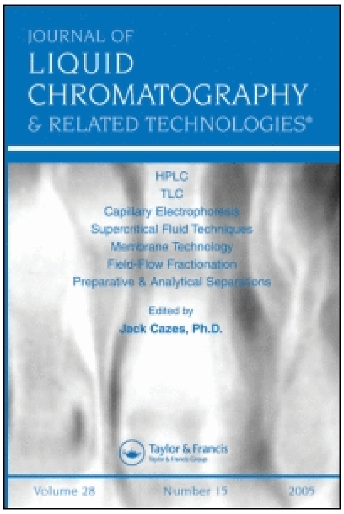

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GAS AND LIQUID CHROMATOGRAPHIC INVESTIGATIONS INTO ALKYLNITRILE COMPOUNDS CONTAINING AMINE IMPURITIES

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ABSTRACT

The purity of alkylnitrile compounds has been investigated using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC). The investigation was carried out to supplement earlier reported work using the alkylnitrile compounds. hexanenitrile and valeronitrile, as mobile phase additives in HPLC. GC impurity profiling and GC-MS experiments showed the presence of low level nitrogenous impurities in the alkylnitrile compounds. Analytical interrogation and chemical isolation were employed to identify the impurities as alkylamines. Tri-n-butylamine (TBA) and di-n-pentylamine (DPA) impurities were found in batches of hexanenitrile and valeronitrile respectively and it was found that it was the amine impurities, at very low levels, which caused the observed chromatographic improvements for basic solutes when using the alkylnitrile compounds as mobile phase additives in reversed-phase HPLC. The amines themselves were subsequently used at extremely low levels (ppm) in the mobile phase and were found to be very effective in their action.

INTRODUCTION

The chromatography of basic solutes has always presented problems on silica-based reverse phase columns because of the residual silanol groups resident on the silica substrate.[1-5] It is well known that silanol groups cause undesired secondary retention processes and distorted peak shapes.[2,6-8] Many techniques are employed to overcome these problems e.g. use of specialised base-deactivated column packings and polymer coated phases,[9-11] amine additives[12-14] and ion suppression.[2]

In an earlier investigation using aliphatic compounds as mobile phase additives to influence the retention characteristics of solutes on reverse phase media, we reported the successful use of alkylnitrile compounds to improve the chromatography of basic solutes.[15,16] Our recent studies have shed further light on this area and the results are reported here. Di-n-pentylamine (DPA) impurities were found in batches of valeronitrile at very low levels using a chemical isolation procedure and GC-MS analysis. Similarily, tri-n-butylamine (TBA) was found in batches of hexanenitrile. Both DPA and TBA were shown to be effective mobile phase additives in that only very low quantities (ppm) were required to bring about good chromatography for some basic solutes.

This paper describes these recent findings which show that our initial explanation for the observed chromatographic effects needs to be revised.

EXPERIMENTAL

Materials

HPLC

A Hewlett-Packard 1090M HPLC system (Stockport, UK) was used. Data were collected and reduced using the Waters 860 Expert Ease data system (Millipore, Watford, UK). HPLC grade solvents (Romil Chemicals, Loughborough, UK) and purified water were used for the investigations. All

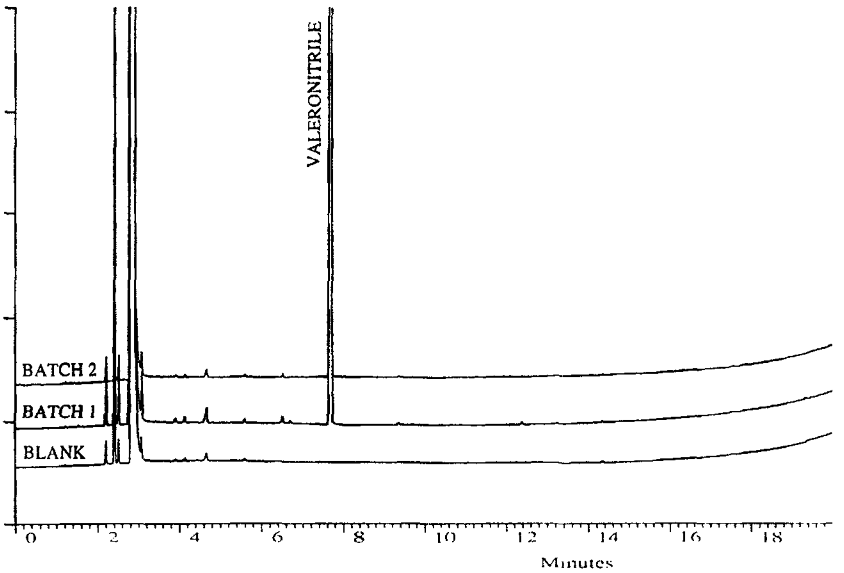

Figure 1. GC Impurity profile chromatograms of two valeronitrile batches. [Column DB 624, 30mx0.32mm, carrier gas:helium at 2.5 mLmin^{-1}, split flow 50mLmin^{-1}; Program: 10°Cmin^{-1} from 40° to 240°C,injection: 1.0 μL at 200°C, Detection: FID at 250°C].

chemicals were of analytical grade (Aldrich, Gillingham, UK). Chromatographic separations were performed on Spherisorb C8, 3 μm, 3 cm x 4.6 mm I.D. columns (Phase Separations, Deeside, UK) thermostatted at 40°C. The flow-rate was 1.0 mLmin^{-1} and UV detection set at 254 nm. The injection volume was 1.0 μL.

Mobile phases were prepared from methanol-water (50:50 w/w) and the additive and degassed with helium before use. The test solutes aniline, N-methylaniline, and N, N-dimethylaniline were prepared in methanol at 0.5 mgcm^{-3} concentration. All separations were carried out in isocratic mode.

GC and GC-MS

A Hewlett-Packard 5890 gas chromatograph (Stockport, UK) was used for the GC experiments. A DB-624, 30m x 0.32mm, 1.8 μm film capillary column (J&W Scientific, Folsom, USA) was used. Dichloromethane was used as the

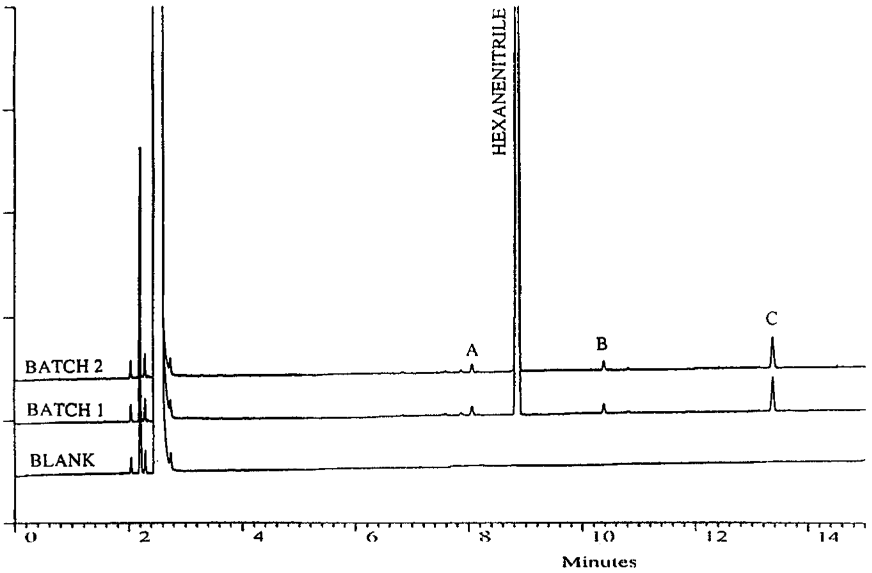

Figure 2. GC Impurity profile chromatograms of two hexanenitrile batches. Conditions as in Figure 1, except program:10°Cmin^{-1} from 50° to 200°C]

sample diluent. GC-MS was performed using a Hewlett-Packard 5890 GC coupled to a VG Trio-2 single quadrupole mass spectrometer (Altringham, UK).

METHODS

Isolation of Impurities from and Purification of Alkylnitrile Compounds

Hexanenitrile and valeronitrile were purified individually by washing the compound with 2M HCl three times. The organic phase was then washed with aq. $NaHCO_3$ three times to neutralise residual acid followed by three washings of aq. NaCl until neutral. The organic phase was dried over anhydrous $MgSO_4$ and filtered to give the purified compound. The acidic aqueous washings from the purification procedure were then adjusted to pH 12 with aq. NaOH and then another extraction with dichloromethane performed. This dichloromethane extract was analysed by GC.

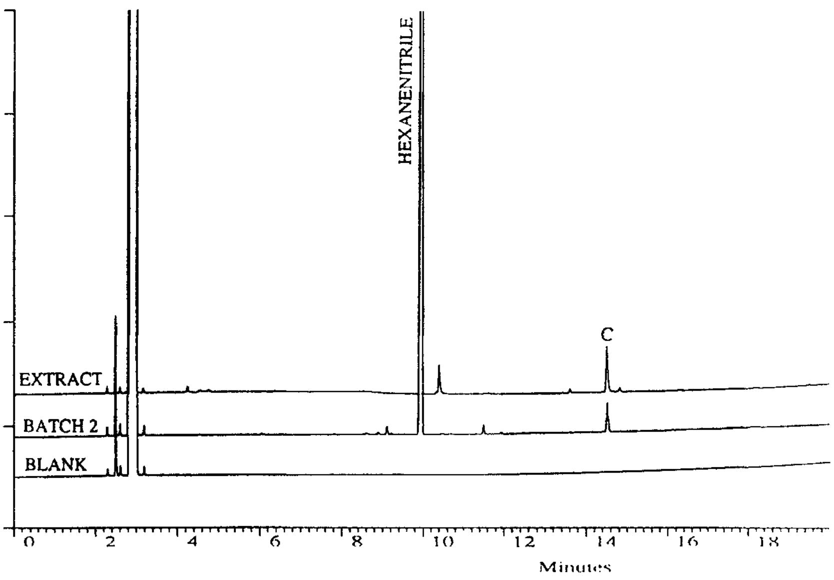

Figure 3. GC Chromatograms showing the presence of Impurity C in hexanenitrile and its dichloromethane extract. [Conditions as in Figure 1].

RESULTS AND DISCUSSION

The chromatography of basic solutes has always been problematical on silica-based reverse phase columns because of the residual silanol groups resident on the silica substrate.[1-5] It is well known that silanol groups cause undesired secondary retention processes and give rise to distorted peak shapes.[2,6-8]

Many techniques are employed to overcome these problems e.g. use of specialised base-deactivated column packings and polymer coated phases,[9-11] amine additives[12-14] and ion suppression.[2] In our earlier work we suggested the use of the alkylnitrile additives, hexanenitrile and valeronitrile, for use as mobile phase additives as an additional approach for the reverse phase chromatography of basic solutes.[15,16]

Our subsequent investigations have revealed that the chemical purity of the alkylnitrile additives employed is in question. Impurities, in some cases at trace levels, in the additives have been found to be responsible for our initial findings as described in the following sections.

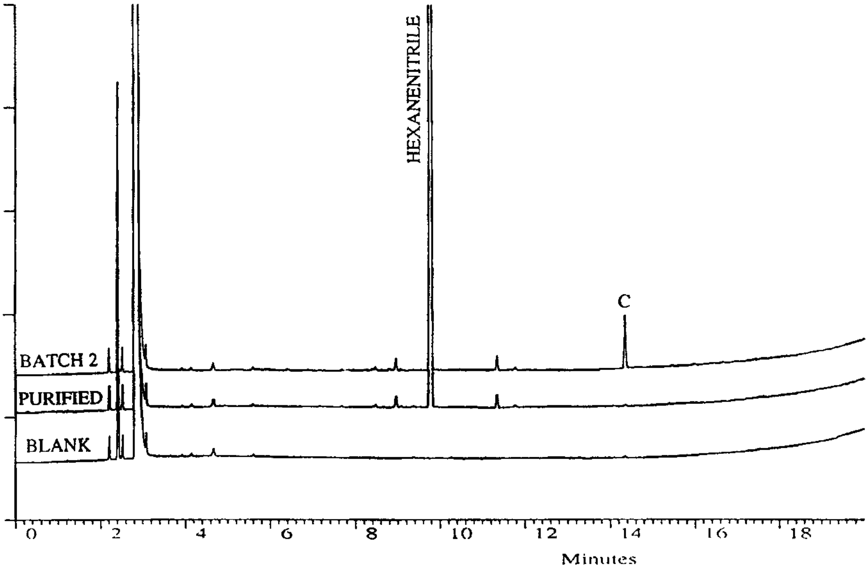

Figure 4. GC Chromatograms comparing parent and purified hexanenitrile. [Conditions as in Figure 1].

GC and GC-MS Investigation of Alkylnitrile Compounds

In the course of our earlier investigations into the use of valeronitrile as a mobile phase additive the chemical purity of the valeronitrile was determined to supplement the study. Valeronitrile was found to have high chemical purity (> 99.8% by area) using GC impurity profiling. Figure 1 shows the impurity profiles of two valeronitrile batches.

In our subsequent investigations into the use of hexanenitrile, its purity was determined similarly by GC impurity profiling. Hexanenitrile was found to be considerably less pure (98.0% by area for two batches) than valeronitrile as shown by the GC chromatograms in Figure 2. Some impurities (**A, B** and **C**) in hexanenitrile were deemed significant. To identify the impurities GC-MS was employed. Results showed that Impurities A and B had molecular masses of 114 and 128 respectively but were not identified. More importantly, MS data for Impurity C showed that its molecular mass was 185 and its molecular formula to be $C_{12}H_{27}N$. It was likely that Impurity C could be an amine, possibly di-n-hexylamine (DHA) or more probably tri-n-butylamine (TBA). Impurity C was found in both batches (1.2% by area). The presence of a

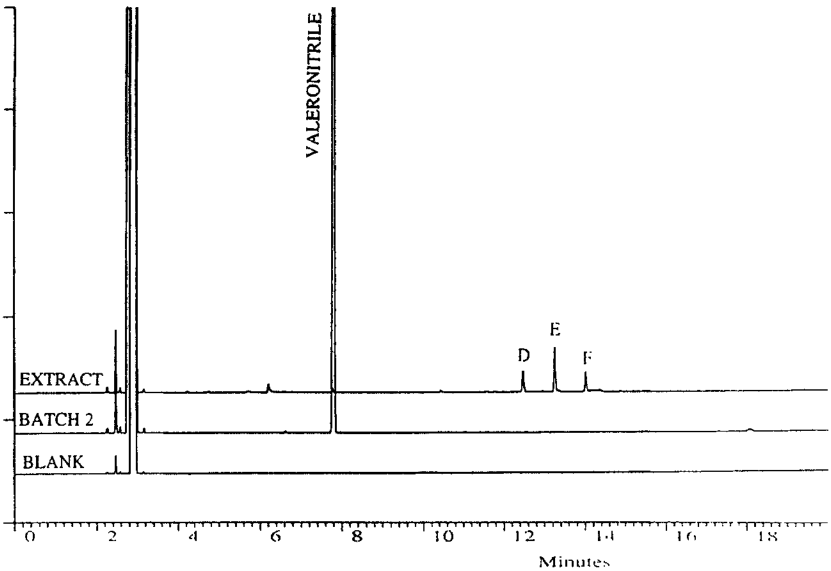

Figure 5. GC Chromatograms comparing valeronitrile with its dichloromethane extract. [conditions as in Figure 1].

nitrogen containing compound prompted further investigation. Work was performed to isolate any possible nitrogen impurities from the hexanenitrile and to purify it using an acid washing and extraction procedure (see Methods). Using the acid washing procedure any nitrogen containing impurities were converted to their respective HCl salts and could be removed from the hexanenitrile. The acid washings were neutralised, rendered basic and extracted with dichloromethane. The nitrogenous impurities (in their non-protonated form) were isolated in the dichloromethane extract. The extract was subsequently analysed by GC and GC-MS. The GC chromatogram of the extract was compared with the hexanenitrile impurity profile (Figure 3). The main component of the extract was found to correspond to Impurity **C**. Impurity **C** was confirmed as tri-n-butylamine from its mass spectrum and by use of an authentic specimen as a GC retention marker. No other significant impurities found in the extract were identified as amines. Figure 4 shows a comparison of the parent and the purified hexanenitrile. The level of TBA was found to be 0.03% (by area) in the purified material thus showing that the purity of the hexanenitrile had been upgraded significantly.

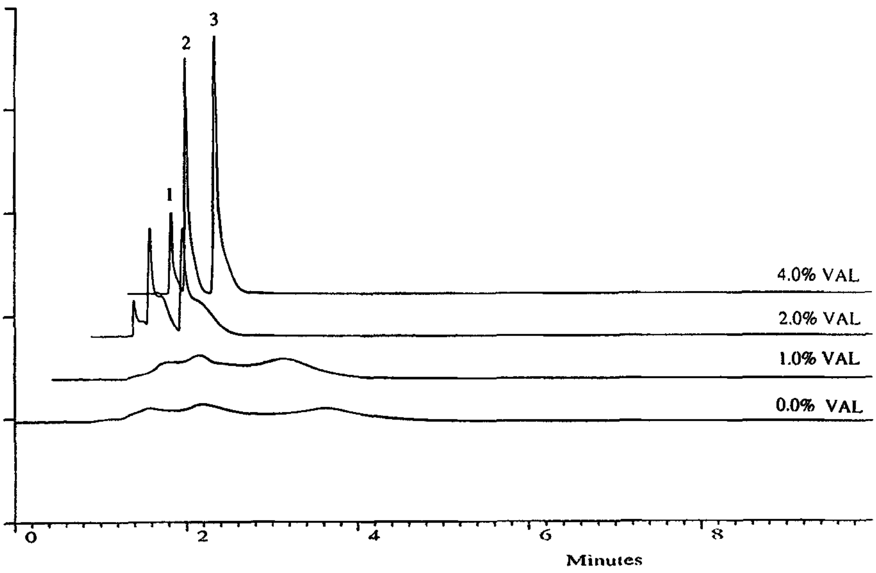

Figure 6. HPLC Chromatograms showing the effect of purified valeronitrile in the chromatography of aniline solutes in a methanol-water mobile phase.
1 = Aniline, 2 = N-methylaniline, 3 = N, N-dimethylaniline [conditions: Column: 3µ Spherisorb C_8, 30mm x 4.6mm ID, thermostatted at 40°C, Flow rate: $1cm^3$ min^{-1} methanol-water (50:50 ww) plus additive].

The presence of amine in hexanenitrile was a cause for concern in light of the HPLC mobile phase additive investigations so the purity of valeronitrile was re-examined. Valeronitrile was subjected to the same acid washing and extraction procedure as hexanenitrile. The extract was analysed using GC and GC-MS with chemical ionization (ammonia gas) employed to maximise detection of nitrogen compounds. Three significant components (**D**, **E** and **F**) were identified in the extract using GC analysis (Figure 5). Using GC-MS components **D**, **E** and **F** were found to have the same molecular mass (157) and molecular formula, $C_{10}H_{23}N$. The components were isomers and had identical fragmentation patterns. MS could not distinguish the isomers but they were tentatively assigned as di-n-pentylamine isomers. Using an authentic mixture of di-n-pentylamines the components (impurities) **D**, **E** and **F** were identified as bis-2-methylbutylamine, pentyl-2-methylbutylamine and di-N-n-pentylamine respectively. The retention of these compounds was compared with the GC chromatogram of the parent valeronitrile from which the extract was derived. The presence of di-n-pentylamines could not be detected in the parent

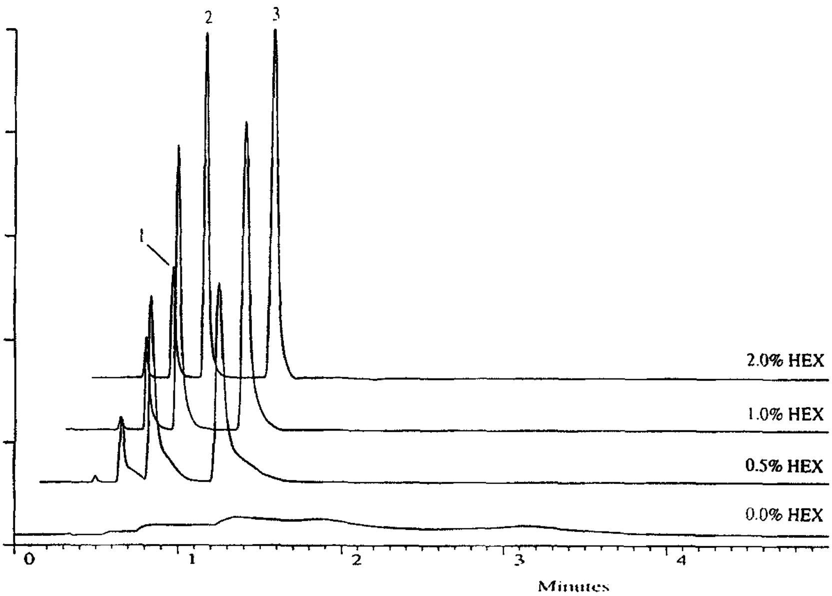

Figure 7.. HPLC Chromatograms showing the effect of purified hexanenitrile on the chromatography of aniline solutes in a methanol-water mobile phase. [Peak identification and conditions as in Figure 6].

valeronitrile under the method conditions used. The levels of di-n-pentylamines in the parent valeronitrile were thus exceedingly small (limit of detection (0.02% by area), however, a trace of impurity D (0.04% by area) could be determined in one valeronitrile batch.

HPLC - Use of Purified Valeronitrile and Hexanenitrile

HPLC chromatograms were obtained using purified and untreated valeronitrile and hexanenitrile as mobile phase additives and the results compared. It was found that substantial quantities of the valeronitrile (> 4% w/w) and hexanenitrile (2.0% w/w) were required to bring about the chromatographic improvements (Figures 6 and 7 respectively). Previous work[15,16] had indicated that only small quantities of valeronitrile (ca. 0.2% w/w) and hexanenitrile (ca. 0.05% w/w) were needed to improve the peak shapes of basic materials. It is clear that the purified additives are very much less effective at reducing peak tailing and that the previously observed chromatographic improvements should be attributed to the presence of amines

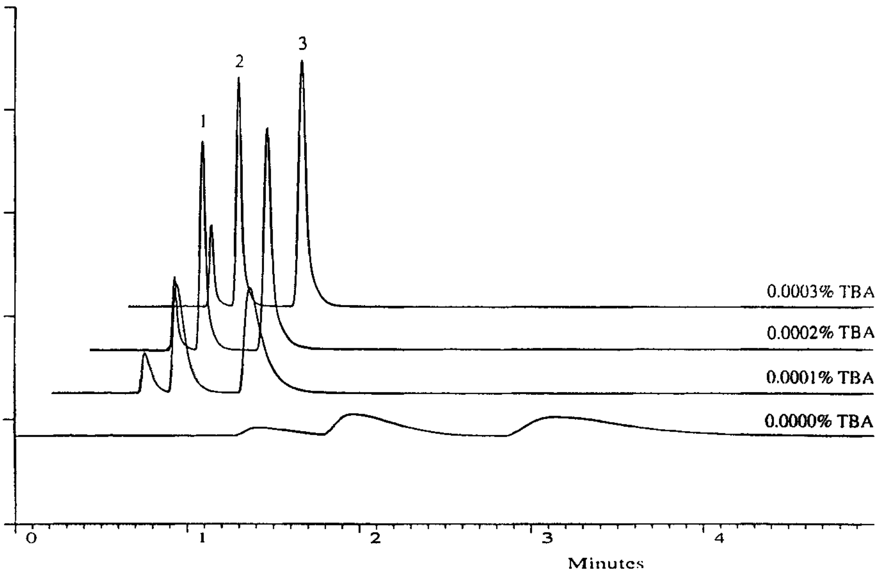

Figure 8. HPLC Chromatograms showing the use of tri-n-butylamine (TBA) as an additive in a methanol-water mobile phase.[Peak identification and condiitons as in Figure 6].

in the mobile phase. In our previous work, the action of the alkylnitrile compounds was attributed to the possible adsorption of the alkylnitrile molecules effectively covering the surface of the reverse phase preventing solute-silanol interactions.[16] Since it was not possible to totally eliminate TBA from hexanenitrile the observed chromatographic improvements in Figure 7 are probably due to the presence of this small quantity of amine in hexanenitrile rather than from the effect of hexanenitrile itself.

HPLC - Use of Amines as Additives

TBA and DPA were used separately as mobile phase additives for the reverse phase chromatography of the aniline test solutes. DPA was used as a mixture of three isomeric di-pentylamines (available commercially). It was found that only very low levels (ppm) of TBA and DPA in the mobile phase were required to bring about good peak shapes for aniline solutes (Figures 8 and 9 respectively). Removal of the amine from the mobile phase returned the chromatography to its previous state.

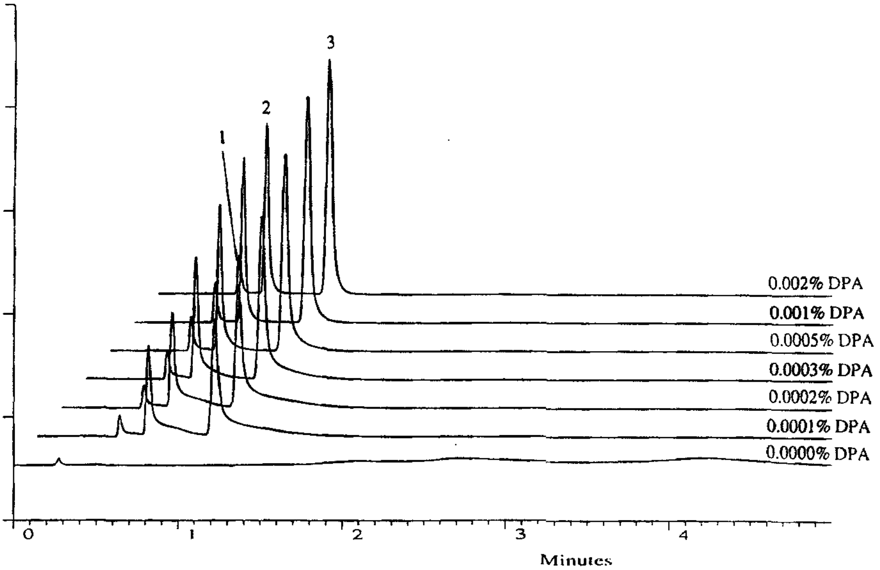

Figure 9. HPLC Chromatograms showing the use of di-n-pentylamines (DPA) as additives in a methanol-water mobile phase.[Peak identification and conditions as in Figure 6]

The efficacy of both DPA and TBA is high because of the properties of the compounds i.e. high basicity and low aqueous solubility. Literature pK_a values for DPA (water) and TBA (in aqueous ethanol) are 11.2 and 9.9 respectively.[17] These values are in reasonable agreement with calculated pKa data of 12.0 for DPA and 10.1 for TBA (programme: PALLAS v.1.1, Compudrug Chem. Ltd). Both amines are strongly basic and depending on the apparent pH of the mobile phase (approximate neutrality) could be ionized i.e. will have a high affinity for silanol groups on the silica substrate. Owing to the lipophilicity of the compounds, molecules of DPA and TBA will also have a strong affinity for the reverse phase which will augment their retention and contribute to their efficacy as additives. Wehrli et al. showed in a study on the effect of amines on the dissolution of silica that there was an order of "aggressivity" of the amine toward silica.[18] It was shown that the type of alkyl substituent on the amine affected the rate of column deterioration i.e. propyl substitution was found to be less aggressive than ethyl substitution and so forth. It follows that TBA and DPA should be even less severe towards silica-based packings and because of the low concentration required may offer an advantage over other commonly employed amines.

Origin of Impurities

The origin of the impurities is not clear, but it is possible that the impurities could be formed from large-scale production of nitrile compounds from an ammoxidation reaction using ammonia in the presence of an alkyl reagent such as an alcohol.[19,20] Proprietary information regarding the synthesis for both valeronitrile or hexanenitrile could not be obtained. Information on potential impurities in valeronitrile was obtained but no details were given for expected amine contamination. No information could be obtained regarding potential impurities in hexanenitrile. The work reported here and previously reported work, was undertaken using the highest quality chemicals that could be commercially purchased and accepted in good faith. This work has shown that for some studies it may be necessary to further purify chemicals in-house or consider the effect of impurities (if known) on study results.

CONCLUSION

It has been shown that the observed chromatographic improvements in the peak shapes for aniline solutes, when using valeronitrile and hexanenitrile as mobile phase additives, can be attributed to the presence of low level amine impurities in the additives and not to the adsorption of the alkylnitrile compounds on to the reverse phase. DPA impurities were found in batches of valeronitrile at very low levels using a chemical isolation procedure and GC-MS analysis. TBA was found in batches of hexanenitrile using GC-MS analysis. Both DPA and TBA were shown to be effective mobile phase additives in that very low quantities were required to bring about good chromatography for some basic solutes.

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