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STUDY OF RETENTION BEHAVIOUR OF PRIMARY, SECONDARY AND TERTIARY ANILINES IN NORMAL- AND REVERSED-PHASE LIQUID CHROMATOGRAPHY

L.-Å. TRUEDSSON* and B. E. F. SMITH
Department of Technical Analytical Chemistry, Lund Institute of Technology, P.O. Box 740, S-220 07 Lund 7 (Sweden)
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SUMMARY

A series of alkyl-substituted primary, secondary and tertiary anilines were chromatographed on three liquid chromatographic systems: one normal-phase system on a nitrile stationary phase and two reversed-phase systems on octadecyl- and octylsilane. On the nitrile stationary phase the elution order is tertiary, secondary and primary anilines and within each group the retention is mainly determined by the base strength, the number and size of *ortho*-alkyl groups and the size of the alkyl groups substituted at the nitrogen atom. On the reversed phases the anilines within each group are mainly eluted in order of increasing alkyl carbon number.

The relationship between the pK_b values of the anilines and log k' on the nitrile stationary phase is discussed and a comparison is made between k' for primary anilines and the corresponding phenols on the same stationary phase. Identification of different kinds of anilines by means of a two-phase plot is discussed and a method is described for the systematic separation of alkylanilines into structural types and individual compounds.

INTRODUCTION

In a previous paper[1], liquid chromatography (LC) was evaluated for the analysis of a series of alkyl-substituted phenols and some useful relationships were established between structure and elution order on various stationary phases. As an extension of this work, a similar study on alkylanilines is reported in this paper. It involved about 40 primary, secondary and tertiary alkylanilines, using both normal- and reversed-phase systems.

Previously, little attention has been paid to bonded-phase LC assay of these kinds of amines, in spite of the fact that they are widely used in the chemical industry and that their toxicity makes it essential to be able to control their occurrence in the environment. In the first successful application of a bonded stationary phase to the LC analysis of anilines, some phenylenediamines were separated on an "ether"-bonded phase[2].

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It was concluded that this stationary phase in combination with cyclopentane–methanol as carrier was useful for the separation of compounds containing NH groups. Later, Sleight[3] made a systematic survey of the elution of some phenols and a few alkylanilines from Durapak OPN and Durapak Carbowax 400 bonded stationary phases. However, these stationary phases are now obsolete for bonded-phase LC. Recently, Frohliger *et al.*[4] reported the separation and quantitation of some aromatic amines that occur in the working environment by means of ion-exchange chromatography, using a surface-sulphonated cation exchanger and perchloric acid as the eluent.

EXPERIMENTAL

Apparatus

The LC pump used was a Varian Model 4100 (Varian, Palo Alto, CA, U.S.A.) and the detector was a Laboratory Data Control Model 1285 UV monitor (Laboratory Data Control, Riviera Beach, FL, U.S.A.) used at 280 nm. Sample application was accomplished by a valve injector (Rheodyne, Berkeley, CA, U.S.A.) with a 20-μl loop.

Columns

The bonded-phase packing materials were the commercially available LiChrosorb RP-8 (10 μm) (E. Merck, Darmstadt, G.F.R.), Nucleosil C_{18} (5 μm) and Nucleosil CN (5 μm) (Machery, Nagel & Co., Düren, G.F.R.). LiChrosorb RP-8 was packed by the balanced density technique using tetrabromoethane. Nucleosil C_{18} and Nucleosil CN were packed in accordance with the upward-slurry packing technique[5,6]. All columns consisted of precision-bore stainless-steel tubing (200 × 4.4 mm I.D.). The columns were used at room temperature.

For accurate work it is necessary to reactivate the columns regularly, especially the nitrile column. This was done according to recommended procedures. The stability of the columns was tested daily using a mixture of phenol, 2,6-dimethylphenol and 4-*tert.*-butylphenol for the reversed phases and a mixture of diphenylamine, triphenylamine and N-methyl-2-methylaniline for the nitrile phase.

Chemicals

Isooctane (certified ACS grade; Fischer Scientific, Fairlawn, NJ, U.S.A.), methanol (analytical-reagent grade; May & Baker, Dagenham, Great Britain), 2-propanol (pro analysi grade; E. Merck, Darmstadt, G.F.R.), sodium dihydrogen orthophosphate, $NaH_2PO_4 \cdot 2H_2O$ (99%) (BDH, Poole, Great Britain), disodium hydrogen orthophosphate, $Na_2HPO_4 \cdot 2H_2O$ (according to Sörensen; E. Merck) and orthophosphoric acid (pro analysi grade; E. Merck) were used for preparing the LC eluents.

The anilines were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. N,N-Dimethyl-2,6-dimethylaniline, N,N-diethyl-2-ethylaniline and N-ethyl-2-ethylaniline were prepared in this labotarory.

Mobile phases

For reversed-phase LC on C_8 and C_{18} stationary phases methanol–aqueous buffer was used and for normal-phase LC on the nitrile stationary phase isooctane containing 0.2% (v/v) of 2-propanol was applied.

Methanol–aqueous buffer (60:40) (pH 7.0). A 15-ml volume of 0.025 *M* Na_2HPO_4 + 250 ml of 0.025 *M* NaH_2PO_4 + 397.5 ml of methanol; the pH was adjusted to 7.0 with small amounts of orthophosphoric acid and 1 *M* sodium hydroxide solution.

Methanol–aqueous buffer (70:30) (pH 7.0). A 15-ml volume of 0.025 *M* Na_2HPO_4 + 250 ml of 0.025 *M* NaH_2PO_4 + 618.33 ml of methanol; the pH was adjusted as above.

Methanol–aqueous buffer (80:20) (pH 7.0). A 15-ml volume of 0.05 *M* Na_2HPO_4 + 250 ml of 0.05 *M* NaH_2PO_4 + 1060 ml of methanol; the pH was adjusted as above.

Procedure

Anilines were dissolved in methanol or isooctane to give concentrations of approximately 0.02–0.04 mg·ml^{-1} and 20 µl of the solutions were injected on to the LC columns. The capacity factors given are mean values from at least three injections with a relative standard deviation of about 3%. Retention times of unretained solutes were determined by injecting *n*-hexane on the nitrile phase and sodium nitrate solution (0.05%, w/w) on the reversed phases. The capacity factor, k', was calculated from the equation

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_R is the retention time of the sample and t_0 that of the unretained solute.

The systematic separation procedure described later involves separation into primary, secondary and tertiary anilines on the nitrile phase, followed by a separation according to alkyl carbon number on the C_{18} phase. The fractions of anilines in isooctane solution collected from the nitrile phase cannot be introduced directly on to the C_{18} phase, but have to be transferred to methanol before injection. About 200 µl of methanol were added and the mixture was shaken in order to extract the anilines. The methanolic solution was then injected on to the C_{18} phase.

Acetylation procedure for the separation of secondary and tertiary anilines

The mixture of secondary and tertiary anilines eluted from the nitrile phase with isooctane was collected in a test-tube. The volume was reduced by about 75% by blowing a gentle stream of nitrogen over the surface at room temperature. A 1-ml volume of acetic anhydride was added and the mixture heated over a small flame nearly to boiling for 1 min. The excess of acetic anhydride was then removed with a stream of nitrogen as above nearly to dryness.

In order to separate the tertiary anilines from the acetylated secondary anilines, *ca.* 100 µl of isooctane were added and the solution was injected on top of a short glass column (50 × 4 mm), filled with nitrile stationary phase (slurry packed without pressure). Tertiary anilines were eluted with 2–3 ml of isooctane, then acetylated secondary anilines with the same volume of ethanol, with separate collection.

The tertiary aniline fraction can be injected on to the nitrile phase after concentration, but the acetylated secondary anilines have to be hydrolysed first. A 2-ml volume of 50 % sulphuric acid was added and the mixture heated nearly to boiling for 10 min. After cooling, the acid was neutralized with excess of dilute sodium hydroxide solution and the free secondary anilines were extracted with a few millilitres of isooctane. After concentration with a stream of nitrogen as before, the solution is ready for injection on to the nitrile phase.

RESULTS AND DISCUSSION

The liquid chromatographic investigation involved three chemically bonded stationary phases: one normal-phase system with a nitrile phase and two reversed-phase systems with octyl- and octadecylsilane. With the reversed-phase systems, two eluents with different proportions of methanol and aqueous buffer were investigated.

Normal-phase liquid chromatography

The results of the runs on the nitrile phase are given in Table I. Within the group of primary anilines, the compounds are listed in order of increasing number of alkyl groups on the benzene ring, and within the secondary and tertiary groups of anilines in order of increasing size of substituents on the nitrogen atom.

It can be seen from Table I and from Fig. 7 that primary anilines travel more slowly on the nitrile phase than any of the secondary and tertiary anilines, and secondary more slowly than tertiary anilines, with a few exceptions. This result indicates that the migration is governed by the ease of access to the nitrogen atom. This conclusion is further confirmed by an examination of the order of elution within each group of anilines.

For primary anilines, *i.e.*, anilines without alkyl groups on the nitrogen atom, the order of elution is determined mainly by the number and size of *ortho*-alkyl groups. Thus, primary anilines with two *ortho*-alkyl groups have the lowest retentions, and those without such groups the highest. Among the latter, *para*-substituted anilines travel more slowly than the corresponding *meta*-substituted anilines and the size of the alkyl group seems to be of minor importance for the retention. This fact is demonstrated by the series of 4-alkyl-substituted primary anilines.

The difference in retention between *para*-substituted primary anilines without *ortho*-substituents and the corresponding *meta*-substituted anilines can be ascribed to the fact that anilines belonging to the former group are stronger bases and accordingly more ionized in the eluent than are the members in the latter group.

The influence of *ortho*-substitution on the interaction of primary anilines with the nitrile phase is demonstrated in Fig. 1, where log k' is plotted against alkyl carbon number. It can be seen that the anilines fall into three groups according to the number of *ortho*-substituents, *viz.*, non-*ortho*-, mono-*ortho*- and di-*ortho*-substituted compounds, separated by the broken lines in Fig. 1. In a previous study the same relationship was found to apply to phenols[1].

For secondary anilines the retention on the nitrile phase is mainly governed by two factors, *viz.*, the size of the alkyl group substituted on the nitrogen atom and the presence or absence of *ortho*-alkyl groups and by their size. Thus, log k' decreases linearly with N-alkyl carbon number for N-alkylanilines without nuclear substituents

TABLE I

CAPACITY FACTORS (k') FOR ALKYLANILINES IN FOUR LC SYSTEMS

Normal-phase system: Nucleosil CN. Mobile phase: 0.2% (v/v) 2-propanol in isooctane. Reversed-phase systems: (a) Nucleosil C_{18}, mobile phase methanol–aqueous buffer (80:20 and 70:30) (pH 7.0); (b) LiChrosorb RP-8, mobile phase methanol–aqueous buffer (60:40) (pH 7.0).

No.	*Aniline (substituent)*	k'			
		Nitrile phase	C_{18} *phase*		C_8 *phase, (60:40) eluent*
			(80:20) eluent	*(70:30) eluent*	
	Primary anilines				
1	None	7.53	0.41	0.55	0.60
2	2-Methyl	4.84	0.55	0.85	0.92
3	2-Ethyl	3.65	0.71	1.20	1.40
4	2-Isopropyl	3.14	0.86	1.63	2.07
5	3-Methyl	8.07	0.51	0.83	0.94
6	3-Ethyl	7.02	0.67	1.20	1.49
7	4-Methyl	9.62	0.54	0.85	0.97
8	4-Ethyl	8.72	0.71	1.26	1.59
9	4-Isopropyl	8.87	0.90	1.75	2.46
10	4-*n*-Butyl	8.72	1.38	3.11	4.84
11	2,3-Dimethyl	5.48	0.74	1.18	1.31
12	2,4-Dimethyl	6.04	0.78	1.25	1.49
13	2-Methyl-4-*n*-butyl	5.07	1.91	4.71	7.40
14	2,5-Dimethyl	4.45	0.75	1.26	1.43
15	2,6-Dimethyl	2.83	0.78	1.31	1.43
16	3,4-Dimethyl	9.51	0.69	1.16	1.40
17	2,4,6-Trimethyl	2.96	1.08	1.99	2.34
	Secondary anilines				
18	N-Methyl	2.32	0.68	1.12	1.22
19	N-Methyl-2-methyl	1.43	0.97	1.68	1.86
20	N-Methyl-3-methyl	2.16	0.89	1.62	1.86
21	N-Methyl-4-methyl	2.73	0.90	1.63	1.93
22	N-Ethyl	1.49	0.87	1.54	1.77
23	N-Ethyl-2-methyl	0.82	1.34	2.57	2.91
24	N-Ethyl-3-methyl	1.40	1.14	2.26	2.71
25	N-Ethyl-4-methyl	1.72	1.15	2.26	2.73
26	N-Ethyl-2-ethyl	0.67	1.86	3.80	4.61
27	N-Acetyl		0.41	0.62	0.71
28	N-Acetyl-4-methyl		0.54	0.87	1.09
29	N-Propyl	1.06	1.23	2.35	3.00
30	N-*n*-Butyl	0.90	1.69	3.70	5.04
31	N-Phenyl	2.50	1.52	3.45	4.86
32	N-Benzyl	1.65	1.50	3.42	4.79
	Tertiary anilines				
33	N,N-Dimethyl	0.65	1.35	2.42	2.68
34	N,N-Dimethyl-2-methyl	0.45	1.76	3.48	3.99
35	N,N-Dimethyl-3-methyl	0.68	1.82	3.61	4.17
36	N,N-Dimethyl-4-methyl	0.79	1.80	3.64	4.17
37	N,N-Dimethyl-2,6-dimethyl	0.14	4.20	10.1	
38	N,N-Diethyl	0.50	2.32	5.21	6.44
39	N,N-Diethyl-2-methyl	0.29	3.45	7.71	8.89
40	N,N-Diethyl-3-methyl	0.54	3.08	7.31	9.18
41	N,N-Diethyl-4-methyl	0.90	2.95	6.81	8.06
42	N,N-Diethyl-2-ethyl	0.21	5.21	12.8	16.8
43	N,N-Diphenyl	0.39	7.40	25.9	

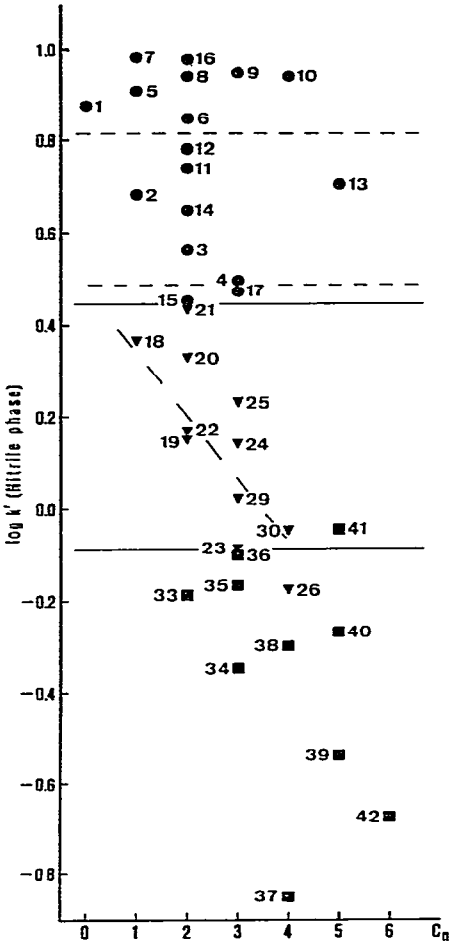

Fig. 1. Relationship between alkyl carbon number (C_n) and log k' of alkylanilines on the nitrile stationary phase (Nucleosil CN). Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. ● = Primary anilines; ▼ = secondary anilines; ■ = tertiary anilines. The numbers refer to Table I.

(N-alkyl = methyl to butyl, Nos. 18, 22, 29 and 30 in Fig. 1), and a further decrease then results from *ortho*-substitution of hydrogen for alkyl groups. As for primary anilines, the effect of *meta*-substitution on retention is slight while *para*-substitution increases retention.

For the retention of tertiary anilines on the nitrile phase the same rules apply as for secondary anilines. Accordingly, retention is a function of the size of the alkyl groups on the nitrogen atom, decreasing with increasing size. In the same way, *ortho*-substitution of alkyl groups decreases retention. Among tertiary anilines, those with one or two *ortho*-alkyl groups can be clearly distinguished from secondary anilines by their lower retention, whereas there is some mixing between other kinds of tertiary and secondary anilines (Fig. 1).

Relationship between pK_b of anilines and log k' on the nitrile phase

It is interesting that if log k' on the nitrile phase is plotted against pK_b of the primary anilines[7–9], a semi-linear correlation is obtained (Fig. 2). A similar relationship between pK_b and log k' on silica gel was reported for toluidines and phenylenediamines[10]. Among secondary anilines, N-methylanilines form a group of their own, whereas N-alkylanilines with ethyl and larger alkyl groups align themselves around a lower line.

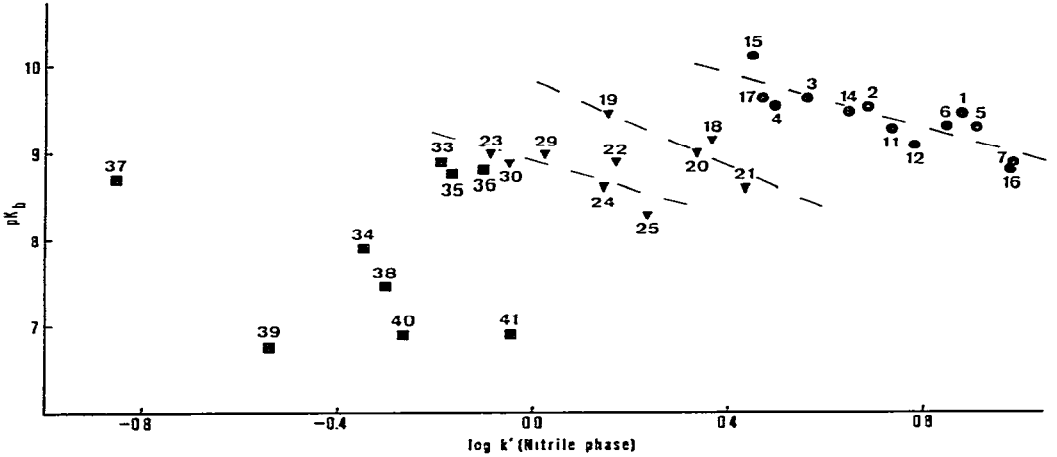

Fig. 2. Relationship between pK_b and log k' of alkylanilines on the nitrile stationary phase (Nucleosil CN). Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. ● = Primary anilines; ▼ = secondary anilines; ■ = tertiary anilines. The numbers refer to Table I.

To this group also belong some tertiary anilines, *viz.*, N,N-dimethylanilines without *ortho*-alkyl groups. For tertiary anilines with greater steric hindrance around the nitrogen atom, which appear at the bottom of the plot, the spread of points is considerable. This group consists of N,N-dimethylanilines with *ortho*-methyl groups and N,N-diethylanilines.

Within each of the above-mentioned groups of anilines there is a general trend of increasing k' values with decreasing pK_b values, *i.e.*, with increasing base strength of the compounds. This would be expected as the pK_b value is a measure of the ability of the amino group to accept a proton and the normal-phase k' value is mainly a measure of its ability to interact with a nitrile group in the bonded phase.

Because of the different steric requirements of the proton and the nitrile group, the semi-linear relationship between pK_b and log k' is only valid for sterically equivalent anilines, which causes a subdivision into structural groups as illustrated in Fig. 2. Accordingly, the k' value of an alkylaniline on the nitrile phase system is primarily a function of its base strength and of the substitution pattern around the nitrogen atom, the latter mainly determining the subdivision into structural groups, whereas the position of an aniline within a group is governed by both the base strength and steric factors.

Reversed-phase liquid chromatography

Three systems were studied, two on a C_{18} stationary phase with methanol–

aqueous buffer (80:20 and 70:30) as eluents and one on a C_8 stationary phase with methanol–aqueous buffer (60:40) as eluent. In all instances the pH was adjusted to 7.0. The results are given in Table I. It is evident that for primary anilines the alkyl carbon number determines the retention whereas the position of the alkyl groups in the benzene ring is of minor importance.

On moving alkyl groups from the nucleus to the nitrogen atom a distinct increase in retention occurs. Thus, secondary anilines invariably have higher retentions than primary anilines with the same total alkyl carbon number, and the same applies to tertiary *vs.* secondary anilines. Accordingly, if retention on the C_{18} phase is plotted against total alkyl carbon number, primary, secondary and tertiary anilines align themselves along three parallel lines (Fig. 3).

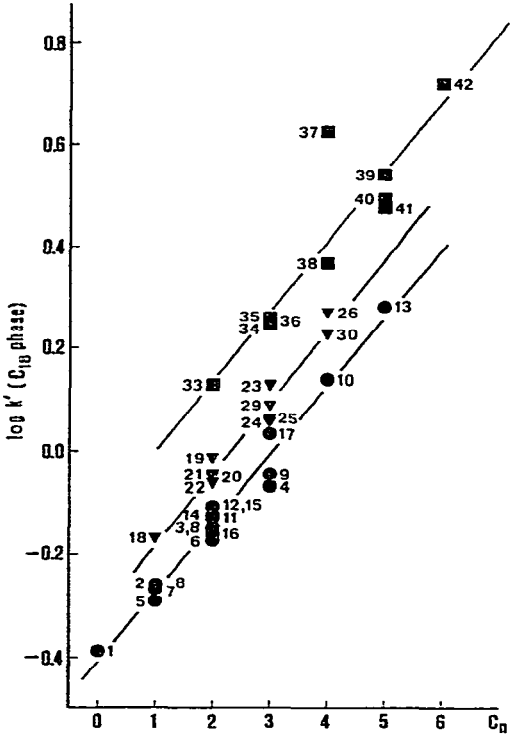

Fig. 3. Relationship between alkyl carbon number (C_n) and log k' of alkylanilines on the C_{18} stationary phase (Nucleosil C_{18}). Mobile phase, methanol–aqueous buffer (80:20) (pH 7.0); 45 ml h^{-1}. ● = Primary anilines; ▼ = secondary anilines; ■ = tertiary anilines. The numbers refer to Table I.

Of the two solvent systems used for the C_{18} phase, methanol–aqueous buffer (80:20) elutes lower primary anilines too rapidly for a good resolution to be obtained, and methanol–aqueous buffer (70:30) elutes higher tertiary anilines too slowly to be of practical value. A compromise between the two solvent systems should therefore best meet the case of a more universal isocratic solvent for alkylanilines on the C_{18} phase.

Using the C_8 stationary phase with methanol–aqueous buffer (60:40) (pH 7.0)

as eluent, alkylanilines behave much as on the C_{18} phase with methanol–aqueous buffer (70:30), the retentions being slightly higher (Table I).

Comparison between primary anilines and phenols

Primary anilines are structurally analogous to phenols and it is therefore not surprising to find their behaviour in LC to be similar. For phenols it was concluded that differences in the strength of hydrogen bonding to the proton-accepting cyano group was responsible for the division into non-*ortho*-, mono-*ortho*- and di-*ortho*-substituted compounds, when run on the nitrile phase[1]. The same explanation should be valid for the corresponding primary anilines, although the division into structural classes is less sharp.

In Fig. 4, k' values for alkylphenols on a nitrile stationary phase, taken from ref. 1, are plotted against k' values for the corresponding primary anilines, taken from Table I. It can be seen that there is a considerable spread of points, indicating different behaviours of primary anilines and phenols on the nitrile phase. Although the two nitrile phases used are different, it is felt that certain conclusions can be drawn from Fig. 4.

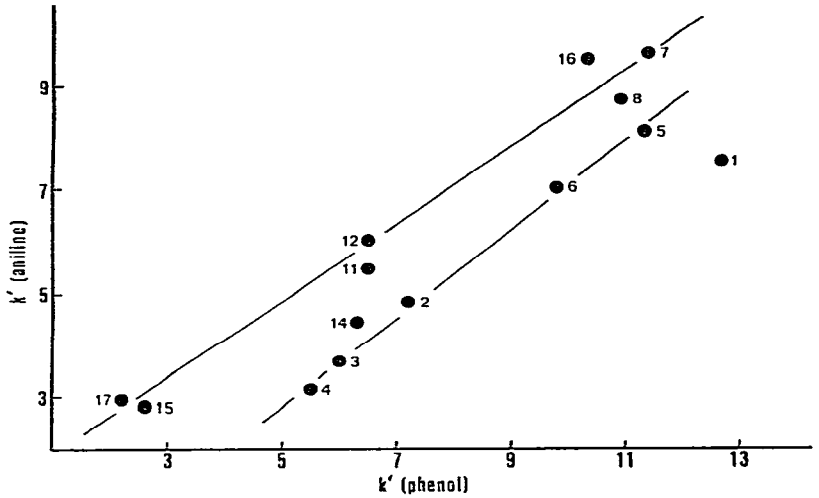

Fig. 4. Relationship between k' values of alkylanilines and k'values of corresponding alkylphenols on nitrile phases. Anilines: stationary phase, Nucleosil CN; mobile phase, 0.2 % (v/v) 2-propanol in isooctane; 45 ml h^{-1}. Phenols: stationary phase, Cyano Sil-X-1; mobile phase, 0.5 % (v/v) 2-propanol in isooctane; 40 ml h^{-1}. The numbers refer to Table I.

The points are more or less aligned along two straight lines. Along the upper line are mainly found di-*ortho*-alkyl-substituted compounds and compounds having a *para*-alkyl group. For these compounds there is no great difference between the k' values of corresponding anilines and phenols. In the former instance this can be ascribed to the fact that the *ortho*-alkyl groups partly inhibit hydrogen bonding, making the chromatographic properties of the aniline and the phenol more similar. A levelling effect on the hydrogen bonding properties would also be exerted by a *para*-alkyl group, which tends to weaken the acidic properties of phenols and strengthen the basic properties of anilines[7].

Along the lower line are mainly compounds with a single *ortho*- or *meta*-alkyl group. For these compounds there is a considerable difference between the k' values for anilines and the corresponding phenols. In this instance the levelling effect of the alkyl groups is smaller, and the stronger hydrogen bonding of the phenolic hydroxyl group causes phenols to move more slowly than the corresponding anilines on the nitrile phase. This difference is most pronounced for phenol and aniline themselves with their points lying far outside the two regions discussed above, as can be seen from Fig. 4.

In reversed-phase chromatography of primary anilines and phenols the influence of the two functional groups is small, retention being mainly determined by the number of alkyl groups. This fact is demonstrated in Fig. 5, which shows k' values on the C_{18} phase for corresponding compounds. The slightly higher k' values of anilines on this phase can be ascribed to the presence of residual acidic silanol groups in the C_{18} phase, which interact with the basic amino groups, and to the fact that phenols are stronger electrolytes.

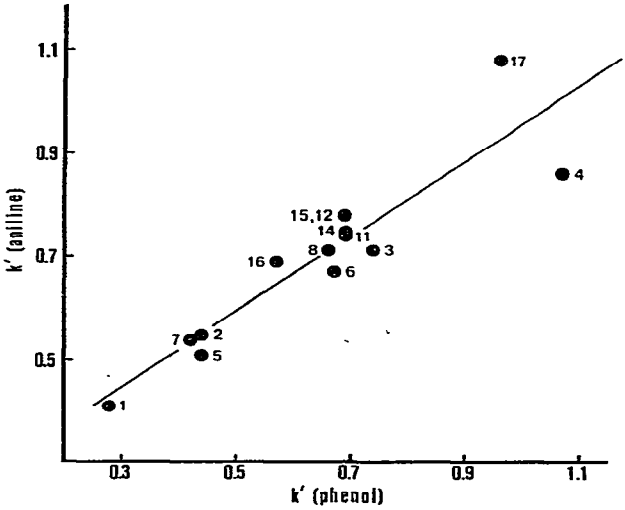

Fig. 5. Relationship between k' values of alkylanilines and k' values of corresponding alkylphenols on C_{18} phases. Anilines: stationary phase, Nucleosil C_{18}; mobile phase, methanol–aqueous buffer (80:20) (pH 7.0); 45 ml h^{-1}. Phenols: stationary phase, μBondapak C_{18}; mobile phase, ethanol–water (60:40); 20 ml h^{-1}. The numbers refer to Table I.

Use of two-phase plot for the identification of alkylanilines

In Fig. 6, log k' for the C_{18} phase system is plotted against log k' for the nitrile stationary phase system. It appears that the plot can be divided into zones for different kinds of anilines. Thus, all primary anilines fall to the right of vertical line 1 (zone P), and the horizontal line divides the secondary and tertiary anilines into two zones below and above the line (zones S and T, respectively). The P zone may in turn be divided by two vertical lines into zones for non-*ortho*-substituted compounds (to the right of line 3), mono-*ortho*-substituted compounds (between lines 2 and 3) and di-*ortho*-substituted compounds (between lines 1 and 2).

Although the boundaries between some of the zones are rather narrow and

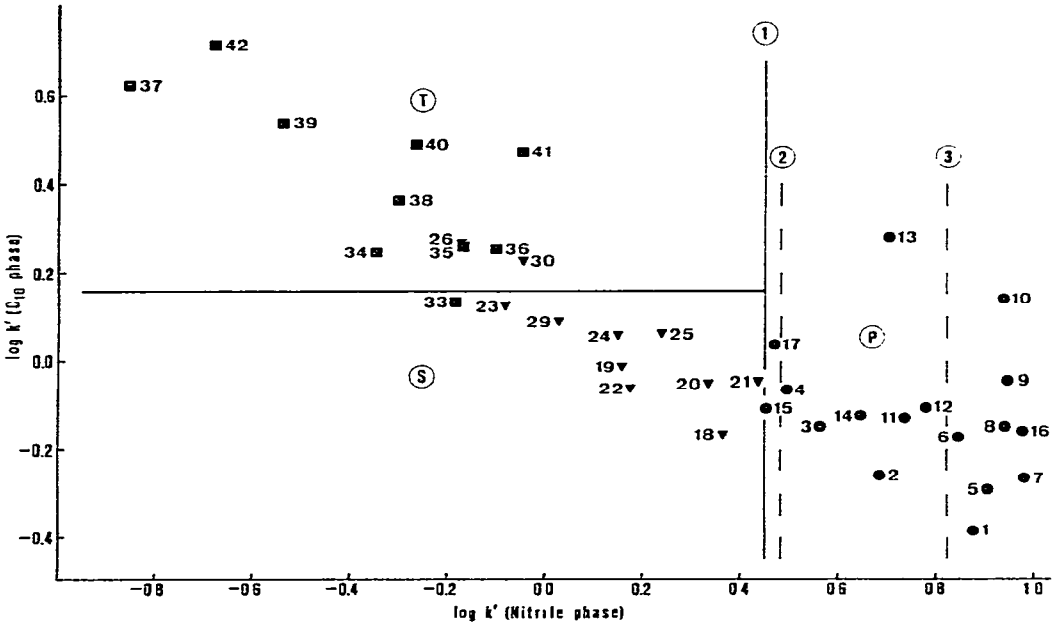

Fig. 6. Two-phase plot of log k' of alkylanilines on reversed phase [Nucleosil C_{18}, methanol–aqueous buffer (80:20) (pH 7.0); 45 ml h^{-1}] *versus* log k' on normal phase [Nucleosil CN, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}]. ● and P = primary anilines; ▼ and S = secondary anilines; ■ and T = tertiary anilines. The numbers refer to Table I.

some mixing of compounds occurs, it is felt that Fig. 6 would be useful for identification purposes. The fact that different kinds of alkylanilines fall into different zones will make it possible to distinguish to a great extent between primary, secondary and tertiary alkylanilines and between the three structural classes of primary anilines. In addition, the zone diagram gives information about alkyl carbon number, anilines with the lowest carbon number appearing at the bottom of each zone and those with the highest carbon number at the top.

There is just one tertiary aniline falling into the S zone; as it is the lowest member in the series of tertiary anilines, *viz.*, N,N-dimethylaniline, it is safe to conclude that no other tertiary aniline will appear in this zone. The T zone contains some secondary anilines. This is unavoidable, as the horizontal separation between secondary and tertiary anilines, *i.e.*, the separation on the nitrile phase, is insufficient and the vertical position of a secondary aniline in the diagram is determined solely by the alkyl carbon number.

It can be concluded that it is impossible to decide whether an aniline falling in the T zone is in fact a secondary aniline. For this purpose other methods must be resorted to. A simple test to distinguish between the two classes of amines is to heat the sample for about 2 min with an excess of acetic anhydride and then chromatograph the product on the nitrile phase system. If no change in retention is observed, the aniline is tertiary and, if no peak is obtained, or the retention time is much increased, the amine is secondary. The reason for this behaviour is that only secondary anilines react with acetic anhydride and that the acetylated amine travels very slowly on the nitrile column (see below).

Systematic separation of alkylanilines into primary, secondary and tertiary anilines and individual compounds

As pointed out previously, the investigated primary alkylanilines may be separated as a class from secondary and tertiary anilines on the nitrile phase, and the latter two classes are also largely resolved on the same phase. Accordingly, the problem of separating an unknown mixture of alkylanilines by LC should be solved by first running the mixture on the nitrile phase, isolating the compounds according to class, and then running the three classes separately on the C_{18} phase, where a separation according to alkyl carbon number is achieved. When this procedure was applied to the 41 anilines (Nos. 1–26 and 29–43) listed in Table I, the chromatograms shown in Figs. 7 and 8 were obtained.

If the contents in the peaks representing the different carbon numbers are collected from the C_{18} phase and re-run on the nitrile phase, the peaks are largely resolved into individual compounds, as demonstrated in Figs. 9 and 10. In order to distinguish between primary, secondary and tertiary anilines, 2,6-dimethylaniline (No. 15) and N-propylaniline (No. 29) are admixed for marking the boundaries between the three structural classes.

In Fig. 8 the tertiary aniline fraction was run as obtained from the nitrile phase, which means that it contains some secondary anilines. However, for mixtures of unknown composition it is recommended first to separate the two groups of anilines by the acetylation method described below, and then to run them separately on the C_{18} phase.

The acetylation procedure, as detailed under Experimental, involves treatment

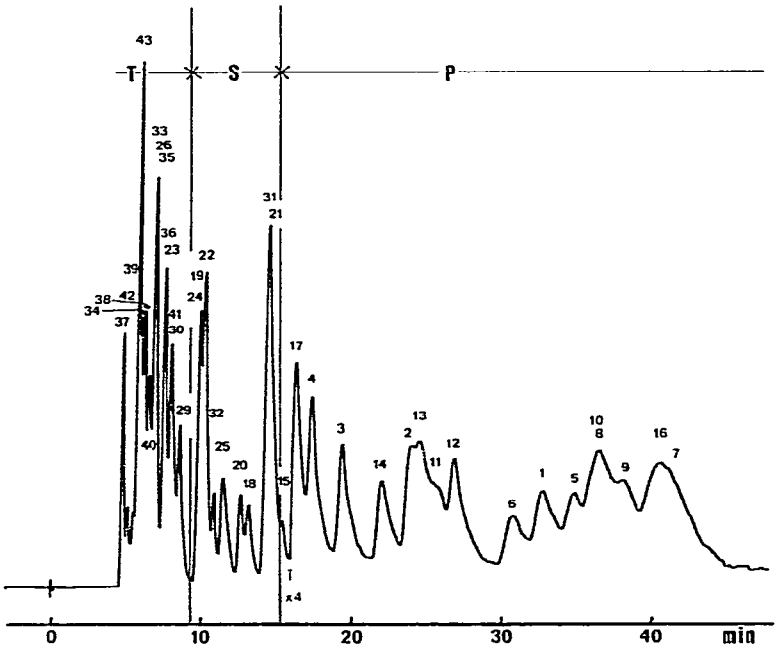

Fig. 7. Class separation of a mixture of primary, secondary and tertiary alkylanilines on the nitrile stationary phase (Nucleosil CN). Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. P = primary anilines; S = secondary anilines; T = tertiary anilines. The numbers refer to Table I.

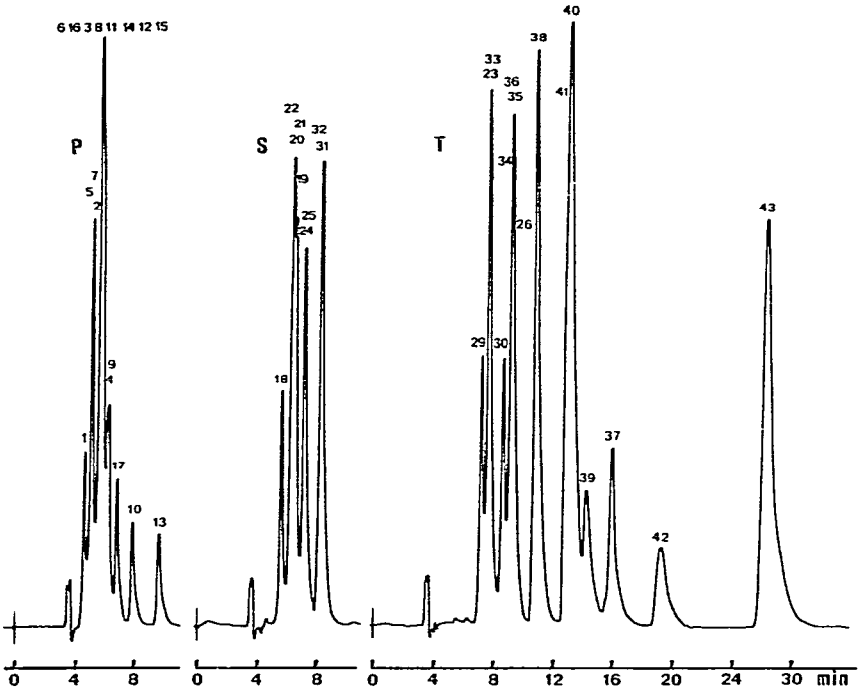

Fig. 8. Carbon number separation on the C_{18} stationary phase (Nucleosil C_{18}) of primary, secondary and tertiary anilines isolated from the run on the total mixture on the nitrile phase (Fig. 7). Mobile phase, methanol–aqueous buffer (80:20) (pH 7.0); 45 ml h^{-1}. P = primary anilines; S = secondary anilines; T = tertiary anilines. The numbers refer to Table I.

of the mixture of secondary and tertiary anilines with acetic anhydride in order to convert the secondary anilines into acetyl derivatives. The mixture is then passed through a short column packed with nitrile phase. Unchanged tertiary anilines are rapidly eluted with isooctane, whereas acetylated secondary anilines travel more slowly and are eluted in a second step with a stronger solvent, ethanol. On hydrolysis of the latter mixture, free secondary anilines result.

The application of the acetylation procedure to a mixture of eight secondary

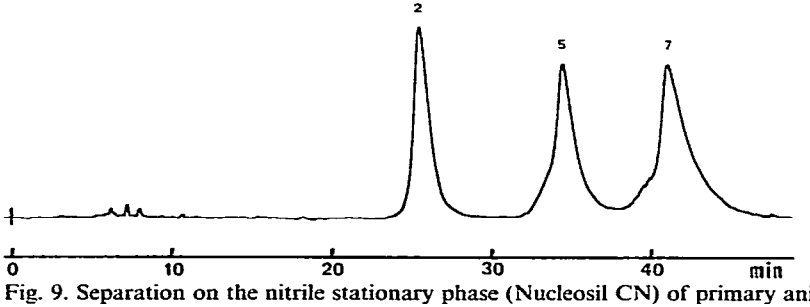

Fig. 9. Separation on the nitrile stationary phase (Nucleosil CN) of primary anilines present in the peak with carbon number 1, isolated from the carbon number run on primary anilines (Fig. 8). Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. The numbers refer to Table I.

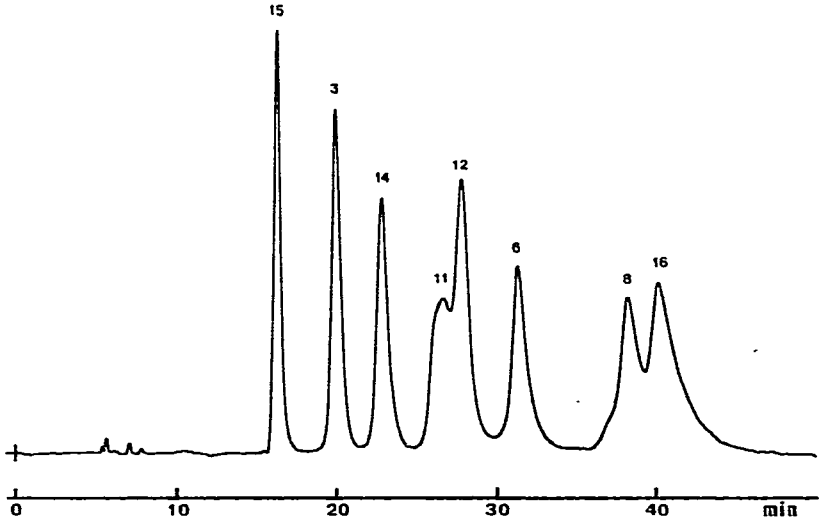

Fig. 10. Separation on the nitrile stationary phase (Nucleosil CN) of primary anilines present in the peak with carbon number 2, isolated from the carbon number run on primary anilines (Fig. 8). Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. The numbers refer to Table I.

and tertiary anilines is demonstrated in Fig. 11. This mixture contains all four secondary anilines (Nos. 23, 26, 29 and 30) that fall into the tertiary group in the original class separation on the nitrile phase (Fig. 8). The chromatogram of the total mixture on the nitrile phase is given in Fig. 11 (S + T). After acetylation, separation and hydrolysis, the two chromatograms in T and S for tertiary and secondary anilines, respectively, result. It can be seen that the class separation achieved is complete. The separation procedure outlined above will permit the class separation of complex mixtures of primary, secondary and tertiary anilines and will also largely resolve the mixtures into individual compounds. In addition, information is obtained about alkyl carbon number and, to a certain extent, about the number and position of nuclear alkyl groups.

As shown by the chromatograms in Fig. 8, the investigated primary and secondary anilines are eluted from the C_{18} phase in order of increasing alkyl carbon number. For tertiary anilines there is some deviation from this rule, as N,N-dimethyl-2,6- dimethylaniline (No. 37) with an alkyl carbon number of 4 appears after the three tertiary anilines Nos. 39–41 with an alkyl carbon number of 5. It seems that a high degree of steric hindrance around the nitrogen atom will increase the retention on the C_{18} phase, resulting in a disturbance of the regular elution according to alkyl carbon number.

N-Phenyl- and N-benzylanilines

The investigated material contains some anilines with aromatic groups substituted on the amino group. These anilines appear together with other secondary and tertiary anilines in the class separation on the nitrile phase (Fig. 7). On the following carbon number separation on the C_{18} phase they are eluted last of the investigated compounds in their respective groups (Fig. 8). In the two-phase plot in Fig. 6 these

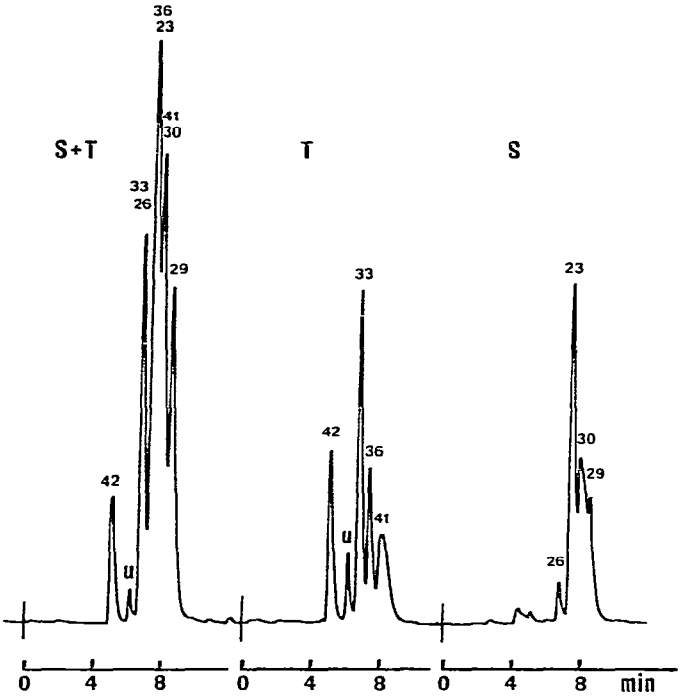

Fig. 11. Application of the acetylation procedure to a mixture of secondary (S) and tertiary (T) anilines. (S + T) = chromatogram of mixture on the nitrile stationary phase (Nucleosil CN); S and T = chromatograms of the isolated secondary and tertiary anilines, respectively, on the nitrile phase. Mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml h^{-1}. The numbers refer to Table I. u = Unknown.

compounds would appear in the T zone and cannot be distinguished from other kinds of secondary and tertiary anilines.

CONCLUSIONS

Bonded-phase normal- and reversed-phase LC, using nitrile and C_{18} stationary phases, can be applied to the separation of complex mixtures of alkylanilines and the identification of individual compounds. The separation on the nitrile phase proceeds in the order tertiary, secondary and primary anilines, and these classes can then be resolved on the C_{18} phase in order of increasing alkyl carbon number.

For confirming the structure of an alkylaniline, several useful relationships are available, *viz.*, two-phase plots and correlations between log k' and carbon number and between log k' and pK_b values of the anilines. By these means it is possible to ascertain the identity of an alkylaniline in terms of class and general structure with a fair degree of certainty.

ACKNOWLEDGEMENT

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REFERENCES

1 K. Callmer, L.-E. Edholm and B. E. F. Smith, *J. Chromatogr.*, 136 (1977) 45.
2 J. J. Kirkland, *J. Chromatogr. Sci.*, 2 (1971) 206.
3 R. B. Sleight, *Chromatographia*, 6 (1973) 3.
4 J. O. Frohliger, K. S. Booth and N. Kotsko, *ACS Symp. Ser.*, No. 120 (1980) 115.
5 P. A. Bristow, P. N. Brittain, C. M. Riley and B. F. Williamsson, *J. Chromatogr.*, 131 (1977) 57.
6 C. Hansson, L.-E. Edholm, G. Agrup, M. Rorsman, A.-M. Rosengren and E. Rosengren, *Clin. Chim. Acta*, 88 (1978) 419.
7 J. W. Smith, in S. Patai (Editor), *The Chemistry of the Amino Group*, Wiley, Chichester, 1968, pp. 180*ff*.
8 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
9 I. Bacaloglu, R. Bacaloglu, H. Glatt, C. Viragh and G. Ostrogvich, *J. Chem. Soc., Perkin Trans. 2*, (1976) 524.
10 P. R. Young and H. M. McNair, *Anal. Chem.*, 47 (1975) 756.