Journal of Chromatography, 289 (1984) 31–41
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 292

CHANGE OF CHROMATOGRAPHIC PROPERTIES OF GAS–LIQUID CHROMATOGRAPHY COLUMNS BY ADDITION OF ORGANIC VAPOR TO THE CARRIER GAS

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SUMMARY

Advances in the technique of bonding liquid phases to solid supports have made possible columns that are both more thermostable and more resistant to the effect of organic vapor. Since not all liquid phases can be bonded with the same permanence, it was thought of interest to explore the possibility of changing the relative separation factors of columns temporarily by adding volatile organic compounds to the carrier gas. Coupling an ultraviolet absorbance or fluorescence detector to the gas–liquid chromatography column permitted us to study this possibility.

Organic liquid was delivered to the gas–liquid chromatography oven inlet either by a 50-ml syringe pump at *ca.* 0.010 ml/min for addition to the carrier gas as a vapor or by an high-performance liquid chromatography pump at *ca.* 0.2 ml/min for a complete carrier gas. Mixtures of aromatic acids, hydrocarbons, and alcohols were separated on columns containing Tenax stationary phase coupled to ultraviolet absorbance detectors. Primary alkyl amines and polyamines were separated on the same columns and detected by their fluorescence after post-column reaction with *o*-phthalaldehyde. Addition of methanol, ethanol, acetonitrile or hexane shortened retention times of polar more than non-polar compounds. The effect was reversible; flushing the column with helium overnight increased the retention times to their original values.

INTRODUCTION

A gas chromatography (GC) detector that is not affected by compounds to which it does not respond offers the possibility of changing the chromatographic separation by altering the composition of the carrier gas. The hydrogen flame ionization detector is insensitive in this way to carbon dioxide, ammonia and water vapor. These gases can therefore be added to the carrier for specific analytical purposes. In 1962 Desty and co-workers reported that the retention times of hydrocarbons were affected by changes in the carrier gas from hydrogen to nitrogen to carbon dioxide[1]. They attributed the changes to interactions between the carrier gas and the solute molecules in the gas phase. In the same year we demonstrated that substituting carbon dioxide for nitrogen carrier gas resulted in both lower retention times and improved chromatographic behavior (*viz.* greater symmetry of peaks) of long-chain fatty acids on polyester columns[2]. We postulated that the more polar carrier gas

0021-9673/84/$03.00

acted primarily by modifying the stationary phase and support. We later analyzed amino acid esters on similar columns by adding ammonia to the carrier gas[3]. A steam generator was offered commercially in the 1960s as a portable source of carrier gas; the steam also modified the chromatographic behavior of the column. Since ultraviolet absorbance and fluorescence detectors and at least some of the post-column reactors are similarly insensitive to solvents that do not have the property detected, application of these detectors to GC offers the possibility of adding a wide variety of organic vapors to the carrier gas[4,5].

EXPERIMENTAL

Apparatus

Two gas chromatographs were used in this study: a Shimadzu Model GC-3BF and a Perkin-Elmer Model 3920, both equipped with flame-ionization detectors. The columns used were 2 ft. × 1/8 in. O.D. stainless steel, packed with Tenax GC, 80–100 mesh (from Supelco, Bellefonte, PA, U.S.A.), or μBondapak C_{18}, Porasil C, 120–150 mesh, from Waters Assoc., Milford, MA, U.S.A. To generate carrier vapor, organic solvents were pumped with one of the pumps of a dual Milton Roy Model 396 HPLC minipump into a 34-cm length of 1/8 in. O.D. stainless-steel tube in the column oven. The vapor generated was delivered first to the first injector port of the Perkin-Elmer gas–liquid chromatograph, which was maintained at 250–300°C, and then to and through the second injector port to the analytical column. The outlet of each column was fitted with a 5-μm frit (Supelco). Effluent then passed through a 10 cm × 1/16 in. O.D., 0.03 in. I.D., stainless-steel tube to a 1/16-in. stainless-steel Swagelok tee connector. "Make-up" solvent or post-column reagent was delivered to a second arm of the tee with the second pump of the "Minipump" at 0.24–0.69 ml/min. The combined liquid–vapor stream emerging from the third port of the tee was delivered to a mixing–condensing coil consisting of 24 cm × 1/16 in. O.D. tubing immersed in an ambient temperature water reservoir. For detecting aromatics, the effluent of the mixing coil was passed through the 8-μl flow cell of a Perkin-Elmer UV–visible digital spectrophotometer detector, Model LC55, connected to either a linear strip chart recorder or a Model 4416 data system from Nelson Analytical, Cupertino, CA, U.S.A. For detecting primary amines by fluorescence after post-column reaction with *o*-phthalaldehyde, the 18-μl flow cell of an ARPCO fluorometer Model A4-2001, from American Research Products, Kensington, MD, U.S.A., was substituted for the UV detector.

Reagents

The reagent for detecting amines consisted of: 1,2-benzyl-dicarboxaldehyde (OPA), from Aldrich (Milwaukee, WI, U.S.A.); buffered borate, prepared by dissolving 24 g of boric acid in 1 l of distilled water and adjusting the pH to 9.5 with potassium hydroxide. OPA was dissolved in ethanol (800 mg/20 ml) and filtered into the buffer with stirring. Two ml of mercaptoethanol followed by 10 ml of ethylene glycol were then added.

Aromatic hydrocarbons; benzene (thiophene free), Lot No. 706657 was from Fisher Scientific, Fair Lawn, NJ, U.S.A.; naphthalene (recrystallized), from Eastman Kodak, Rochester, NY, U.S.A.); α-naphthol, reagent grade, from Amend Drug &

Chemical, New York, NY, U.S.A.; anthracene from Packard, Downers Grove, IL, U.S.A.; benzyl and phenethyl alcohols, 3-phenyl-1-propanol, 4-phenyl-1-butanol, *n*-amylamine, *n*-hexylamine, *n*-heptylamine, and *n*-octylamine from Aldrich.

Procedures

Test mixtures consisting of methanol solutions of mixtures of members of different families of compounds were chromatographed on the Tenax or Porapak columns using nitrogen carrier gas and flame ionization detection (FID). After chromatographic conditions were established, the organic vapors were substituted for the nitrogen, followed by the appropriate detector. When the change in chromatography became apparent, the temperature and flow rate were reoptimized.

RESULTS

Our first experiments used Tenax columns with helium carrier gas doped with small concentrations of ethanol. The retention of phenols and benzoates decreased with higher concentrations of organic modifier (Fig. 1). The magnitude of the effect on the different compounds differed.

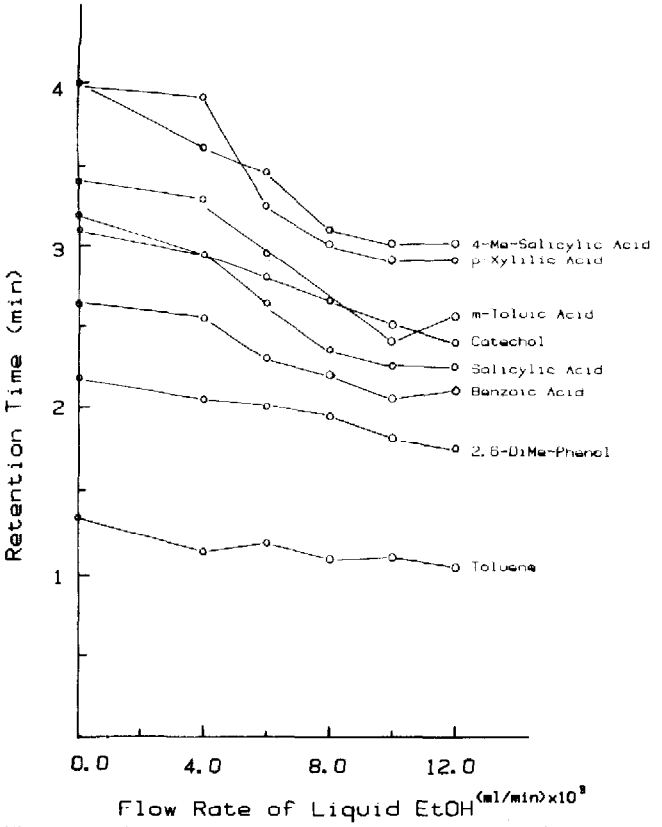

Fig.1. Retention of phenols and benzoates as a function of the ethanol organic modifier concentration in nitrogen.

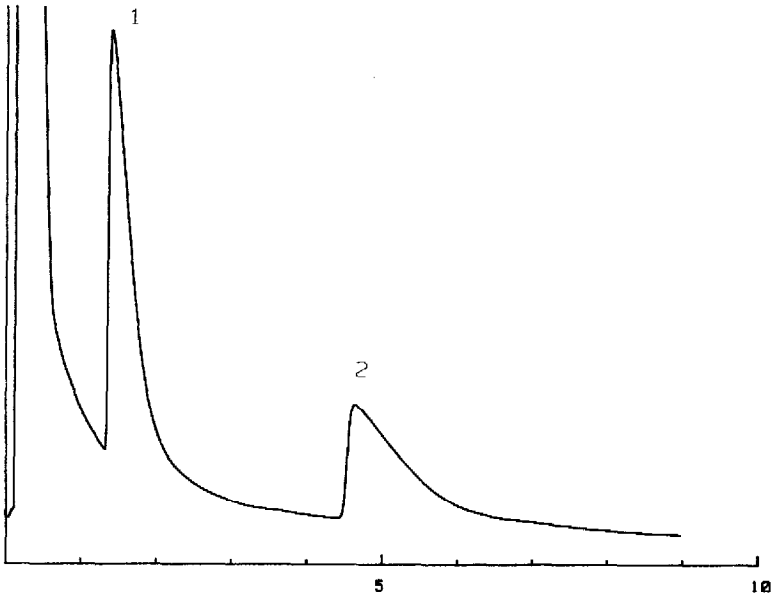

Fig. 2. Separation of aromatic hydrocarbons on Tenax-GC. Column temperature 200°C; nitrogen flow-rate 37.5 ml/min. Peaks: 1 = phenol; 2 = naphthalene.

The aromatic hydrocarbons, benzene, naphthalene, and anthracene, and naphthol are strongly retarded on Tenax (Fig. 2). Totally replacing nitrogen by methanol dramatically reduced their retention (Fig. 3). Pure acetonitrile vapor carrier gave an excellent separation of the aromatics in 5 min (Fig. 4). The dependence of retention time on temperature is shown in Table I.

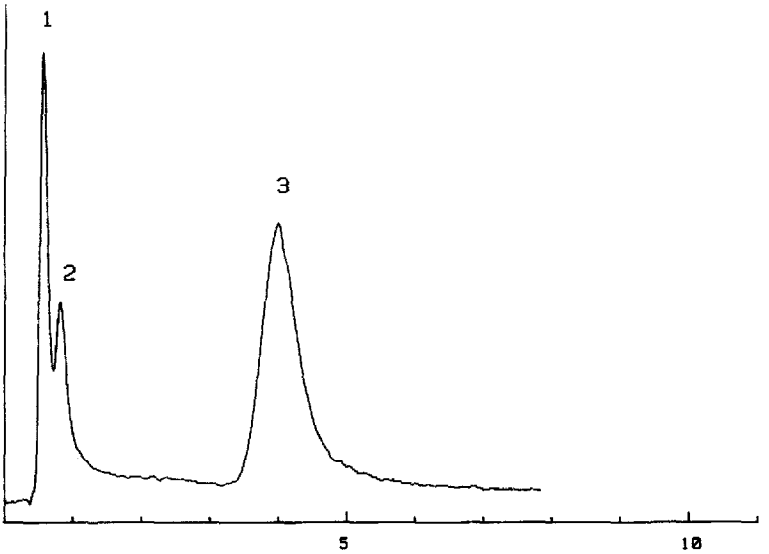

Fig. 3. Separation of aromatic hydrocarbons on Tenax-GC. Column temperature 225°C; methanol liquid flow-rate at the inlet 0.39 ml/min. Peaks: 1 = benzene; 2 = naphthalene; 3 = anthracene.

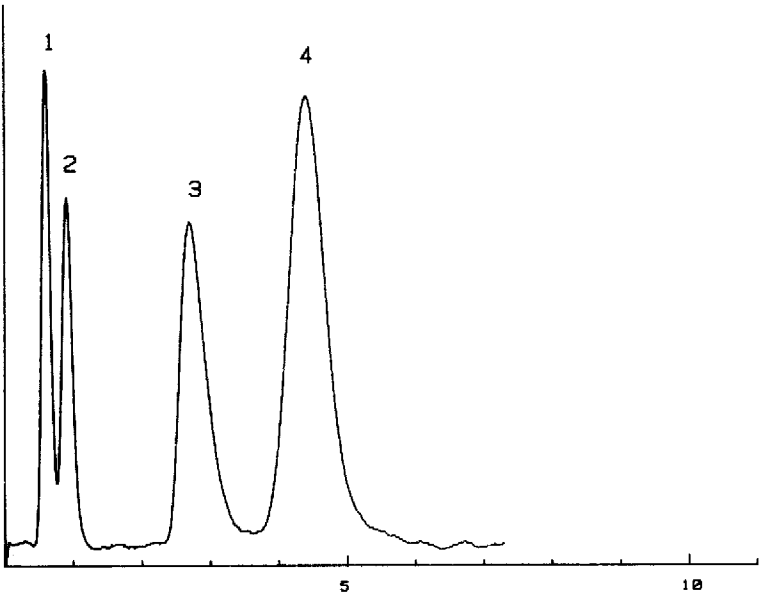

Fig. 4. Separation of aromatic hydrocarbons on Tenax-GC. Column temperature 225°C; acetonitrile liquid flow-rate at the inlet 0.39 ml/min. Peaks: 1 = benzene; 2 = naphthalene; 3 = naphthol; 4 = anthracene.

The phenyl alcohol homologues were strongly retarded on Tenax. Temperature programming was required to elute the mixtures in a reasonable time with nitrogen carrier gas (Fig. 5). With either pure methanol or acetonitrile carrier vapor the solutes

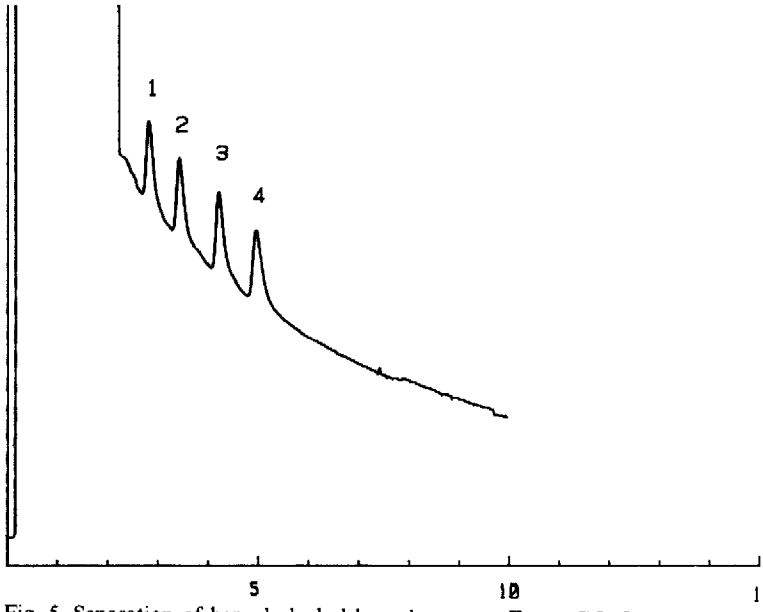

Fig. 5. Separation of benzyl alcohol homologues on Tenax-GC. Temperature programming from 180–250°C at 16°C/min; nitrogen flow-rate 37.5 ml/min. Peaks: 1 = benzyl alcohol; 2 = phenethyl alcohol; 3 = 3-phenyl-1-propanol; 4 = 4-phenyl-1-butanol.

TABLE I

RETENTION TIME IN MINUTES OF AROMATICS AT DIFFERENT COLUMN TEMPERATURES

Organic liquid flow-rate at 0.24 ml/min. CH_3OH = methanol; ACN = acetonitrile.

Aromatic	*125°C*			*150°C*			*175°C*			*200°C*			*225°C*		
	CH_3OH	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*
Benzene	0.84	0.87	1.11	0.83	0.87	1.02	0.73	0.87	0.93	0.75	0.76	0.88	0.66	0.74	0.86
Phenol	3.96	2.76	3.53	2.24	1.59	2.12	1.32	1.21	1.50	1.00	0.99	–	0.94	0.89	–
Naphthalene	8.98	4.55	8.92	5.34	3.60	4.48	2.42	1.76	2.64	1.39	1.35	1.80	1.09	1.08	1.34
Naphthol	–	–	–	–	–	–	–	–	–	4.29	3.92	6.10	2.24	2.19	3.21
Anthracene	–	–	–	–	–	–	–	–	–	12.05	10.05	17.14	5.32	4.59	7.46

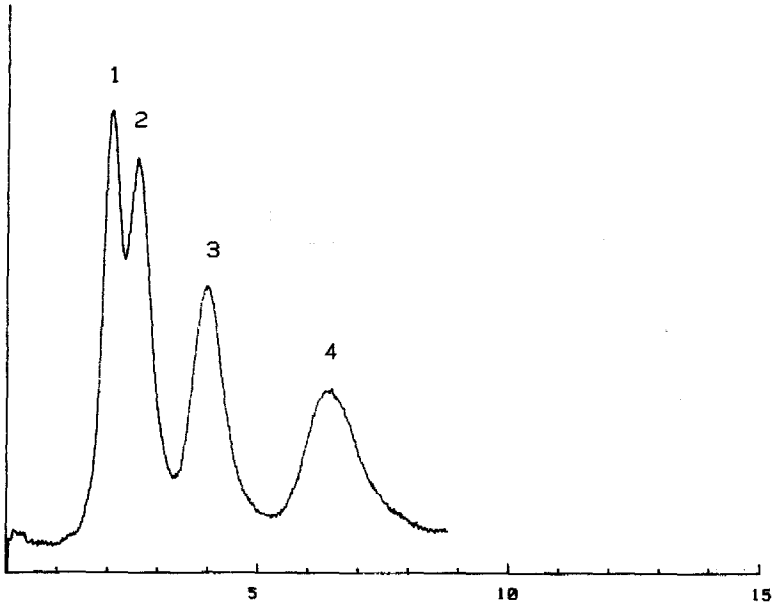

Fig. 6. Separation of benzyl alcohol homologues on Tenax-GC. Column temperature 160°C; methanol liquid flow-rate at the inlet 0.39 ml/min. Peaks: 1 = benzyl alcohol; 2 = phenethyl alcohol; 3 = 3-phenyl-1-propanol; 4 = 4-phenyl-1-butanol.

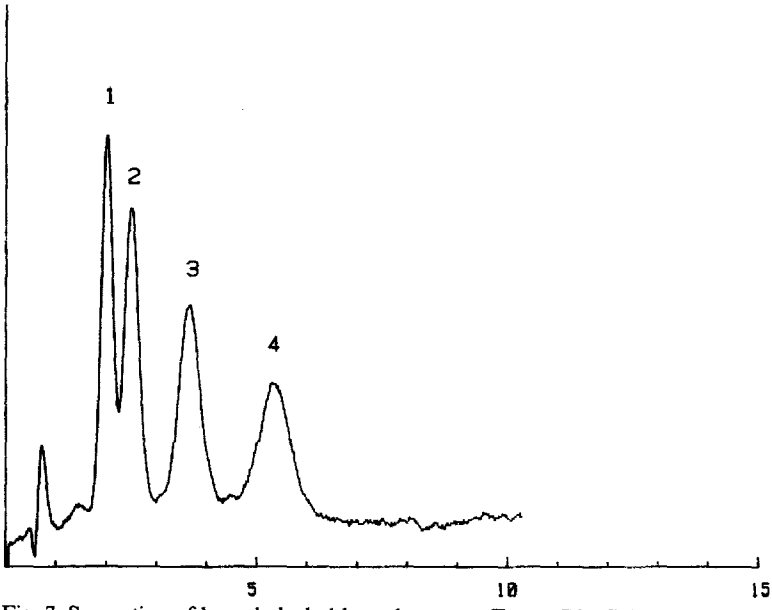

Fig. 7. Separation of benzyl alcohol homologues on Tenax-GC. Column temperature 160°C; acetonitrile liquid flow-rate at the inlet 0.39 ml/min. Peaks: 1 = benzyl alcohol; 2 = phenethyl alcohol; 3 = 3-phenyl-1-propanol; 4 = 4-phenyl-1-butanol.

TABLE II

RETENTION TIMES IN MINUTES OF PHENYL ALCOHOLS AT VARIOUS COLUMN TEMPERATURES

Organic liquid flow-rate at 0.24 ml/min. CH_3OH = methanol; ACN = acetonitrile.

Phenyl alcohol	*140°C*			*160°C*			*180°C*		
	CH_3OH	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*	*CH_3OH*	*ACN*	*Hexane*
Benzyl alcohol	3.43	2.22	3.50	2.25	1.63	2.33	1.52	1.19	1.69
Phenethyl alcohol	5.21	2.84	4.59	3.06	2.02	2.89	1.82	1.51	1.97
3-Phenyl-1-propanol	9.91	4.49	7.59	5.02	2.91	4.36	2.70	2.02	2.70
4-Phenyl-1-butanol	18.77	7.37	12.86	8.61	4.42	6.69	4.02	2.82	3.83

eluted in the same time frame at a much lower temperature (Figs. 6 and 7, Table II).

Acetonitrile carrier vapor proved to reduce the peak asymmetry noted with many compounds with permanent gas carriers. In analyses of alkyl amines and polyamines, chosen for their polarity and high boiling points, temperature programming was needed with nitrogen carrier (Figs. 8 and 9). These amines were chromatographed and resolved at a much lower temperature with the acetonitrile carrier. The alkyl amines were isothermally separated at 120°C in 7 min (Fig. 10). The diamines were chromatographed by temperature programming from 133–160°C in 5 min (Fig. 11).

The acetonitrile did not interfere with fluorescence detection after post-column reaction of the amines with OPA.

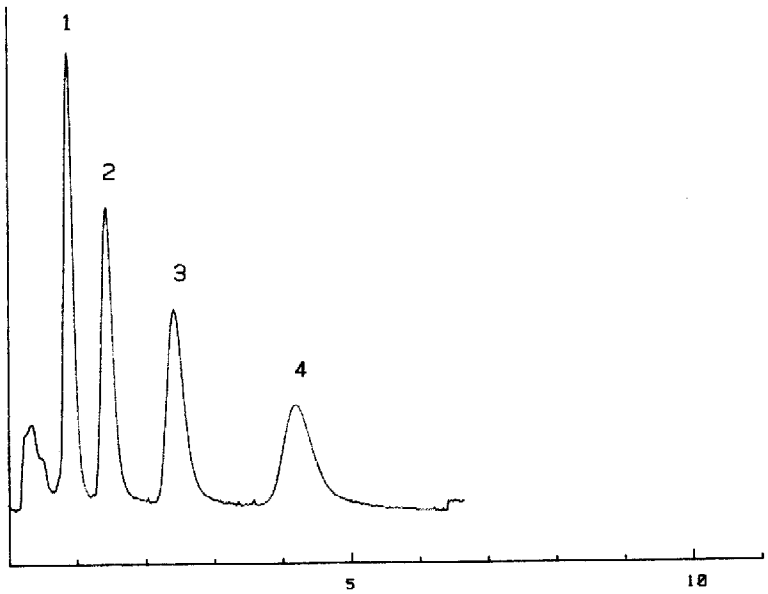

Fig. 8. Separation of primary amines. Column temperature 180°C; nitrogen flow-rate 37.5 ml/min. Peaks: 1 = *n*-amylamine; 2 = *n*-hexylamine; 3 = *n*-heptylamine; 4 = *n*-octylamine.

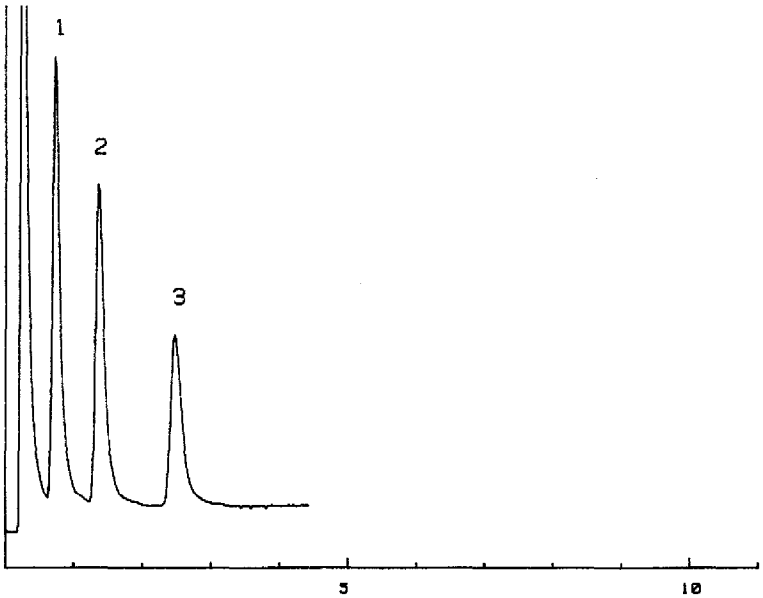

Fig. 9. Separation of diamines. Temperature programming 220–250°C at 8°C/min; nitrogen flow-rate 37.5 ml/min. Peaks: 1 = 1,4-diaminobutane; 2 = 1,6-diaminohexane; 3 = 1,8-diaminooctane.

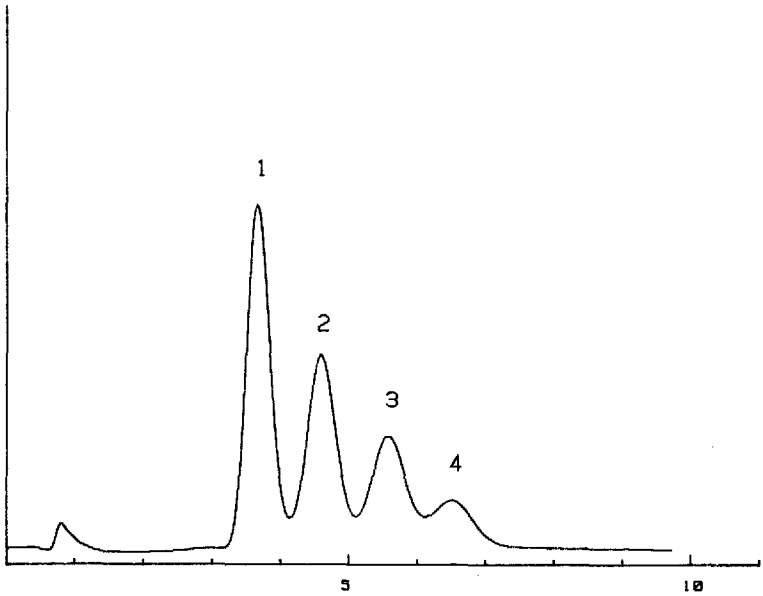

Fig. 10. Separation of primary amines. Column temperature 120°C; acetonitrile liquid flow-rate at the inlet 0.39 ml/min. Peaks: 1 = *n*-amylamine; 2 = *n*-hexylamine; 3 = *n*-heptylamine; 4 = *n*-octylamine.

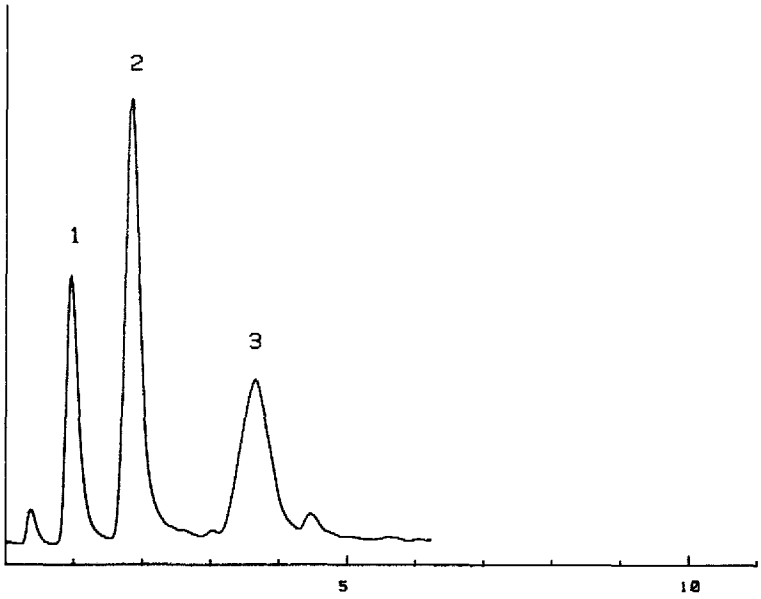

Fig. 11. Separation of diamines. Temperature programming 135–160°C at 4°C/min; acetonitrile liquid flow-rate at the inlet 0.55 ml/min. Peaks: 1 = 1,4-diaminobutane; 2 = 1,6-diaminohexane; 3 = 1,8-diaminooctane.

DISCUSSION

In GC the carrier gas is usually considered not to affect the chromatographic separation to any great extent. Separations are thought to result primarily from different interactions between analytes and stationary phase and the volatility of the analyte. As shown here, marked changes in chromatographic behavior can be achieved and an extra degree of chromatographic selectivity can be achieved by adding organic vapor to the carrier gas. Assuming that the carrier gas is relatively inert, and does not react or form complexes with the analyte, the effect on chromatography must be attributable to change in the stationary phase. In our experiments with Tenax stationary phase and helium carrier gas doped with small amounts of ethanol (0–12 μl/min), the retention of phenols and benzoates not only decreased with increase in ethanol delivery rate, but the order of elution of solute pairs such as *m*-toluic acid and catechol, *m*-salicylic and *p*-xylilic acids, was reversed with increasing ethanol concentration. This effect may be due to the reduction of hydrogen bonding of the solutes to the Tenax by the adsorbed ethanol. The largest change in retention was observed with the benzoates, which form strong hydrogen bonds, while the smallest was with toluene, which forms weaker bonds.

Replacing the carrier gas completely with organic vapor offered the increased convenience of avoiding the separation of gas and liquid phases prior to detection. Replacing the nitrogen carrier gas with methanol dramatically reduced the retention of the aromatics. Acetonitrile vapor yielded excellent separations of benzene, naphthalene, naphthol and anthracene in 5 min. Some of these effects on retention time may have been attributable to change in carrier gas flow-rate which was difficult to

measure experimentally. Estimation of the flow from the retention time of presumably unretained methanol showed little difference from one carrier vapor to the other, but this estimation is admittedly imprecise. We thought the effect impressive: chromatography of the more polar and more strongly retained phenyl alcohols, with nitrogen carrier gas, required temperature programming from 180–250°C. An alcohol or acetonitrile vapor carrier phase facilitated the separation at 160°C. The acetonitrile carrier vapor yielded results superior to those with methanol in all of the analyses of aromatics studied.

The alkylamines chromatographed with acetonitrile carrier vapor at a much lower temperature, better efficiency and selectivity. The diamines could be separated with a temperature gradient much lower than that required in GC with nitrogen. The underivatized amine peaks were symmetric without the "tailing" usually encountered with most stationary phases. The acetonitrile did not interfere with the sensitive detection of the amines by fluorescence after reaction with *o*-phthalaldehyde.

Addition of organic vapor to the carrier gas, or substituting organic vapor for carrier entirely, thus proved a practical method for obtaining an extra degree of selectivity in gas chromatography. The organic vapor certainly acts by forming an adsorbed layer on the solid support, thus modifying the support in a manner analagous to that achieved by adding various polar compounds in the formulation of the liquid phase. While modifying it this way does not have the apparent convenience of including an additive to the liquid phase or pre-coating an active stationary support material with a coating more tenacious than the liquid phase chosen, it should offer the increased permanence of a coating that is constantly replaced. Its effect is reversible by outgassing the column and is presumably adjustable after the column is prepared by adjusting the composition of the vapor. Its use with polymeric supports such as Tenax improves separation of both moderately polar compounds such as benzene and highly polar polyamines. Achievement of satisfactory chromatography at lower temperatures should also permit more temperature-sensitive compounds to be chromatographed more effectively.

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