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Effect of some mobile phase additives on the retention characteristics of different solute types on reversed-phase media. II

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Abstract

In this paper, the use of aliphatic additives for mediating the retention characteristics of general solutes on reversed-phases using high-performance liquid chromatography is described. Aliphatic nitrile compounds have been investigated as potential mobile phase additives in simple mobile phases consisting of organic modifier–water mixtures. A range of amine solutes have been chromatographed at various levels of additive concentration on commercial bonded reversed-phases and their retention characteristics compared. Satisfactory separations were only obtained when additives such as valeronitrile and hexanenitrile were used in the mobile phase. The additives were effective in dramatically reducing peak tailing using a range of mobile phase compositions and commercial reversed-phases. The results obtained show that relatively small amounts of additive can cause significant changes in retention and can significantly improve the chromatography of organic amines.

1. Introduction

Reversed-phase high-performance liquid chromatography on bonded-phase silica is the preferred means of separating polar and non-polar solutes. The most common form of bonded-phase is silica covalently bonded with alkyl ligands. The retention characteristics of a reversed-phase are governed by the number of ligands bonded to the silica and the number of non-bonded surface silanol groups. The relative number of bonded-phase to silanol groups is dependent on the nature of the silica substrate, the chemical structure of the alkyl ligand and the chemistry of the bonding process.

Large differences in chromatographic reten-

tion can be observed between commercial columns and between different batches of the same brand of column packing. Marked differences were noted between some commercial C₁₈ columns in a recent comparative investigation [1].

In particular, polar compounds, e.g. acids and amines, have been shown to be difficult to chromatograph satisfactorily on reversed-phases which have a high proportion of residual silanol groups [2,3]. The choice of a suitable column for any application is a difficult one and still relies on the experience and judgement of the analyst. However, some chromatographic tests can be carried out to ascertain the nature and retention character of reversed-phases [4–6].

Methods of circumventing the problem of column irreproducibility have been sought, e.g. the method of dynamically modifying silica. An

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in-situ coating of stationary phase can be created on the silica surface by using mobile phase additives, usually polar long-chain aliphatic compounds, that are adsorbed on to the silica surface. The column takes on the retention characteristics of a reversed-phase. A reproducible 'reversed' phase can thus be created on any type of silica provided that equilibrium is achieved [7]. An analogous approach to eliminate column variability by using mobile phase additives to modify bonded-phases has been investigated by Lau and Simpson [8]. Aliphatic compounds having different functional groups may be used to modify the retention characteristics of the bonded-phase and has been demonstrated by a number of applications [8–11].

This investigation focuses on the use of aliphatic nitrile compounds as additives in reversed-phase high-performance liquid chromatography and supplements investigations previously carried out by the authors [10].

2. Experimental

A Hewlett-Packard 1090M HPLC system (Stockport, UK) was used. Data were collected at 10 points per second and reduced using the Waters 860 Expert Ease data system (Millipore, Watford, UK). HPLC grade methanol, acetonitrile and tetrahydrofuran (Romil Chemicals, Loughborough, UK) and purified water were used for the investigations. All chemicals were of analytical grade (Aldrich, Gillingham, UK). Chromatographic separations were performed on Spherisorb C_8 , 3 μm , 3 cm \times 4.6 mm I.D. columns (Phase Separations, Deeside, UK); Ultrasphere C_{18} , C_8 , and CN 3 μm , 7.5 cm \times 4.6 mm I.D. columns (Beckman, Fullerton, USA); and a Brownlee Velosep C_8 , 4 cm \times 3.2 mm I.D. column (Applied Biosystems, San Jose, CA, USA) thermostatted at 40°C. The flow-rate was 1.00 ± 0.02 ml/min and detection was UV at 254 nm (6 mm pathlength flow-cell). An Erma CR Model ERC-7515A refractive index detector (Tokyo, Japan) was used to determine retention time of the additives. The injection volume was 1.0 μl and replicates performed as necessary.

Base-solvents consisting of organic modifier–water mixtures were prepared in fixed weight ratios. Mobile phases were then prepared from known weights of base-solvents and additive. Mobile phases were degassed with helium before use. The test solutes aniline, N-methylaniline, and N,N-dimethylaniline (pK_a values 4.6, 4.8 and 5.2, respectively) were prepared in methanol, acetonitrile and tetrahydrofuran at 0.5 mg/ml concentration. For refractive index measurements solutes were prepared in mobile phase at ca. 1 mg/ml concentration. Dead volume measurements were determined with sodium nitrate dissolved in base-solvent (ca. 0.02 mg/ml) at 220 nm for UV detection and ca. 1 mg/ml for refractive index detection. All separations were carried out isocratically after equilibrium had been established.

3. Results and discussion

3.1. Chromatography of amine solutes using alkylnitrile additives in methanol–water

Valeronitrile was found to be an effective mobile phase additive for improving peak shapes in the reversed-phase chromatography of some organic amines using aqueous methanol mobile phases [10]. As a corollary to this study other aliphatic nitrile compounds (butyronitrile and hexanenitrile) were tested as potential additives under the same chromatographic conditions. Aniline test compounds were separated using mobile phases consisting of mixtures of methanol–water containing additive on a commercial C_8 reversed-phase column. Fig. 1 shows the separation of the aniline test mixture using increasing amounts of hexanenitrile in the mobile phase. Excellent chromatography is obtained when the concentration of hexanenitrile is 0.05% (w/w). Fig. 2 shows a comparison of the separations obtained using butyronitrile, valeronitrile and hexanenitrile as additives (at the 0.05% w/w level) in methanol–water. The separations show the relative efficacy of the additives under the same experimental conditions and clearly demonstrate a trend, i.e. efficacy in-

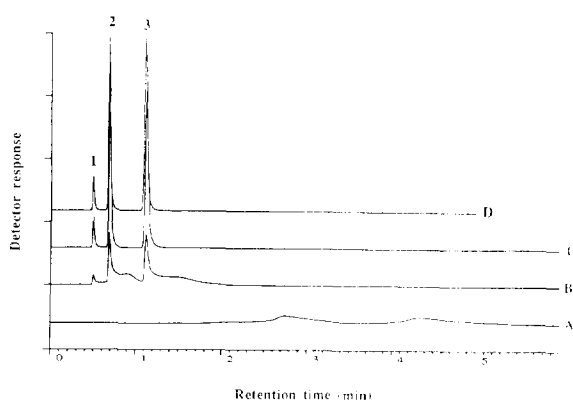


Fig. 1. Separation of anilines using methanol–water (50:50, w/w) with added hexanenitrile. Column: Spherisorb C_{18} , 3 μm , 3 cm \times 4.6 mm I.D. Peaks: 1 = aniline; 2 = N-methylaniline; 3 = N,N-dimethylaniline. (A) No additive; (B) hexanenitrile at 0.01% (w/w); (C) hexanenitrile at 0.02% (w/w); (D) hexanenitrile at 0.05% (w/w).

creases with increasing chain-length of the additive, $C_5H_{11}CN > C_4H_9CN > C_3H_7CN$. For valeronitrile, approximately 0.2% (w/w) is required in the mobile phase to give good separation and peak shapes; for butyronitrile, up to 2% (w/w) was added but even so poor peak shapes were observed. The affinity of the additive for the stationary phase is controlled by the distribution coefficient of the additive between the stationary and mobile phases in the same way as general solutes behave. As the additive chain-length is

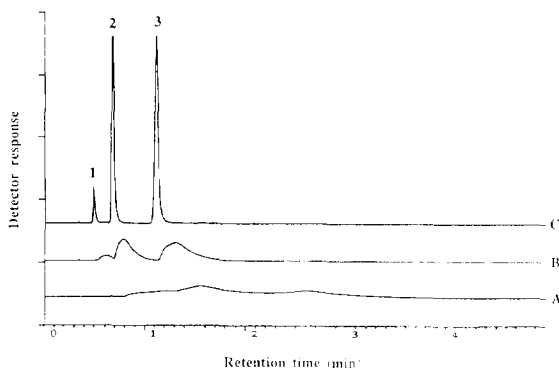


Fig. 2. Separation of anilines using methanol–water (50:50, w/w) with added butyronitrile, valeronitrile and hexanenitrile at the 0.05% (w/w) level. Peaks and column as in Fig. 1. (A) Butyronitrile; (B) valeronitrile; (C) hexanenitrile.

increased its adsorption strength is increased and its efficacy as a moderator enhanced. It is not possible to chromatograph anilines satisfactorily without the use of valeronitrile and hexanenitrile additives in methanol–water. Butyronitrile is not an effective additive owing to its high solubility in methanol–water. Similar improvements in chromatography using valeronitrile as the mobile phase additive have also been observed for isomeric toluidines and pyridine separations [10].

3.2. Chromatography of amine solutes using alkylnitrile additives in acetonitrile–water and tetrahydrofuran–water

Hexanenitrile was used as an additive in mobile phases consisting of water and other organic modifiers having different properties to methanol. Mobile phases of acetonitrile–water and tetrahydrofuran–water were prepared on a weight basis with small amounts of hexanenitrile added. The separations of anilines using acetonitrile and tetrahydrofuran as organic modifiers are shown in Fig. 3 and Fig. 4, respectively. Good peak shape is realised at about the 0.05% (w/w) level added hexanenitrile. The results demonstrate that hexanenitrile is an effective additive in common aqueous mobile phases. Further work showed that similar additive efficacy is

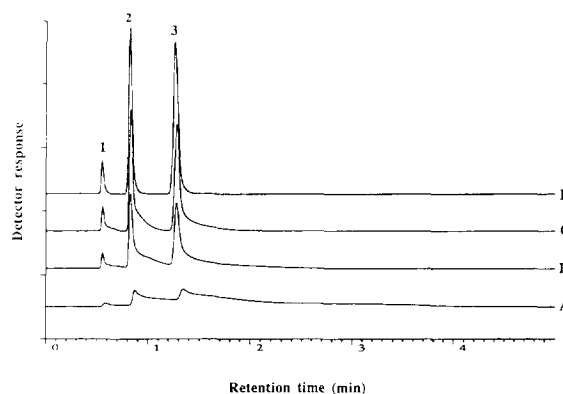


Fig. 3. Separation of anilines using acetonitrile–water (40:60, w/w) with added hexanenitrile. Peaks and column as in Fig. 1. (A) No additive; (B) hexanenitrile at 0.01% (w/w); (C) hexanenitrile at 0.02% (w/w); (D) hexanenitrile at 0.05% (w/w).

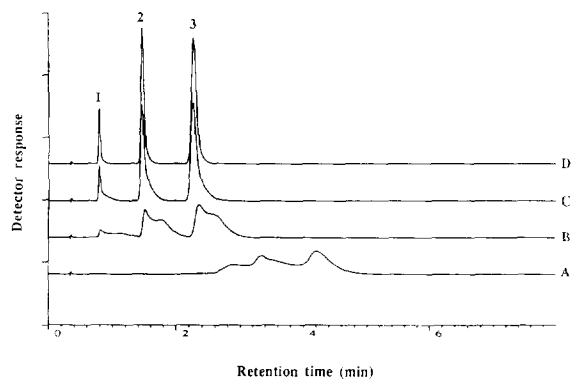


Fig. 4. Separation of anilines using tetrahydrofuran–water (30:70, w/w) with added hexanenitrile. Peaks and column as in Fig. 1. (A) No additive; (B) hexanenitrile at 0.01% (w/w); (C) hexanenitrile at 0.02% (w/w); (D) hexanenitrile at 0.05% (w/w).

obtained when using different ratios of water to organic modifier in the mobile phase (Fig. 5).

3.3. Chromatography of amine solutes using alkylnitrile additives in methanol–water on commercial reversed-phase columns

The chromatography of the anilines on different reversed-phase columns was studied. A second C_8 commercial column was tested with and without hexanenitrile added to a methanol–water mobile phase. Fig. 6 shows the chromatography when hexanenitrile is added to the mobile

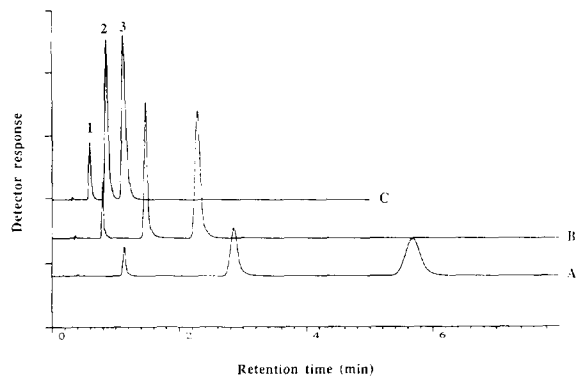


Fig. 5. Separation of anilines using tetrahydrofuran–water (20:80), (30:70) and (40:60, w/w) with added 0.05% (w/w) hexanenitrile. Peaks and column as in Fig. 1. (A) 20:80; (B) 30:70; (C) 40:60.

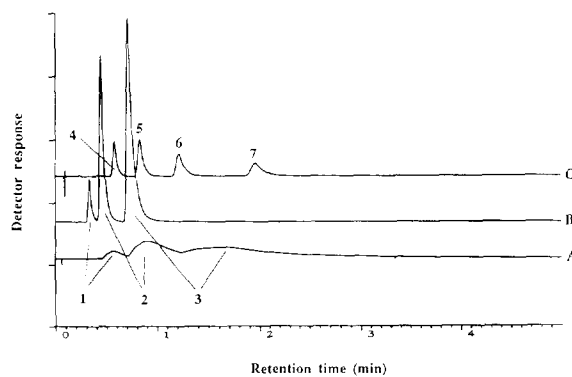


Fig. 6. Separations using methanol–water (50:50, w/w). Column: Brownlee Velosep C_8 , 3 μm , 4 cm \times 3.2 mm I.D. Peaks 1–3 as in Fig. 1; 4 = benzene; 5 = toluene; 6 = ethylbenzene; 7 = *n*-propylbenzene. (A) Anilines separation no additive; (B) anilines separation using hexanenitrile at 0.05% (w/w); (C) benzenes separation no additive.

phase at the 0.05% (w/w) level. The anilines peak shapes are improved though some tailing is still observed but this is due to the column performance as shown by the separation of some aromatic carbons (Fig. 6).

The study was extended to include C_{18} and cyano columns. Fig. 7 is a comparison of aniline separations performed on two reversed-phases (C_8 and C_{18}) and a cyano phase from the same manufacturer. Good separations are obtained on

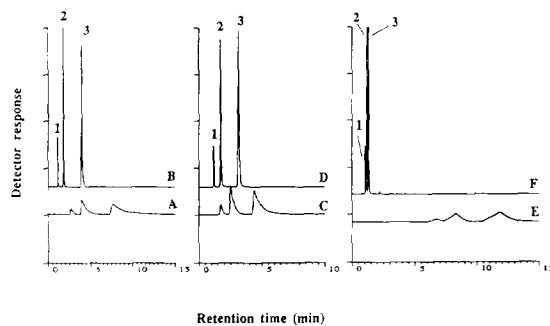


Fig. 7. Separation of anilines on Ultrasphere XL, 3 μm , 7.5 cm \times 4.6 mm I.D. columns using methanol–water (50:50, w/w) and methanol–water (50:50, w/w) with added hexanenitrile. Peaks as in Fig. 1. (A) C_{18} column no additive; (B) C_{18} column 0.05% (w/w) hexanenitrile; (C) C_8 column no additive; (D) C_8 column 0.05% (w/w) hexanenitrile; (E) CN column no additive; (F) CN column 0.1% (w/w) hexanenitrile.

all three of the stationary phases when hexanenitrile is added to the mobile phase.

3.4. Adsorption of alkylnitrile additives and their mechanism of action

The chromatography of amines on reversed-phase media has always been problematical owing to amine molecules interacting with residual silanol groups. Many ways of eliminating these interactions have been devised; use of ion suppression [2]; competing amines [12] and speciality base-deactivated columns [13]. However the use of ion-pairing necessitates the use of careful pH control, competing amines destroy the column with continued use and the speciality base-deactivated columns have not been shown to perform adequately in other reports [14,15] necessitating the inclusion of additives (e.g. N,N-dimethyloctylamine and tetrabutylammonium hydrogensulphate) to obtain adequate performance.

The work described in this paper demonstrates that the presence of small quantities of alkylnitrile additives can afford many advantages over other common approaches for chromatographing amines. The alkyl additives act by either presenting a new stationary phase to the solute molecules or by preventing access to residual silanol groups. The effect of the additive on the mobile phase strength might also be a contributing factor. These effects are currently being investigated.

The chromatography of the anilines clearly involves two mechanisms at hexanenitrile concentrations below 0.02% (w/w) as is shown by examining the amine peak shapes in Figs. 1, 3 and 4. The solute molecules may undergo dispersive interactions with the C_8 ligands and also hydrogen bonding interactions with the residual silanol groups which produces the distorted peak shape.

In an effort to determine the mechanism of action of the additives the retention of hexanenitrile was measured in methanol–water mobile phase containing added hexanenitrile. A composition of methanol–water base mobile phase (20:80) was required so that the retention

time of hexanenitrile could be measured accurately using a 3 cm long column. The hexanenitrile retention decreases as the hexanenitrile concentration of the mobile phase increases (Fig. 8). This decrease in retention is also observed for the anilines chromatographed in this study and in previous work [10] when pyridine and the isomeric toluidines were studied. This observation suggests that a more polar in-situ stationary phase is being formed. The orientation of the hexanenitrile molecules at the stationary phase surface is thought to be akin to the adsorption of common ion-pairing agents such as alkyl sulphonates, i.e. the hydrocarbon alkyl chain of the molecule is partitioned into the C_8 ligands while the polar group is oriented toward the mobile phase. This has been supported by some recent spectroscopic evidence for the orientation of long-chain (C_7 – C_{10}) *n*-alcohols adsorbed onto a C_{18} reversed-phase [16]. The retention of hexanenitrile passes through a minimum and then increases ($>0.2\%$ w/w hexanenitrile in the mobile phase). At this point there is a reversal of the retention character of the in-situ stationary phase, i.e. it becomes less polar and is possibly caused by the formation of hexanenitrile multilayers. Further, it was possible that the critical micelle concentration for hexanenitrile may have exceeded at the minimum of the curve leading to a micellar mechanism for retention

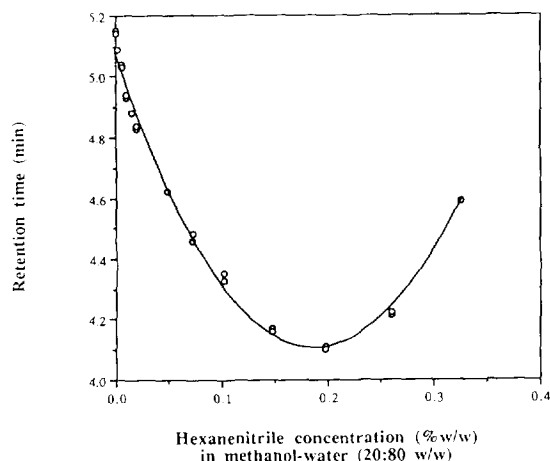


Fig. 8. Retention of hexanenitrile in methanol–water (20:80, w/w) with added hexanenitrile. Column as Fig. 1.

but NMR experimentation gave no evidence for this hypothesis. However, the possibility of micelle formation cannot be excluded. It is thought that the nitrile groups of the second layer of hexanenitrile molecules are oriented toward the nitrile groups of the monolayer. This could account for the increase in retention observed and has been suggested by Daucik et al. in their paper describing the use of hexanenitrile to influence the separation of some isomeric substituted tributylphenols [9]. Further work is in hand to provide a rationale for these effects and will be reported in a subsequent paper.

4. Conclusions

It has been shown that valeritrile and hexanenitrile are effective mobile phase additives for the reversed-phase separations of amines but butyitrile is ineffective. The efficacy of the additive is dependent on the alkyl chain-length and hexanenitrile was shown to be the most efficacious. Good chromatographic separations for amines were obtained using aqueous mobile phases having methanol, acetonitrile or tetrahydrofuran organic modifiers and containing hexanenitrile at low concentration. Equivalent effects were realised on reversed-phase columns from different sources when using the additives. To eliminate the undesired effects of silanol groups in reversed-phase chromatography these simple mobile phase additives offer several advantages over standard practices of separating organic amines in that the column performance

does not deteriorate with use and the UV cut-off is of the same order as acetonitrile. Hexanenitrile is insoluble in pure water but is soluble up to about 0.5% (w/w) in aqueous–organic solvents.

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