	U.V.	pH	partition coefficient (H_2O/org.solv.)	observed and calculated	
	ref. 41		ref. 42		
phenol	10.02	10.08		9.95(obs.)	
o-cresol	10.32	10.20		10.28	
m-cresol	10.09	10.01		10.08(uncorrected)	
p-cresol	10.27	10.25		10.19	
o-ethylphenol			10.2	10.2(uncorrected)	
m-ethylphenol			9.9	9.9(uncorrected)	
p-ethylphenol			10.0	10.0(uncorrected)	
	U.V.	pot		obs.	calc.
2,3-xylenol	10.54	10.49		10.50	10.41
2,4-xylenol	10.60	10.50		10.47	10.52
2,5-xylenol	10.41	10.18		10.32	10.41
2,6-xylenol	10.63	10.58		10.60	10.61
3,4-xylenol	10.36	10.25		10.32	10.32
3,5-xylenol	10.19	10.18		10.17	10.21

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alkylphenols were carried out by high-performance liquid chromatography [16~35,37~40]. However the separation of xylenols has not been completed.

In normal-phase liquid chromatography(NPLC),the separation of *o*-, *m*- and *p*-ethylphenols is sufficient, but not that of xylenols.

Reversed-phase liquid chromatography(RPLC) generally lends to separate homologues of solutes. The elution order of di-subtituted structural isomers in RPLC are usually *m*-<*p*-<*o*-, but the elution order of *p*- and *m*-substitutes was reversed on μ-Bondapak C_{18} column [13].

It is expected that the various commercially available packings have very different properties such as pore size, silanization group, carbon loading, etc. They play distinctively in chromatography of structural isomers, especially that of tri-substituted compounds such as xylenols. Previously, the separation of the nine structural isomers of alkylanilines was accomplished by ion-pair liquid chromatography [36]. The dissociation constants of alkylanilines are less than 4 and those of alkylphenols are more than 10 as given in TABLE 1. According to these values, it seems to be effective to separate alkylphenols by addition of ion-pair reagent. But increase of retention of these solutes was not observed with ion-pair reagent.

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TABLE 2 The Column Packings and the Composition of the Eluent.

packing material	constitution	particle diameter (μm)	eluent composition (v/v%) in experiments
Develosil TMS-3	C_1	3	MeOH:H_2O=10~20:90~80 $*^1$
Develosil C_8-3	C_8 $*^3$	3	MeOH:H_2O=30~45:70~55 $*^1$
Develosil C_{10}-3	C_{10} $*^3$	3	MeOH:H_2O=30~40:70~60 $*^1$
Develosil ODS-3	ODS $*^3$	3	MeOH:H_2O=30~50:70~50 $*^1$
Develosil C_8-5	C_8 $*^2$	5	MeOH:H_2O=20~30:80~70 $*^1$
Develosil C_8-5	C_8 $*^3$	5	MeOH:H_2O=20~30:80~70 $*^1$

$*^1$ with 0.1~0.3% (v/v) of triethylamine.
$*^2$ not end capped $*^3$ end capped

The difference of the retention of xylenols at high pH suggested the possibility of their separation depending upon the difference of their dissociation constants. It is well known that alkyl-bonded silica gel packings are usually unstable in strong alkali with hydroxide ion, such as pH range 10~12 Kolthoff's buffer. Then a trace of triethylamine was added instead of hydroxide ion in the eluent. Thus the separation of nine strcuctural isomers of carbon number two alkylphenols was accomplished.

EXPERIMENTAL

The chromatograph, UV-detector, recorder and column temperature controller used in this study were the same as described in previous report [37].

Phenol, *o*- and *p*-cresol and, *o*- and *p*-ethylphenol, 2,3- and 2,5-xylenol, and *o*-*iso*-propylphenol were purchased from Wako Pure Chem. Ind.,Ltd.(Osaka,Japan). And 2,4-, 2,6-, 3,4- and 3,5-xylenol from Nacalai Tesque(Kyoto,Japan),and *m*-cresol,*m*-ethylphenol,and *o*-*n*-propylphenol were purchased from Aldrich Chem.Ind.,Ltd.(Milwaukee,USA). *p*-*iso*-Propylphenol, and *p*-*n*-propylphenol were purchased from Tokyo Kasei Co.,Ltd.(Tokyo,Japan).

These phenol derivatives were purified by reduced pressure distillation under nitrogen stream. Deionized water was distilled and further sub-boiling distilled with quartz sub-boiling still. HPLC grade methanol, special grade potassium dihydrogen phosphate, sodium hydroxide and triethylamine were purchased from Wako Pure Chem.Ind., Ltd. Ion-pair liquid chromatography reagent grade tetraethylammonium bromide, tetrabutylammonium phosphate were purchased from Chemco Sci. Co.,Ltd.(Osaka,Japan).

TABLE 3 The comparison of capacity ratio of xylenols.

xylenol	TMS	C$_8$		C$_{10}$	ODS
	—	—	TEA*	TEA*	TEA*
3,5-	2.73	9.83	3.86	4.63	4.63
3,4-	2.36	8.60	4.19	5.10	4.93
2,5-	2,73	10.30	5.57	6.72	6.43
2,3-	2.36	9.30	5.82	6.95	6.82
2,6-	2.08	8.92	6.15	7.22	7.22
2,4-	2.57	10.30	6.71	7.92	7.87

Column: 10cm×4.0mm i.d.
Eluent:methanol:water=40:60(v/v)
Fc: 0.5mℓ/min * with 0.2%TEA

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The column packings were purchased from Nomura Chem. Co., Ltd. (Seto,Japan). The eluent composition in this study were described in TABLE 2. The packings were packed in stainless steel tube by slurry packing method using Chemco Slurry Packing Apparatus Model 124A from Chemco Sci.Co.,Ltd. under 6.06×10^7Pa(N/m^2) pressured by nitrogen gas.

RESULTS AND DISCUSSION

The alkyl chain length effect of bonded phase was studied among Develosil TMS(C$_1$), C$_8$, C$_{10}$ and ODS(C$_{18}$). The eluent composition were settled to obtain the capacity ratio of xylenols among 3 to 10 in the experiment. The capacity ratios on TMS column in TABLE 3 were extrapolated from the capacity ratio with lower methanol concentration. Because those capacity ratios on TMS column were too small to compare with the other packings, as shown in Fig.1.

The elution order of di-substituted alkylphenols with alkyl-bonded silica gel packings is usually in *m*-<*p*-<*o*-. If this elution order will be able to extend to tri-substituted compounds of alkylphenol, the elution order of xylenols will be expected in di *m*-(3,5-) <*m* ,*p*-(3,4-)<*o*-,*m*-(2,5- and 2,3-)<*o*-,*p*-(2,4-)<di *o*-(2,6-). The elution order of them was different on Develosil TMS and C$_8$ without triethylamine (TEA) in the eluent, and the retention of di *o*-

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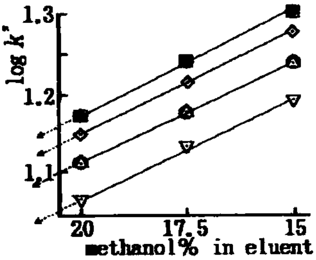

Fig.1 Variation of log k' of xylenols related to methanol ratio in eluent.
Column: 15cm×4.6mm i.d. packed Develosil TMS-3
Eluent:methanol:water(v/v)
Fc: 0.6ml/min
Column temp.: 25℃
Sample: ▽:2,6- △:2,3-
⊙:3,4- ◇:2,4-
◆:2,5- □:3,5-

TABLE 4 The effect of ion-pair reagent on the capacity ratio of xylenols.

xylenol	k'			
	TEA·Br (pH* : 11.53)	TBA·H_3PO_4 (pH* : 11.56)^{*2}	Kolthoff's buffer^{*3}	with TEA (pH* : 11.50)
3,5-	2.18	2.33	2.45	2.82
3,4-	2.45	2.53	2.71	3.12
2,5-	2.78	2.81	3.18	3.66
2,3-	3.16	3.10	3.47	3.99
2,6-	3.29	3.29	3.77	4.34
2,4-	3.94	3.70	4.21	4.84

Column: 10cm×4.0mm i.d. packed Develosil C_8-3
Eluent: methanol:water=35:65(v/v) *Fc*: 0.5ml/min
Column temp.: 25℃
* apparent pH ^{*2} 40% methanol ^{*3} pH : 11.50

(2,6-) compound was the weakest. This fact should be explained the stereo hindrance of phenolic hydroxyl group by two *ortho* substituted methyl groups.

The retention of the solutes on ODS column in 40% methanol without TEA was too strong to measure their retention with precision, and the same result was obtained on the other packings described in TABLE 4. The results of some attempts of ion-pair liquid chromato-

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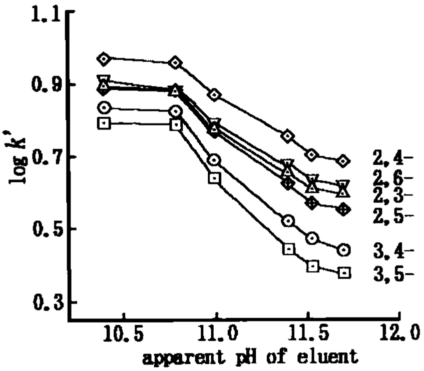

Fig.2-A Variation of log k' of xylenols with change of apparent pH of eluent.

Column: 10cm×4.6 i.d. packed Develosil C_8-3
Eluent: methanol:water=35:65(v/v) pH was adjusted with Kolthoff's buffer solution.
Column temp.: 25℃
Sample size: each 2 μℓ of 2mM solution

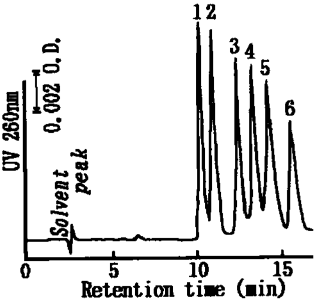

Fig.2-B Chromatogram of xylenols on Develosil C_8-3 at pH 11.53 (apparent).

Column: 10cm×4.6mm i.d.
Eluent: methanol:water=35:65 (v/v)
pH buffer: Kolthoff's buffer
Fc: 0.5mℓ/min
Column temp.: 25℃
Sample: 1:3,5- 2:3,4- 3:2,5- 4:2,3- 5:2,6- 6:2,4-
Sample size: each 2 μℓ of 2mM solution

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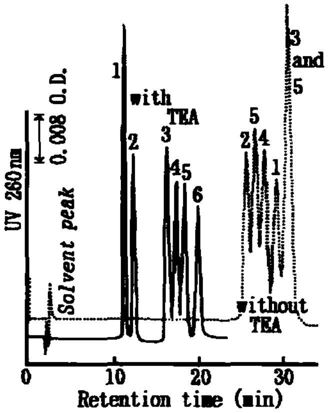

Fig.3 The comparison of the chromatograms with triethylamine and without in eluent.

Column: 10cm×4.6mm i.d. packed
Develosil C_8-3
Eluent:methanol:water=40:60(v/v)
Fc: 0.60ml/min
Column temp.: 25°C
Sample:
1:3,5-xylenol 2:3,4-xylenol
3:2,5-xylenol 4:2,3-xylenol
5:2,6-xylenol 6:2,4-xylenol
Sample size: with TEA each 1µl
and without TEA 2µl
of 0.2mM solution

graphy of alkylphenols are shown in TABLE 4, compared with the result measured with Kolthoff's buffer only. Capacity ratios of ion-paired compounds should be larger on C_{18} than C_8.

However, the capacity ratios measured on both packings were similar, and increase of them was not found with ion-pair reagent.

The variation of log *k'* of xylenols measured in Kolthoff's buffer is shown in Fig.2-A, and a chromatogram of them at apparent pH 11.53 is shown in Fig.2-B. Six xylenols were separated with Kolthoff's buffer. Kolthoff's buffer at pH near 11 contains hydroxide ion, but alkyl-bonded silica gel is unstable with hydroxide ion. Therefore, TEA was added to the eluent instead of Kolthoff's buffer. The comparison of chromatograms between with TEA and without TEA is shown in Fig.3.

The full line shows the chromatogram with TEA and the dotted line shows that without TEA.

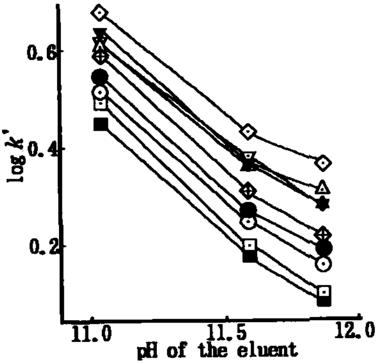

Fig. 4-A Variation of log k' of ethylphenols and xylenols with change of pH of eluent.

Column: the same as Fig. 3
Eluent: the same as Fig. 3, with 0.2%TEA
Sample:

◇ : 2,4-xylenol ⊡ : 3,5-xylenol
▽ : 2,6-xylenol ▼ : *o*-ethylphenol
△ : 2,3-xylenol
⊕ : 2,5-xylenol ■ : *m*-ethylphenol
⊙ : 3,4-xylenol ● : *p*-ethylphenol

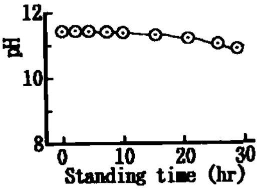

Fig. 4-B Stability of eluent.

Eluent: methanol:water:TEA
=35.0:65.0:0.2(v/v)
Temperature: 25℃

The capacity ratios of nine structural isomers, ethylphenols and xylenols, were varied with the pH change of the eluent as seen in Fig. 4-A. The chromatogram of these mixture is shown in Fig. 5.

The pH variation of the eluent related to the passage of the time was rather small as seen in Fig. 4-B. The pH of the eluent with TEA decreased very slowly in a few hours.

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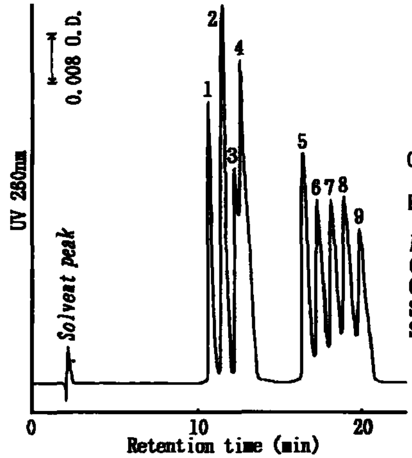
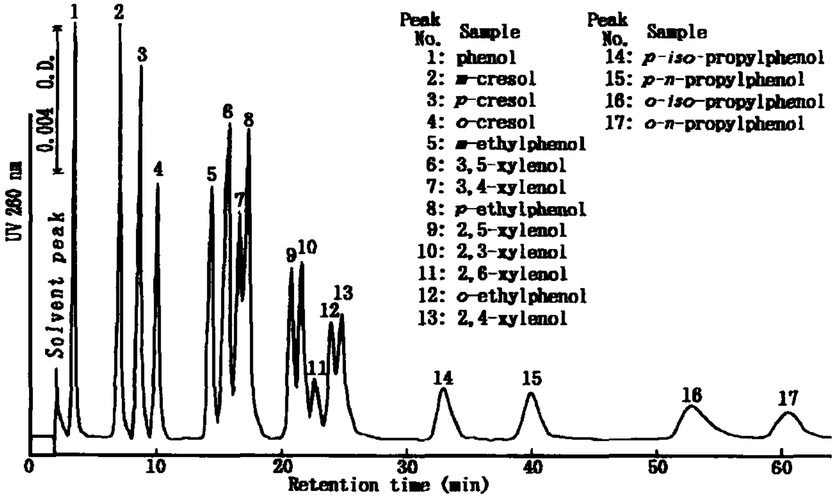


Column: 10cm×4.6mm i.d. packed Develosil C_8-3
Eluent: methanol:triethylamine:water= 40:0.2:59.8(v/v%)
Fc: 0.60ml/min
Column inlet press.: 1.606×10^7 Pa(N/m^2)
Column temp.: 25℃
Sample size: each 3μl of 0.2mM solution
Sample: 1:*m*-ethyl., 2:3,5-xyl., 3:3,4-xyl., 4:*p*-ethyl., 5:2,5-xyl., 6:2,3-xyl., 7:2,6-xyl., 8:*o*-ethyl., 9:2,4-xyl.

Fig.5 Chromatogram of ethylphenols and xylenols.



Peak No.	Sample	Peak No.	Sample
1:	phenol	14:	*p-iso*-propylphenol
2:	*m*-cresol	15:	*p-n*-propylphenol
3:	*p*-cresol	16:	*o-iso*-propylphenol
4:	*o*-cresol	17:	*o-n*-propylphenol
5:	*m*-ethylphenol		
6:	3,5-xylenol		
7:	3,4-xylenol		
8:	*p*-ethylphenol		
9:	2,5-xylenol		
10:	2,3-xylenol		
11:	2,6-xylenol		
12:	*o*-ethylphenol		
13:	2,4-xylenol		

Fig.6 Chromatogram of phenol and alkylphenols on Develosil C_8-3 using methanol-triethylamine-water (40:0.15:59.85) as eluent.

Column: 15(+1)cm×4.6mm i.d. *Fc*:0.6ml/min Column temp.: 25℃
Sample size: each 2μl of 0.1 mM solution of No.1~13
each 2μl of 0.2 mM solution of No.14~17

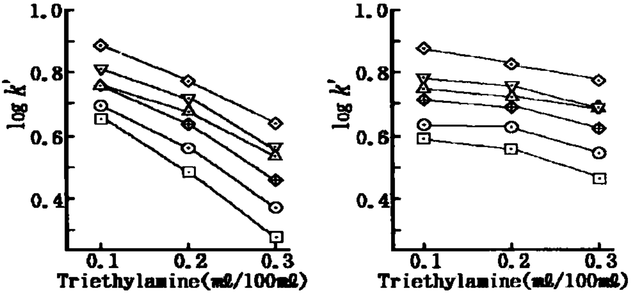


Fig.7-A Fig. 7-B

Variation of log k' of xylenols related to the adding amount of triethylamine.

Column: 15cm×4.6mm i.d. packed Develosil C_8-5 (5μm)
A: not end capped B: end capped
Eluent: methanol:water=20:80(v/v)
Fc: 0.6mℓ/min Column temp.: 25℃
Sample: ◇: 2,4- ▽: 2,6- △: 2,3- ◈: 2,5-
⊙: 3,4- ⊡: 3,5-

It is well known that apparent pH is higher in alcoholic solution than aqueous solution. The optimum pH in this chromatographic system was 0.87~1.31 higher than the *pKa* values described in TABLE 1.

Those *pKa* values were determined electrochemically or spectrophotometrically and some of them were calculated from their structural parameters. These values are agreeable with each others [43,44,45].

The retention order of ethylphenols and xylenols can be related with their *pKa* except 2,6-xylenol. The coefficient of variation of the capacity ratio of *o*-cresol in ten times experiments could afford a good result, 5.78×10^{-3}.

The end capping effect of C_8-5 packing is shown in Fig.7. The results measured on end capped packing is seen as Fig.7-A and those measured on not end capped packing as Fig.7-B. Differenceof capacity ratios measured on the two packings was rather small by addition of 0.1% TEA, and became larger by addition of more TEA. It is presumable that the residual silanol groups on the surface of not end capped packing are affected with TEA at the beginning. Then the TEA layer on silanol groups avoids the succeeding TEA to eluate the solute molecules.

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CONCLUSION

The nine structural isomers of carbon number two alkyl phenols were separated by RPLC depending on their dissociation constants, at about pH 11 controlled by the addition of triethylamine in the eluent. The separation mechanism is assumed depending on the dissociation of the solutes and no formation of ion-paired compound.

The alkyl chain length effect have not been found between eight and eighteen carbon chains.

The not end capped alkyl-bonded packing predominates in the RPLC depending on the dissociation constant of alkylphenols more than end capped packings.

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