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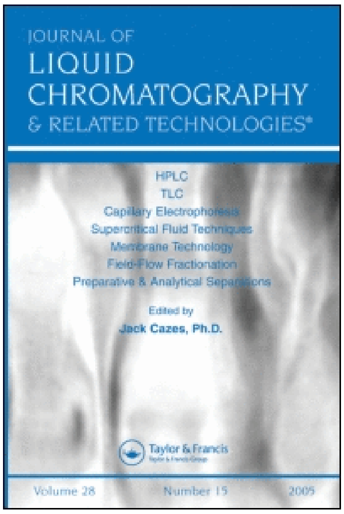

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Ion-Pair Liquid Chromatography of Alkylanilines

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ION-PAIR LIQUID CHROMATOGRAPHY OF ALKYLANILINES

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ABSTRACT

The optimization of ion-pair liquid chromatography of structural isomers of carbon number two alkyl anilines has been studied.

The shorter alkyl chain bonded silica gel demonstrated the stereo selectivity. The alkyl chain length of ion-pair reagent ruled the equilibration of ion-pair formation. The longer chain of ion-pair reagent formed hydrophobic ion-pair and the shorter chain could not form ion-pair, and ionized solute formed ion-pair with polar ion which was a component of eluent. Three ethylanilines and six xylidines mixture was separated on an octadecyl-bonded silica gel column using an eluent containing methanol/water mixture with sodium 1-dodecanesulfonate.

INTRODUCTION

The separation and rapid determination of small amounts of alkylanilines are very important, due to the wide use as starting materials of pharmaceuticals and dyes,and as adduct of rubber products. In addi-

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* partly presented by Nomura,N.,Hara,M. and Ishiji,K. at 25th Sym. Liq. Chromatogr.,Kyoto,Feb.23,1982(Abstract p.75), Nomura,N.,Hara, M. and Yamamoto,A. at 40th Sym. Anal. Chem.of Japan,Kōfu,June 2,1979(Abstract p.99) and Nomura,N. and Hara,M. at 10th Chūbu Assoc. Chem. Soc. of Japan, Shizuoka,Oct.2,1979(Abstract p.158).

tion, alkylanilines cause serious hazards as neurotoxicity[1], formation of methemoglobin[2] and carcinogenics[3].

Gas chromatography of aniline derivatives has been reported[4-7], however the derivatization of the solutes to volatile compounds was required as an indispensable technique. James and Martin[4], James[5], Funasaka[6] and Schmeltz *et al.*[8] studied gas chromatographic separation of some aniline derivatives by their prelabeling. Schmeltz *et al.* separated six xylidine isomers by gas chromatography after trifluoroacetylation[8]. However, they did not try the whole separation of structural isomers of carbon number two alkyl derivatives of aniline.

HPLC is useful separation technique of thermo unstable compounds.

Normal-phase HPLC(NPLC) generally lends to separate structural isomers. McNair and Young pointed out the reflection of the interaction of the basic solutes with the slightly acidic stationary phase to the profile of the solutes[9]. They also postulated an interaction between silanol and lone pair of nitrogen atom of aniline[10]. Schmeltz *et al.* improved the elution characteristics of xylidines by NPLC[8], after the modification of the mobile phase by addition of ethyl acetate in n-hexane to reduce the interaction between the basic solutes and the slightly acidic stationary phase. Chow and Glushka attempted to separate of aniline derivatives by formation of copper amino complex[11] and Kunzru attempted with cadmium impregnated silica gel as stationary phase in NPLC[12].

Reversed-phase liquid chromatography(RPLC) generally lends to separate homologues of solutes depending on the difference of hydrophobicity, and have difficulty to separate by small differences of hydrophobicity. The improvement of column packing material are quite remarkable, however the sufficient separation of alkylanilines has not been still succeeded.

The optimization of ion-pair liquid chromatographic separation of three ethylanilines and six xylidines is described on octadecyl bonded silica gel.

EXPERIMENTAL

The chromatograph is assembled with a model TRIROTOR high pressure pump, a model Uvidec-100 UV spectrophotometric detector, from JASCO Co., Ltd. (Hachioji, Japan) and a Chromatopac C-R1A recorder from Shimadzu Sci.Ind.Co.,Ltd.(Kyoto,Japan). The measurement wavelength was 260 nm. Column temperature was controlled at 25℃ with water jacket circulating Taiyo Thermo-Unit C-550 from Taiyo Sci.Co.Lt.(Tokyo,Japan).

Aniline, *o*-,*m*-, and *p*-toluidine and, *o*- and *p*-ethylaniline, 2,3-, 2,4-,2,5-,2,6-,3,4- and 3,5-xylidine and *N*-ethylaniline were purchased from Nacalai Tesque(Kyoto,Japan) and *m*-ethylaniline was done from Aldrich Chem.Ind.,Ltd.(Milwaukee,USA). These aniline derivatives were purified by reduced distillation under nitrogen stream. Deionized water was distilled and further sub-boiling distilled with quartz sub-boiling still. HPLC grade methanol and acetonitrile and special grade acetic acid and phosphoric acid were purchased from Wako Pure Chem. Ind.,Ldt.(Osaka,Japan). Ion-pair chromatography reagent grade sodium 1-pentanesulfonate, sodium 1-heptanesulfonate and sodium 1-dodecanesulfonate were purchased from Regis Chem.Co.,Ltd.(U.S.A.). Slurry solvent, Chemco slurry mix RP, was obtained from Chemco Sci. Co.,Ltd.(Osaka, Japan). Packing materials, HITACHI GEL 3043, spherical 5μm porous silica gel and HITACHI GEL 3053, octadecylsilylated HITACHI GEL 3043, were supplied from HITACHI Sci.Instr.Co.(Tokyo,Japan). Develosil TMS(trimethylsilyl)-3, Develosil C8(dimethyloctylsilyl)-3 and Develosil ODS(dimethyloctadecylsilyl)-3 were purchased from Nomura Chem.Co., Ltd.(Seto,Japan). The specificity of these packing materials are summarized in TABLE 1.

Columns were packed in 10cm ×4.6mm i.d. stainless steel tube by slurry packing method using Chemco Slurry Packing Apparatus Model 124A from Chemco Sci.Co.,Ltd. under 59.2Pa/m^2 pressured by nitrogen gas.

The pH of aqueous methanol and aqueous acetonitrile were adjusted with 0.1～1.0% of acetic acid to pH3.0～5.2 and with 0.02M phosphoric acid to pH2.1～2.9, and further added 2 mM solution of alkylsulfonate for ion-pair formation.

The concentration of standard samples were 0.2mM in 50 v/v% of aqueous methanol, and injection volume was 5 μℓ each.

RESULTS AND DISCUSSION

Normal-phase liquid chromatographic separation of alkylanilines was achieved using 5μm porous silica gel, HITACHI GEL 3043. The eluent was isopropyl alcohol:isooctane:acetonitrile= 0.99:98.21:0.80(

TABLE 1 The Properties of Column Packing Materials.

LC type	packing material	particle diameter (μm)	surface area (m^2/g)	mean pore diameter (Å)	pore volume (cm^3/g)	carbon content (w%)	end capped
NPLC	HITACHI GEL 3043	5	300	70~80	1.20	—	—
RPLC	HITACHI GEL 3053	5	300	70~80	1.20	9	not
	Develosil TMS-3	3	350	100	1.00	5	yes
	Develosil C8-3	3	350	100	1.00	13	yes
	Develosil ODS-3	3	350	100	1.00	21	yes

v/v%). *N*-Ethylaniline, 2,6-xylidine, *o*-ethylaniline, 2,5-xylidine, 2,4-xylidine, 3,5-xylidine, *m*-toluidine, 3,4-xylidine and *p*-toluidine were separated, but the whole separation of xylidines was not succeeded as seen in Fig. 1-A where *o*-toluidine was co-eluated with 2,4-xylidine, and 2,3- and 3,5-xylidine were co-eluated with *m*-ethylaniline.

These compounds are generally contained in aqueous sample solution, therefore RPLC is suitable for the practical application. First, the separation of these compounds on HITACHI GEL 3053 was investigated in RPLC. A mixture of *m*-toluidine, *p*-toluidine, 2,6-xylidine, 2,5-xylidine, 2,4-xylidine, 3,5-xylidine and 3,4 xylidine was satisfactorily separated in methanol:water=55:45(v/v%) as the eluent, however the separation of all xylidine isomers was not succeeded. The example of chromatogram is shown in Fig. 1-B. Therefore alkyl chain length effect of bonded phase was examined. Log k's of xylidines on Develosil TMS-3 were very close each other, and the separation was unsatisfactorily even high theoretical plate number column packed with $3\mu m$ packing and the Develosil TMS-3 demonstrated the stereo selectivity compared to a chromatogram obtained on Develosil C8-3 as seen in Fig. 2-A and B. The separation was better in methanol/water mixture than acetonitrile/water mixtures, and the addition of tetrahydrofuran did not improve the separation. Log k's of xylidines on Develosil C8-3 and Develosil ODS-3 were apparently larger than those on Develosil TMS-3. However log k's of xylidines on Develosil C8 3 were almost same as on Develosil ODS-3. This means that alkyl chain length effect of bonded phase to retain of xylidines is smaller on TMS and larger on both C8 and ODS as seen in Fig. 3.

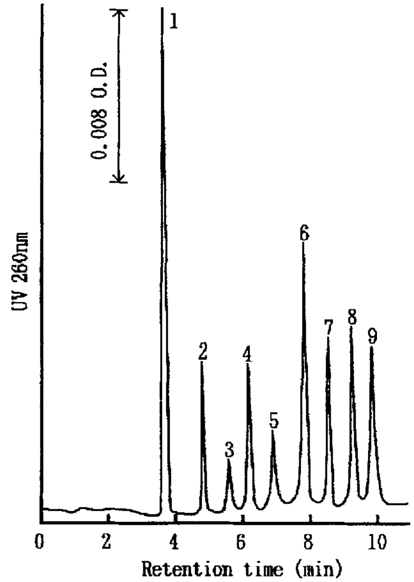

Fig.1-A Chromatograms of alkylanilines.

Column: 25cm×4.0mm i.d. packed HITACHI GEL 3043

Eluent: isopropanol:isooctane:acetonitrile=0.99:98.21:0.80(v/v)

Fc: 0.83mℓ/min

Sample:1:*N*-ethyl, 2:2,6-dimethyl, 3:*o*-ethyl, 4:2,5-dimethyl, 5:2,4-dimethyl, 6:3,5-dimethyl, 7:*m*-methyl, 8:3,4-dimethyl, 9:*p*-methylaniline

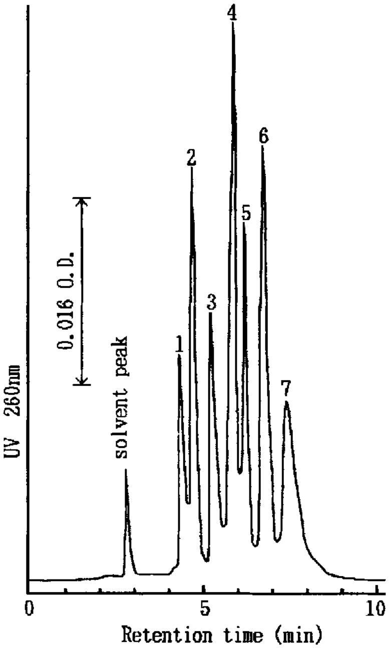

Fig.1-B Chromatograms of alkylanilines.

Column: 15cm×4.0mm i.d. packed HITACHI GEL 3053

Eluent: methanol:water=55:45 (v/v)

Fc: 0.70mℓ/min

Sample:1:*m*-methyl, 2:*p*-methyl, 3:2,6-dimethyl, 4:2,5-dimethl, 5:2,4-dimethyl, 6:3,5-dimethyl, 7:3,4-dimethylaniline

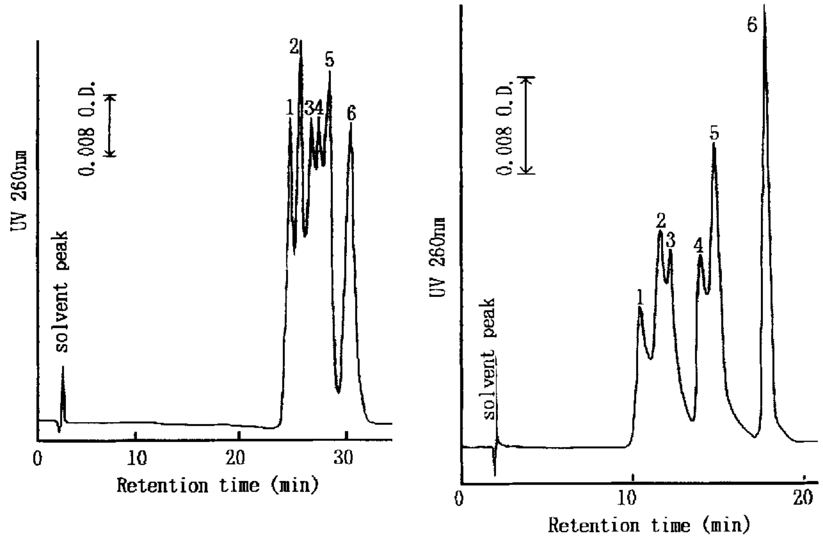

Fig.2-A Fig.2-B

Chromatograms of xylidines.

Fig.2-A	Fig.2-B
Column: 10cm×4.6mm i.d. packed Develosil TMS-3	Column: 10cm×4.6mm i.d. packed Develosil C8-3
Eluent: acetonitrile:water= 15:85(v/v)	Eluent: methanol:water= 35:65(v/v) added 0.09mM of phosphoric acid
Fc: 0.60mℓ/min	*Fc*: 0.60mℓ/min
Column temp.: 20℃	Column temp.: 25℃

Sample size: each 1 μℓ of 2mM solution

Sample: 1:2,3-, 2:2,6-, 3:2,4-, 4:2,5-, 5:3,4-, 6:3,5-	Sample: 1:2,3-, 2:3,4-, 3:2,4-, 4:3,5-, 5:2,5-, 6:2,6-

The variations of log *k*'s of xylidines were improved on Develosil C8-3 in methanol/water, but the stereo selectivity was poor as seen in Fig.2-B for the separation of isomers. Therefore pH effect was examined by addition of acetic acid or phosphoric acid in the eluent, however no clear separation of xylidines was obtained on Develosil ODS-3.

The addition of these acids did not affect the retention of di-*ortho* substituted anilines such as 2,6-xylidine because of the steric hindrance, and did not improve the total separation. Therefore the

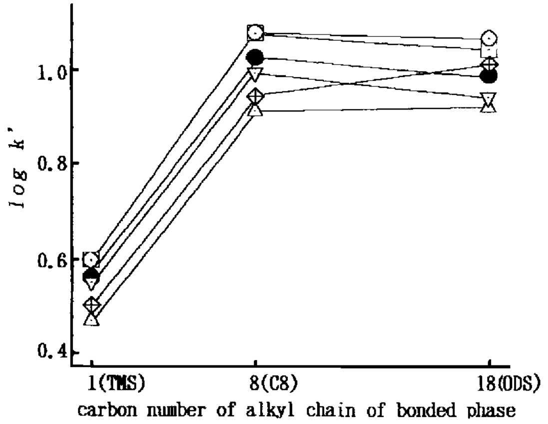

Fig.3 Variation of log *k*' of xylidines with change of carbon number of alkyl chain of bonded phase.

Column: 10cm×4.0mm i.d. packed Develosil 3μm
Eluent: methanol:water=35:65(v/v) *Fc*: 0.60mℓ/min
Column temp.: 25℃ Sample size:each 2μℓ of 2mM solution
Sample: ○: 3,4-, □: 3,5-, ⊕: 2,5-, ●: 2,4-,
▽: 2,6-, △: 2,3-

further separation was studied in ion-pair liquid chromatography. The result of pH effect of ion-pair was not simply understood. 1-Dodecanesulfonate demonstrated the expected result and 1-heptanesulfonate showed a mixed result as seen in Fig.4-A~C. Generally ionized solutes form ion-pair with oppositely charged ion either hydrophobic or polar ion. Ion-pair formation increases the retention time than that of ion-form solute.

The retention time of very hydrophobic ion-pair was longer than the retention time of molecular form solutes as the result obtained by addition of 1-dodecanesulfonate as seen in Fig.4-C. However 1-heptanesulfonate does not seem to make hydrophobic ion-pair at low pH in this condition.

The strong basic ion, phosphate ion, seemed to form ion-pair with xylidines rather than 1-heptanesulfonate at low pH. 1-Pentanesulfonate

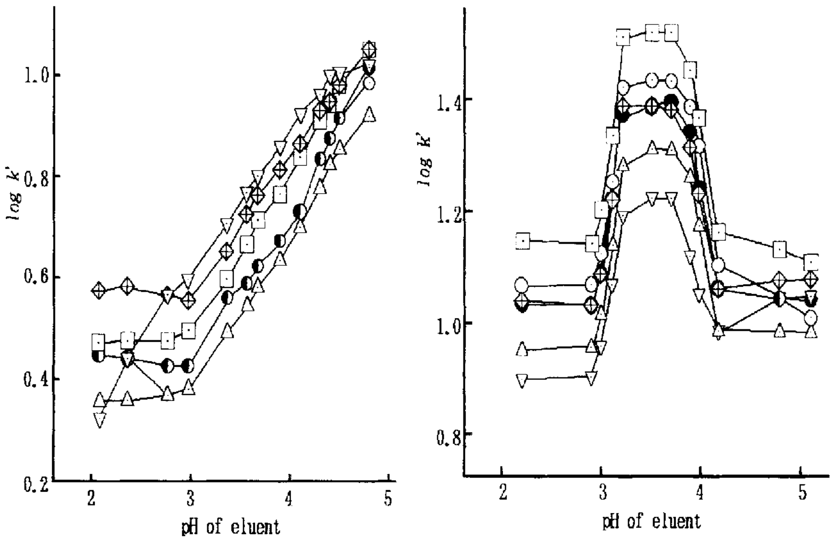

Fig. 4-A Fig. 4-B

Variation of log k' of xylidines related to pH of eluent.

Column: 10cm×4.6mm i.d. packed Develosil ODS-3
Eluent: methanol:water=32.5:67.5(v/v)
pH(3.0~5.1) was adjusted with 0.1~1.0% acetic acid
pH(2.1~2.9) was adjusted with 0.02M phosphoric acid
Ion-pair reagent:
sodium 1-pentanesulfonate 2mM sodium 1-heptanesulfonate 2mM
Sample: ○: 3,4-, □: 3,5-, ●: 2,4-, △: 2,3-, ◈: 2,5 , ▽: 2,6-
◑: 2,4- and 3,4- overlap

was seemed to be powerless to form hydrophobic ion-pair, and the retention behavior of xylidines was the same as the only pH effect of the retention of xylidines. This phenomenon can be explained as the result of strong ionic effect in reversed-phase ion-pair liquid chromatography. The dissociation constant of xylidines is around in 100% water, however that was not around in this system. The differences of dissociation constants in these figures were due to methanol concentration. Measured pH values with organic solvent was always higher than pH values in 100% aqueous solution.

The separation of mixture of three ethylanilines and six xylidines was completed within 20 minutes in ion-pair liquid chromato-

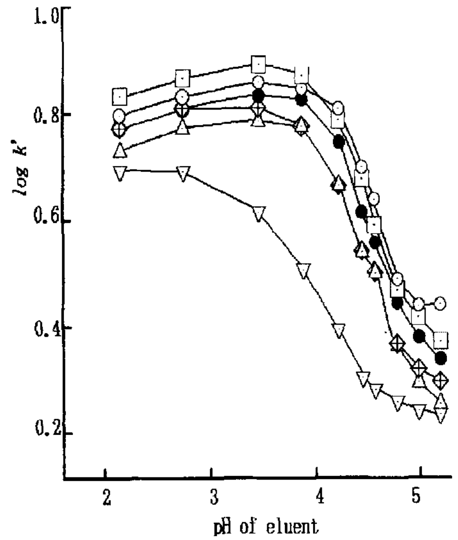

Fig.4-C Variation of log *k*' of xylidines related to pH of eluent.

Column: 10cm×4.6mm i.d. packed Develosil ODS-3

Eluent: methanol:water=60:40(v/v)
pH(3.5~5.2) was adjusted with 0.1~1.0% acetic acid
pH(2.2, 2.8) was adjusted with 0.02M phosphoric acid

Ion-pair reagent: 2mM of sodium 1-dodecanesulfonate

Sample symbols are same as denoted in Fig.3-A.

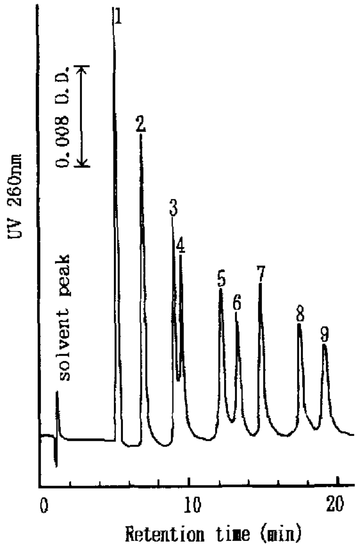

Fig.5 Ion-pairchromatograms of ethylanilines and xylidines.

Eluent: methanol:water=50:50(v/v)
pH4.8 was adjusted with 0.1% acetic acid

Ion-pair reagent: 2mM of sodium 1-dodecanesulfonate

Fc: 1.0mℓ/min Column temp.: 25℃

Column inlet press.: 26.2Pa/m²

Sample size: each 5μℓ of 0.2mM solution

Sample: 1: 2,6-xyl., 2: *o*-ethyl., 3: 2,5-xyl., 4: 2,3-xyl., 5: *m*-ethyl., 6: 2,4-xyl., 7:3,5-xyl., 8: *p*-ethyl., 9: 3,4-xyl.

graphy using 1-dodecanesulfonate on the octadecyl bonded-silica gel as shown in Fig. 5. Their detection limits were several ng in S/N=2 in this system.

CONCLUSION

The selection of alkyl chain length of ion pair reagent was important in reversed-phase ion-pair liquid chromatography[14]. The retention of ion-pair with short chain ion-pair reagent was weaker than the retention of molecular form compounds. Ionized solutes formed polar ion-pairs with strong ion of eluent components at low pH and their retentions were not strong enough for their separation.

The longer alkyl chain of ion-pair reagent forms the more stable ion-pair, and the retention was long enough for the separation on ODS silica gel. Steric hindrance of di-*ortho* substituted compound apparently prevents the formation of ion-pair and the retention was lower than the other ion-pairs, however such steric hindrance was not clearly observed on mono-*ortho* substituted compounds.

The separation of mixture of nine isomers having carbon number two alkyl compound of aniline was completed in ion-pair liquid chromatography.

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