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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH Ag^+ COMPLEXATION IN THE MOBILE PHASE*

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SUMMARY

By reversed-phase high-performance liquid chromatography (HPLC) on chemically bonded phases using common mobile phases (methanol–water, methanol–isopropanol, water–acetonitrile) containing Ag^+, separations with considerably increased selectivity for various types of unsaturated and heterocyclic components can be easily effected. No technical problems arise when using the usual instrumental arrangements with Ag^+-containing mobile phases. Columns could be used for several months without deterioration of the separation efficiency or peak shapes, mainly because of the low Ag^+ concentrations involved.

Reversed-phase HPLC systems give comparatively high separation efficiencies. Moreover, a constant contribution of the CH_2– group to the retention of homologous open-chain compounds is observed. A liquid chromatographic retention index system analogous to the Kováts system in gas chromatography is discussed. It was used successfully for the interpretation of retention behaviour of isomeric compounds as a function of structural features and for identification purposes. Thermodynamic data for the Ag^+ complexation of numerous compounds could easily be measured with our HPLC system.

INTRODUCTION

Chemically bonded phases are important in reversed-phase high-performance liquid chromatography (HPLC) with a mobile phase consisting of mixtures of polar solvents such as water, methanol and acetonitrile, the composition of which can be changed systematically during a chromatographic separation (gradient elution). Particular features of such systems that are important for the work described here are the excellent solubility of the common mobile phases for silver salts such as silver nitrate, perchlorate and tetrafluoroborate and the retention behaviour of homologous and isomeric compounds. The carbon skeleton contributes strongly to retention and a constant increment due to the CH_2– group is observed in homologous series of compounds. Therefore, a retention index in liquid chromatography (LC) can be defined in an analogous manner to that in gas chromatography (GC).

* Thesis, B. Vonach, TU Clausthal-Zellerfeld, 1978.

The chromatographic resolution is proportional only to the square root of the number of theoretical plates, which cannot easily be increased to values higher than about 8000–10,000 in bonded-phase HPLC. These separation efficiencies are still much poorer than those easily attainable in high-resolution capillary column GC and a further increase in efficiency would be desirable for the separation and characterization of certain isomers. This disadvantage in HPLC is only partially compensated for in practice by the possibility of varying the polarity of the mobile phase over a wide range. The influence of the mobile phase on retention can easily be adapted to the chemical nature of the solute pair to be resolved (k' values between 1 and 15 are optimal for practical applications). In reversed-phase HPLC, the solutes of higher polarity remain predominantly in the mobile phase and therefore exhibit short retention times, which are to be preferred in analytical work for several reasons. In GC, for example, strong interactions of the solute with the stationary liquid cause long retention times and broad peak profiles, and require high column temperatures for short chromatograms to be obtained.

In this work we investigated the influence of Ag^+ contained at adequate concentrations in common water, methanol and acetonitrile solvent mixtures on the partition coefficients (k' values) of compounds containing π-electrons in various kinds of double and triple bonds and containing heteroatoms such as N, O and S with lone pairs of electrons.

Olefinic hydrocarbons generally have low polarities compared with other types of compounds, the analysis of which is normally performed with ease by LC. An increase in intermolecular interaction in the mobile phase, including the generation of characteristic selectivities for special types of olefins, is therefore desirable. Silver-containing stationary phases have been used in chromatography for several years in order to improve the separation of isomeric olefins[1,2] (LC) and heterocyclic aromatic hydrocarbons[3] (LC) for analytical and preparative purposes, and also for the determination of thermodynamic data relating to Ag^+ complexation[4,5]. These data provide information on position and/or configuration of a double bond, on the substitution in the vicinity of the double bond and on its conjugation with other π-electrons via their influence on complexation. For heterocyclic hydrocarbons one can ascertain from the complexation constants whether the lone pair of electrons is participating in aromatic electron systems and whether their intermolecular interaction with Ag^+ is especially hindered by shielding substituents.

"Argentation" chromatography, as described in various previous papers[6], is characterized by the fact that silver salts are contained in the stationary phase, either dissolved in a suitable stationary liquid as in GC or impregnated in the surface of typical polar adsorbents such as silica gel and aluminosilicates.

In argentation GC column temperatures above 120° cannot be applied, for the following reasons. Not many solvents with sufficient solubility for silver salts are available with a high enough thermal stability and a low enough vapour pressure for the elution of solutes of low volatility at elevated temperatures. Moreover, the configurational selectivity of olefin complexation diminishes rapidly with increasing temperature, and chemical reaction of the Ag^+ with solutes and solvents occurs. In argentation LSC (including TLC) the following restrictions may be disadvantageous:

(1) The silver concentration in the adsorption-type of stationary phase cannot easily be adapted to the complexation strength of the solute. The chromatographically

effective portion of the amount of silver with which the support has been loaded is not known precisely. The interaction between Ag^+ and the solute takes place on the solid surface of the support, the special properties of which may also contribute to selectivity. A comparison of the retention behaviour of certain solutes in Ag^+-free and Ag^+-loaded chromatographic systems may not be possible because the Ag^+ loading changes the adsorption properties of the support.

(2) The retention behaviour of solutes on silver loaded supports is not as characteristically dependent on the molecular size, *e.g.*, the carbon number of the solute molecules, as in reversed-phase HPLC. The interaction is predominantly influenced by the Ag^+-complexing functional group; the contribution of the carbon skeleton to retention is comparatively low.

(3) The retention times of solutes undergoing the strongest complexation are the longest. The analysis time of these compounds is increased and their limit of detection in quantitative analysis is adversely affected.

EXPERIMENTAL AND RESULTS

Reversed-phase argentation HPLC

From the above considerations, we decided to combine the advantages of argentation chromatography with those of reversed-phase LC on chemically bonded phases by using mobile phases containing silver salts at various concentrations. The mobile phases commonly used in such systems are extremely suitable because of the excellent solubility of silver salts in them. The first paper on reversed-phase argentation HPLC was published in 1975[7], and recently Tscherne and Capitano[8] described the separation of some vitamins and steroids by this method. We report here our further results and experiences in this field.

The special features of HPLC systems with Ag^+ contained in polar mobile phases can be summarized as follows for their characterization:

(1) A linear relationship between log k' and carbon number in series of homologous compounds is observed over a wide range of carbon numbers (Fig. 1).

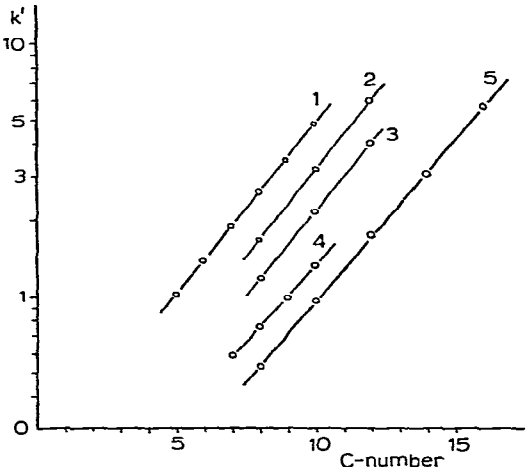


Fig. 1. Log k' *versus* carbon number plots for homologous compounds in reversed-phase HPLC. 1 = Alkanes; 2 = 1-alkenes; 3 = α,ω-alkadienes; 4 = alkylbenzenes; 5 = 1-alkanols.

The retention increment for the CH_2– group is large, especially for the C_{18}-type bonded phases, and illustrates the strong contribution of the carbon skeleton to retention, which is moreover independent of the Ag^+ concentration in the mobile phase. Considering these factors, a LC retention index system can be defined that has been used successfully in this work.

(2) The polarity of the mobile phase, even when containing Ag^+ in the concentration range 10^{-1}–10^{-3} mole/l, has no direct influence on the chromatographic properties of the support. Equilibrium of partition, including the influence of complexation of the solute with the Ag^+ ion, is attained quickly. This permits frequent systematic changes of the "polarity" of the mobile phase also with regard to silver concentration. Gradient elution can be effected easily in such systems.

(3) The absolute and relative reproducibilities of retention with regard to Ag^+ concentration and solvent composition of the mobile phase are very good.

(4) Characteristic selectivity coefficients, $\alpha = k'/k'_{Ag}$, for different types of components undergoing Ag^+ complexation are obtained in argentation HPLC. The separation of configurational olefins isomers can be performed in a wide molecular range of solutes in the same chromatogram even using the isocratic operation mode [see, for example, the chromatograms in Fig. 2 for (*Z*,*E*)-2-alkenes].

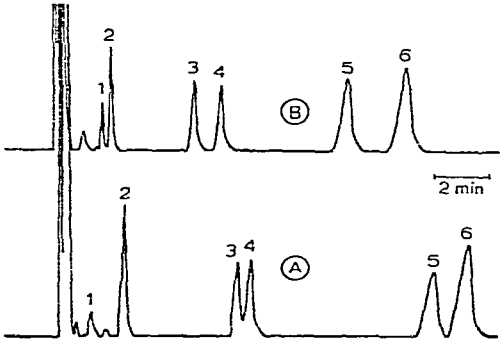


Fig. 2. Separation of homologous (*Z*,*E*)-2-alkenes (C_5, C_8, C_{10}). Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (5:1, v/v); B, A + 10^{-2} *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.7 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 1 = (*Z*)-2-pentene; 2 = (*E*)-2-pentene; 3 = (*Z*)-2-octene; 4 = (*E*)-2-octene; 5 = (*Z*)-2-decene; 6 = (*E*)-2-decene.

(5) A principal analytical advantage of reversed-phase separations is that the more polar solutes are eluted before the less polar. By adjusting the silver concentration in the mobile phase, Ag^+ complexation can be used to shorten the retention times of solutes that show strong Ag^+ complexation.

(6) Low Ag^+ concentrations (*ca.* 10^{-2} mole/l) in the mobile phase are sufficient for the common separations. In Fig. 3, the α_{Ag} data for various unsaturated hydrocarbons as a function of the Ag^+ concentration in the mobile phase are shown. A linear relationship for monofunctional components is obtained, the slope of the lines being proportional to the strength of solute complexation. The complexation of N-heterocyclics via lone pairs of electrons is much stronger than that effected by the π-electrons of double bonds (see Fig. 3). With regard to "preparative" separations of

isomeric unsaturated hydrocarbons or heterocyclics, it is important that the sample capacity should be increased considerably by Ag^+ complexation in the mobile phase. Generally, reversed-phase systems are less affected than the common LC systems because of the absence of adsorption effects[9].

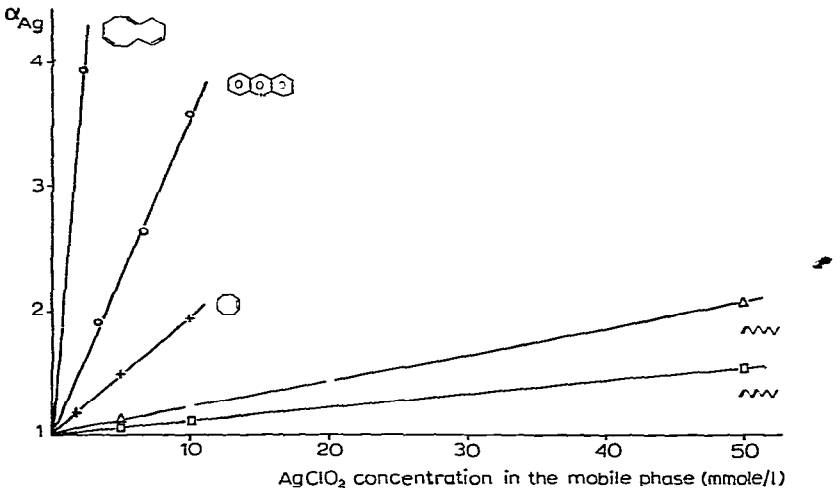


Fig. 3. Reversed-phase HPLC with Ag^+ in the mobile phase. Dependence of selectivity coefficient, α_{Ag} ($= k'/k'_{Ag}$), on Ag^+ concentration for unsaturated and heterocyclic hydrocarbons.

(7) The polarity of the mobile phase with regard to the composition of the solvent mixture used can easily be adapted to the "total" polarity of the solute molecules, which may contain various additional functional groups of different polarity.

(8) It might be expected that Ag^+-containing mobile phases would cause increased corrosion, the deposition of metallic silver in the chromatographic system or irreversible changes of the properties of the support. No severe disturbances occurred during our work in the last 2 years using a Kipp and Zonen LC 771 instrument, an RI detector (Knauer and Siemens) and/or a UV detector (Perkin-Elmer LC 55). The standard columns were 15 cm in length with an I.D. of 4 mm and packed with 5-μm Nucleosil 5 C 18 (Macherey, Nagel & Co.) by a "viscosity–slurry" method. These columns were operated for several months with Ag^+-containing mobile phases without an appreciable decrease in separation efficiency (20–25-μm plate height).

Separation of olefins and heterocyclics

Chromatograms are given that were obtained either with or without Ag^+ in the same mobile phase. In addition, α_{Ag} ($= k'/k'_{Ag}$) values, I, I_{Ag}, ΔI_{Ag} ($= I - I_{Ag}$) and δI_{Ag} values were calculated by applying the above-mentioned LC retention index system, which is based on the linearity of the relationship between log k' and carbon number for homologous series of compounds, primarily *n*-alkanes. Homomorphous and homologous compounds can also be used for standardization, for example saturated fatty acid esters or triglycerides for the interpretation of the retention indices of unsaturated fatty acid esters or triglycerides, respectively.

Open-chain and cyclic mono- and polyunsaturated hydrocarbons

The chromatograms in Fig. 2 (see also the LC retention indices in Tables I and II) illustrate the significant improvement in the *Z,E* selectivity of homologous compounds (2-alkenes). Even the two pentenes could be separated completely, despite their low k' values.

TABLE I

RETENTION INDICES OF UNSATURATED HYDROCARBONS IN REVERSED-PHASE CHROMATOGRAPHY WITHOUT AND WITH 10^{-2} *N* Ag^+

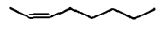
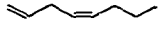
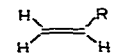
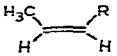
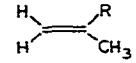
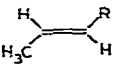
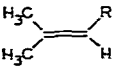
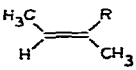
Component	*Structure*	*I*	I_{Ag}	ΔI_{Ag}
1-Octene		682	628	54
(*Z*)-2-Octene		673	625	48
(*E*)-2-Octene		692	678	14
1,4-Octadiene-(*Z*),(*E*)		575	489	86
			515	60
1,7-Octadiene		563	463	100
1-Decene		880	825	55
(*Z*)-2-Decene		869	825	44
(*E*)-2-Decene		893	872	21
(*E*)-4-Decene		875	856	19
1,9-Decadiene		766	668	98
1,(*E*)-4,9-Decatriene		667	557	110

TABLE II

Ag^+ COMPLEXATION OF VARIOUS 1-METHYLALKENES

Compound	ΔI_{Ag}
	55
	44
	37
	21
	14
	10

Separations of internal olefins with different configurations of the double bond can be achieved with high selectivity, whereas open-chain olefins with various positions of the double bond, especially in long carbon chains, do not exhibit characteristic selectivities on Ag^+ complexation. Only substitution at the double bond

causes a strong steric hindrance of Ag^+ complexation. The increment of Ag^+ complexation for a double bond is in the range 10–80 index units (at an Ag^+ concentration of about 10^{-2} mole/l in methanol–water (5:1), depending on the configuration and substitution of the double bond (Table II).

The $\alpha_{(Z,E)}$ ($= k'_Z/k'_E$) or the $\delta I_{(Z,E)}$ ($= I_Z - I_E$) values in the corresponding logarithmic index system are nearly independent of chain length, in a similar manner to the α_{Ag} ($= k'/k'_{Ag}$) and ΔI_{Ag} ($= I - I_{Ag}$) values.

The chromatograms in Figs. 4 and 5 show the separation of a variety of mono- and polyunsaturated cyclooctanes. The order of elution without Ag^+ complexation is roughly determined by the number of double bonds in the ring. With increasing Ag^+ concentration in the mobile phase, the retentions of isomers 3, 4, 5 and 6 are changed significantly. Peak 6 for (*Z*)-cyclooctene, which contains only one double bond but which shows a very strong Ag^+ complexation because of the favourable configuration of the double bond in the ring, changes place with the conjugated 1,3-cyclooctadiene (COD), which shows much weaker complexation, like other conjugated olefins that have been investigated. Compounds 1 (cyclooctatetraene) and 2 (1,3,6-cyclooctatriene) exhibit very strong Ag^+ complexation, which indicates the non-conjugated character of the double bonds. They were not contained in the mixture used to obtain the chromatograms shown in Fig. 5 because their peaks would be obscured by the solvent peak with the given parameters of the phase system.

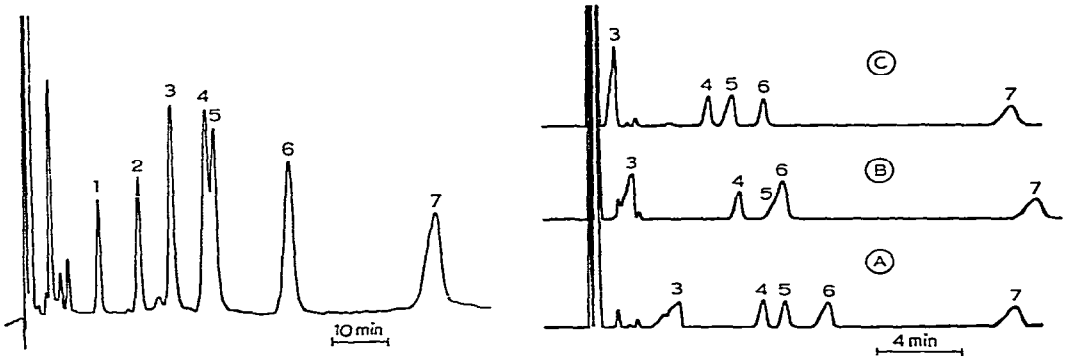


Fig. 4. Separation of unsaturated cyclooctanes. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18; mobile phase, methanol–water (3:1, v/v); pressure, 80 bar; flow-rate, 0.6 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 1 = 1,3,5,7-cyclooctatetraene; 2 = 1,3,5-cyclooctatriene; 3 = 1,5-cyclooctadiene; 4 = 1,4-cyclooctadiene; 5 = 1,3-cyclooctadiene; 6 = cyclooctene; 7 = cyclooctane.

Fig. 5. Separation of unsaturated cyclooctanes. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (3:1, v/v) + $1.7 \cdot 10^{-3}$ *N* $AgClO_4$; B, methanol–water (3:1, v/v) + $5.8 \cdot 10^{-3}$ *N* $AgClO_4$; C, methanol–water (3:1, v/v) + $10 \cdot 10^{-3}$ *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.6 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 3 = 1,5-cyclooctadiene; 4 = 1,4-cyclooctadiene; 5 = 1,3-cyclooctadiene; 6 = cyclooctene; 7 = cyclooctane. *Note:* In part C peak numbers 5 and 6 ought to be interchanged.

The separation of the four (*Z,E*)-isomers of 1,5,9-cyclododecatriene, which differ from each other only with respect to the configuration of three double bonds, is shown in Fig. 6. Without Ag^+, only an incomplete resolution of the four isomers is obtained, whereas with Ag^+ in the mobile phase a tremendous change in the

selectivity of all four isomers occurs and their separation becomes extremely easy. The Ag^+ concentration can be as low as $3 \cdot 10^{-3}$ mole/l (silver perchlorate) in the mobile phase because of the very strong complexation of these compounds. The (*Z,Z,Z*)-isomer is the compound with the highest Ag^+ complexation constant found in our investigations, whereas the (*E,E,E*)-isomer is only weakly complexed.

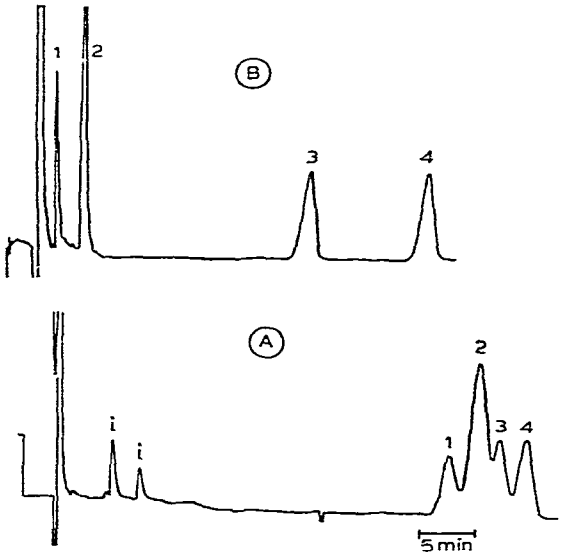


Fig. 6. Separation of isomeric 1,5,9-cyclododecatrienes. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (3:1, v/v); B, A + $3 \cdot 10^{-3}$ *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.7 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 1 = (*Z,Z,Z*)-1,5,9-cyclododecatriene; 2 = (*Z,Z,E*)-1,5,9-cyclododecatriene; 3 = (*Z,E,E*)-1,5,9-cyclododecatriene; 4 = (*E,E,E*)-1,5,9-cyclododecatriene; i = impurity.

Fig. 7 shows the separation of two isomeric sesquiterpenes (δ- and γ-cadinene), both containing two double bonds. Because of the much stronger complexation of the *exo*-double bond of species 1, the separation could be improved so much that a micro-preparative isolation of both isomers for NMR characterization could be carried out after 10-fold repetition of the separation at a sample volume of 50 μl. Some of the reported separations can also be attained by high-resolution capillary column GC with adequate selectivities. Preparative separations cannot be carried out, however, in capillary column GC because of the poor sample capacity of capillary columns. Moreover, the separation of species from the liquid eluates is much easier and can be effected without loss of material. Certain losses arise in our work on the removal of the silver salts. For identification purposes in capillary column chromatography, only a combination with mass spectrometry can be applied. In many instances, olefins are not sufficiently temperature stable for GC, *e.g.*, 1,3,6-cyclooctatriene. LC separation can easily be performed even at sub-ambient temperatures (0°), where Ag^+ complexation is much stronger. The application of LC separations, of course, becomes extremely important for high-molecular-weight components that contain one or more functional groups of strong polarity in addition to double bonds (pheromones, triglycerides, etc.).

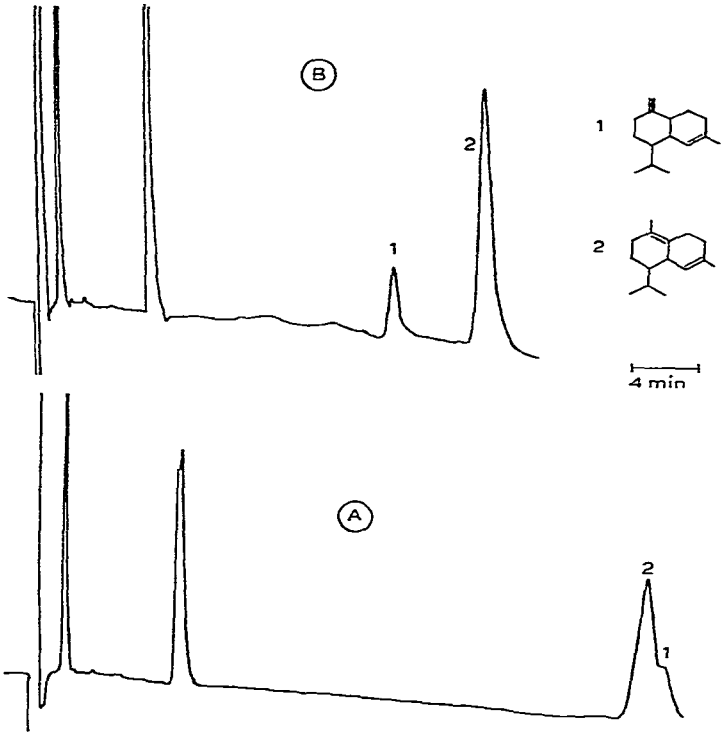


Fig. 7. Separation of sesquiterpenes (δ and γ-cadinene). Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (5:1, v/v); B, A + $8 \cdot 10^{-3}$ *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.7 ml/min; temperature, 24°; detector, RI; attenuation, × 1.

Unsaturated fatty acid esters and triglycerides

The separation of unsaturated C_{18} esters can be improved by using Ag^+ complexation, although the resolution of the two linolenic esters, which differ from each other only by the position of one of the three double bonds, is still incomplete. The (*Z*,*E*)-isomeric oleic and elaidic acid esters are well resolved (Fig. 8).

It has to be pointed out that the (*Z*,*E*)-isomers of the corresponding C_{18} hydrocarbons are eluted with the same characteristic selectivities.

The separation of three authentic unsaturated triglycerides without and with Ag^+ complexation is shown in Fig. 9. The resolution of peaks 2 and 3 is improved although both compounds contain the same number of double bonds (three) but with different configurations. The separation of peak 1 from peaks 2 and 3 is no problem, even without Ag^+ complexation, because the trilinolein contains nine double bonds, which are responsible for strong interactions in the mobile phase. The polarity of the mobile phase had to be decreased for these lipophilic high-molecular-weight components with weak polarities; methanol–isopropanol mixtures proved to be suitable.

The practical application of this separation technique to Spanish olive oil is shown in Fig. 10, where compounds 3 and 4 could be resolved completely by means of Ag^+ complexation. It can also be concluded that the other components are also unsaturated. Both species 3 and 4 were isolated preparatively from eluate fractions

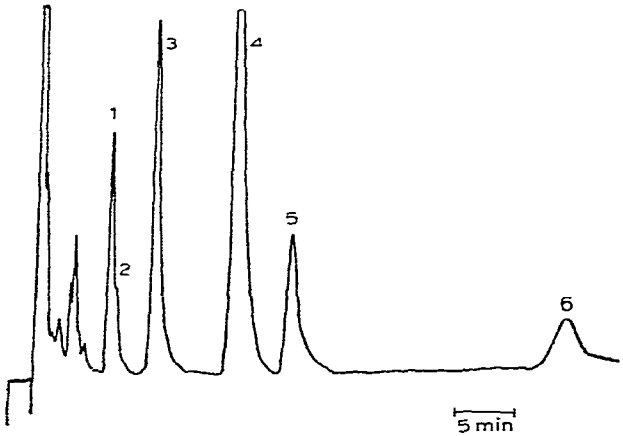


Fig. 8. Separation of unsaturated C_{18} fatty acid methyl esters. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18; mobile phase, methanol–water (5:1, v/v) + 10^{-2} *N* $AgClO_4$; pressure, 80 bar; flow-rate, 0.7 ml/min; temperature, 2°; detector, RI; attenuation, ×4. Peaks of methyl esters: 1 = linolenic acid; 2 = γ-linolenic acid; 3 = linoleic acid; 4 = oleic acid; 5 = elaidic acid; 6 = stearic acid.

of a repetitive separation. From the mass spectra, which were measured after removal of the solvents and the silver perchlorate, compounds 3 and 4 could be identified as triolein and diolein–palmitin, respectively, the molecular weights being found to be 884 and 858. This result was confirmed by the LC retention index interpretation. Table III gives the indices I, I_{Ag} and ΔI_{Ag} standardized on the saturated triglycerides (trimyristin, tripalmitin and tristearin), the I values of which differ, by definition by 600 index units; the I values were assigned to the standard components on the basis of the sums of the carbon atoms of the fatty acids contained in the triglycerides. The retention indices of these components relative to the *n*-alkanes as standards are about 2000 index units (equivalent to 20 CH_2– groups) lower. The retention

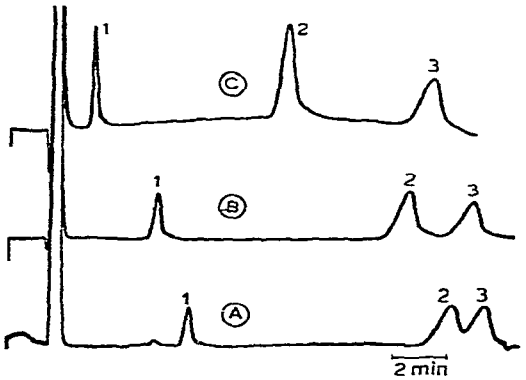


Fig. 9. Separation of unsaturated triglycerides with and without Ag^+. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–isopropanol (3:1, v/v); B, A + 10^{-2} *N* $AgClO_4$; C, A + $5 \cdot 10^{-2}$ *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.9 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 1 = trilinolenin; 2 = triolein (*Z*); 3 = trielaidin (*E*).

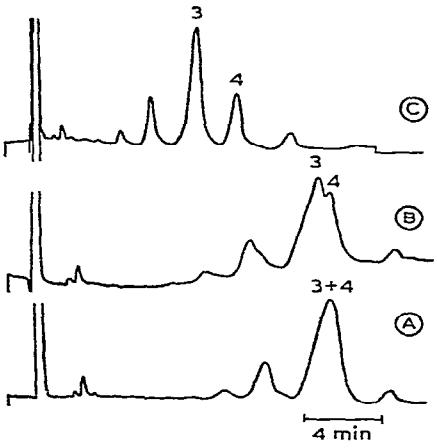


Fig. 10. Separation of Spanish olive oil. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–isopropanol (3:1, v/v); B, A + 10^{-2} *N* $AgClO_4$; C, A + $5 \cdot 10^{-2}$ *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.9 ml/min; temperature, 24°; detector, RI; attenuation, ×4. Peaks: 3 = triolein; 4 = diolein–palmitin.

indices of the triglycerides are lower by these 2000 units because of the three ester groups that are also contained in the molecule. From the retention indices in Table III, the following can be concluded:

(1) Peak 3 has a retention index about the same as that of authentic triolein.

(2) The "characteristic" response ratios, R_{UV}/R_{RI}, for peaks 3 and 4 are the same as those for the triglycerides not containing fatty acids with more than one double bond in the carbon chain. No polyunsaturated fatty acids are contained in the triglycerides.

(3) Without Ag^+ complexation, the retention indices of both components are identical, whereas with Ag^+ a retention difference (δI) of 170 index units is observed. This difference corresponds precisely to the retention increment of one (*Z*)-double bond. Three (*Z*)-double bonds have a ΔI value of 500 (compare triolein). Under the

TABLE III

LC RETENTION INDICES OF UNSATURATED TRIGLYCERIDES (STANDARDIZATION WITH SATURATED TRIGLYCERIDES)

Compound	*Methanol–isopropanol (3:1, v/v)*		*Methanol–isopropanol (3:1, v/v) + 0.05 N $AgClO_4$*	
	I	h_{UV}/h_{RI}	I	ΔI_{Ag}
Trilaurin	3600*	0.3	3600*	0*
Trimyristin	4200*	0.3	4200*	0*
Tripalmitin	4800*	0.3	4800*	0*
Trilinolenin	3880	3.0	2910	970
Trilinolein	4290	1.0	3490	800
Triolein	4760	0.3	4260	500
Trielaidin	4830	0.3	4640	190
Olive oil, peak 3	4810	0.3	4270	530
Olive oil, peak 4	4810	0.3	4440	360

* By definition.

conditions of the phase system used, the ΔI_{Ag} difference corresponds to the retention difference caused by two additional CH_2– groups (200) in the triglycerides. Therefore, compound 3 is triolein and 4 is triolein–palmitin, which confirms the result found by mass spectrometry. These conclusions are valid only if the presence of branched-chain fatty acids in the separated triglycerides can be ruled out.

Heterocyclic hydrocarbons

The separation of N-heterocyclic hydrocarbons with stationary phases of the adsorptive type loaded with silver nitrate has been reported by Vivilecchia *et al.*[3]. Certain N-heterocyclics were found, as expected, to show strong Ag^+ complexation. This can be seen in Fig. 3, where the line for acridine, which is a monofunctional N-heterocyclic species, has a much higher slope than that of a monofunctional 1-alkene. The potential of HPLC with Ag^+ complexation is illustrated by the chromatograms in Figs. 11 and 12, which were measured with a sample containing six N-heterocyclics and some nitrogen-free polyaromatic standard components. The corresponding α ($= k'/k'_{Ag}$) data are given in Table IV.

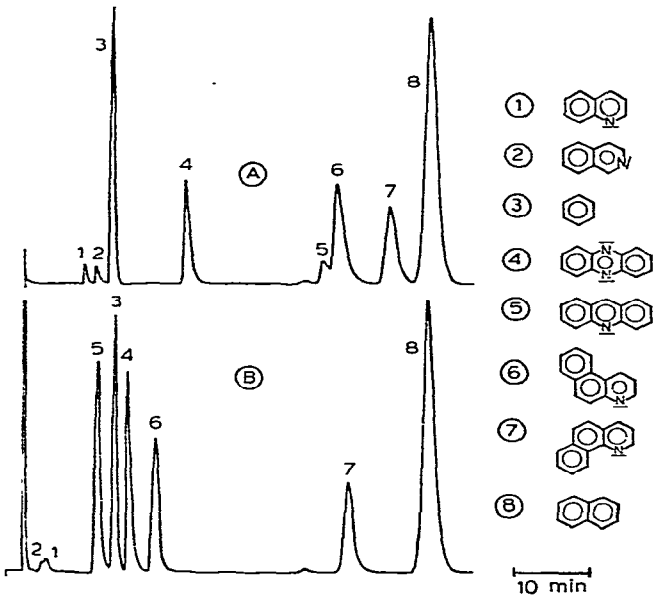


Fig. 11. Separation of polynuclear N-heterocyclics. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (1:1, v/v); B, A + 10^{-2} *N* $AgClO_4$. Pressure, 100 bar; flow-rate, 0.5 ml/min; temperature, 24°; detector, UV (254 nm).

With the exception of the 7,8-benzoquinoline and phenazine, strong Ag^+ complexation occurs. No retention shifts were found for compounds 8 (naphthalene) and 3 (benzene). Although phenazine contains two nitrogen atoms, it exhibits weaker complexation than acridine, because the second nitrogen atom is electron withdrawing and decreases the overall basicity of phenazine in comparison with acridine. With 7,8-benzoquinoline, steric hindrance of the intermolecular interaction explains the weak complexation with Ag^+ (and the basicity), whereas with the two quinolines the basicity and the Ag^+ complexation are different because of electronic effects.

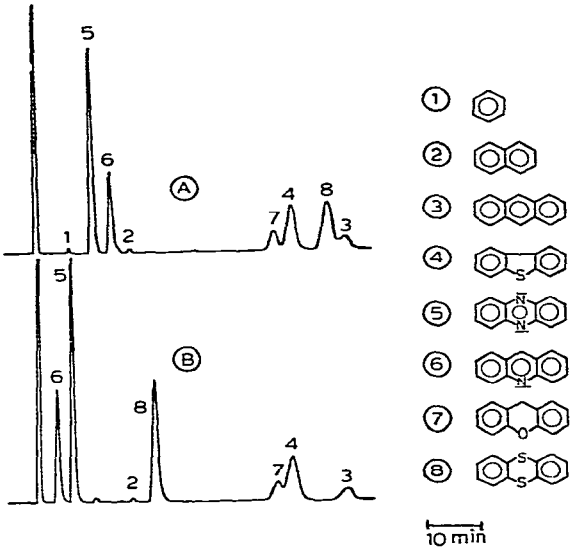


Fig. 12. Separation of polynuclear aza-, oxy- and thio-heterocyclics. Column, 150 × 4 mm I.D.; stationary phase, 5-μm Nucleosil 5 C 18. Mobile phase: A, methanol–water (3:1, v/v); B, A + 10^{-2} *N* $AgClO_4$. Pressure, 80 bar; flow-rate, 0.6 ml/min; temperature, 24°; detector, UV (254 nm).

TABLE IV

CAPACITY COEFFICIENTS OF AROMATIC AND HETEROCYCLIC COMPOUNDS IN REVERSED-PHASE CHROMATOGRAPHY WITHOUT AND WITH 10^{-2} *N* Ag^+

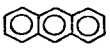
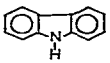
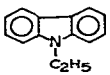
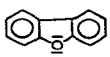
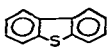
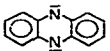
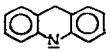
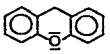
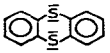
Component	*Structure*	k'	k'_{Ag}	α_{Ag} $(= k'/k'_{Ag})$
Benzene		1.28	1.28	1.0
Naphthalene		3.49	3.49	1.0
Anthracene		11.43	11.42	1.0
Fluorene		8.37	8.30	1.0
Carbazole		2.45	2.41	1.0
N-Ethylcarbazole		8.57	8.30	1.0
Dibenzofuran		6.85	6.89	1.0
Dibenzothiophene		9.45	9.40	1.0
Phenazine		2.12	1.31	1.6
Acridine		2.73	0.73	3.8
Xanthene		8.85	8.89	1.0
Thioanthrene		10.81	4.35	2.5

Fig. 12 illustrates the Ag^+ complexation of heterocyclic compounds containing various heteroatoms (N, O and S). No Ag^+ complexation was observed for the aromatic hydrocarbons (benzene), 2 (naphthalene) and 3 (anthracene), or for 4 (dibenzothiophene) and 7 (xanthene) (Fig. 11). According to the data in Table IV, the heterocyclics fluorene, carbazole, N-ethylcarbazole and dibenzothiophene also do not undergo Ag^+ complexation. Surprisingly strong complexation is found for thianthrene[8].

If methanol is replaced with acetonitrile, the α_{Ag} coefficients decrease, possibly owing to interaction between the nitrile group and Ag^+.

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