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Mechanistic aspects of fatty acid retention in silver ion chromatography

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Abstract

The retention properties of various benzyl, phenacyl, and normal- and branched-chain alkyl esters of 6-18:1, 9-18:1 and 11-18:1 fatty acids have been studied by silver ion high-performance liquid chromatography. The weak electron-donating effect of the alkyl substituents on the carbonyl oxygen in the aliphatic alkyl esters ensures baseline resolution of isopropyl and *tert.*-butyl esters, but not of *n*-alkyl esters. Comparison between benzyl and phenacyl derivatives confirmed that the silver ion interacted simultaneously with the double bond of the fatty acid and the carbonyl oxygen of the phenacyl moiety. This dual interaction enabled the separation of positional isomers. Introduction of electron-withdrawing or electron-donating substituents in the *p*-position in the benzene ring decreased or increased retention, respectively. The substituents strongly affect the resolution, and *p*-methoxyphenacyl esters have not only the highest k' values but also the best resolution of the three positionally isomeric fatty acids, established so far.

Keywords: Silver ion liquid chromatography; Derivatization, LC; Fatty acids

1. Introduction

Silver ion chromatography is certainly one of the most powerful tools in use in lipid analysis. Its utilization and significance have increased greatly in recent years when a stable silver-loaded column for high-performance liquid chromatography (Ag-HPLC) became available [1]. Separation is based on the reversible formation of a weak charge-transfer complex between a silver ion and a double bond. A sigma bond is formed between the occupied 2p π electrons of the olefinic bond and the free 5s and 5p orbitals of the silver ion, and a π acceptor backbone between the occupied 4d orbitals and the free antibonding 2p π^* orbitals of the olefinic bond. Obviously, the strength of the complex should depend on the accessibility of the electrons in the orbitals as well as on the steric inhibition of the orbitals. The result is that it enables the fractionation of lipid molecules according to the number, geometry and position of the double bond(s) in fatty acid moieties [2,3].

The efforts to control better the separation process and to improve the selectivity of resolution require an improved understanding of the mechanism of

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lipid retention. Fatty acid derivatives are suitable simple models, as they are that unit of the lipid structure responsible for the interaction with silver ions and thence for the resolution. In a series of papers [4–8], the retention characteristics of different derivatives of unsaturated fatty acids in Ag-HPLC, silver ion thin-layer chromatography (Ag-TLC), and in combined silver ion/reversed-phase HPLC modes have been reported (results have been summarized in recent reviews [2,3]). It appeared that, in confirmation of older results obtained with Ag-TLC [9], retention of monoenoic fatty acids depends on the position of the double bond in the carbon skeleton. In addition, the nature of the ester moiety (since fatty acids are usually derivatized prior to chromatography) has been found to have a significant effect on retention and resolution, irrespective of the basic chromatographic procedure employed. It became possible to improve greatly the resolution of monoenoic fatty acid positional isomers, for example, by converting them into phenacyl instead of methyl ester derivatives, as was shown with the phenacyl esters of some naturally occurring octadecenoates and eicosenoates [4,6,8]. Vice versa, allyl esters (with a double bond in the alcohol moiety situated near to the ester bond) were held relatively weakly and no resolution of 6-, 9- and 11-18:1 was obtained.

To explain the effects, the formation of a three-centre complex between double bond, silver ion and an electron pair from another electron-rich group in the ester moiety has been assumed. Based on the derivatives examined so far, the carbonyl oxygen of a methyl or other simple alkyl ester, the benzene ring or the additional carbonyl oxygen of the phenacyl ester were considered as possible second reaction sites in fatty acid derivatives. It appeared possible to prove this assumption by introducing electron-donating or electron-withdrawing substituents in the derivatizing moiety. These groups were expected to change the electron density at the second reaction site and thence, to have distinctive effects on the retention of the whole molecule.

A series of benzyl, phenacyl, normal-chain and branched-chain alkyl derivatives of the three naturally occurring C18:1 positional isomers (petroselinic (6-18:1), oleic (9-18:1) and *cis*-vaccenic (11-18:1) acids) were studied.

2. Experimental

2.1. Materials

HPLC-grade or analytical-grade solvents (FSA, Loughborough, UK) were used without further purification. Petroselinic, oleic and *cis*-vaccenic acids and the derivatizing reagents were from Sigma–Aldrich (Poole, UK).

2.2. Derivatization

Methyl, ethyl, *n*-propyl, isopropyl and *n*-butyl esters were produced by acid-catalysed esterification [10], i.e. the free fatty acids were reacted overnight with the respective alcohol containing 1% sulphuric acid in stoppered test tubes at 50°C. 2-Methyl-1-butyl, isoamyl, *tert.*-butyl and *tert.*-amyl esters were produced via the alcohols and the corresponding acid chlorides. Thus, free fatty acids (5 mg) were converted to acid chlorides by reaction with oxalyl chloride (0.5 ml) for 36 h at room temperature. The excess reagent was evaporated, in a stream of nitrogen and then under vacuum. The residue was dissolved in toluene (0.25 ml), and was immediately reacted with 5 mg of the alcohol in toluene (0.5 ml) and pyridine (0.1 ml) at 50°C overnight. The excess solvent and pyridine were removed in a rotary evaporator. Hexane (5 ml) was added to the residue and the solution was washed with water (2×5 ml). Finally, the products were purified by elution through a Florisil column (in a Pasteur pipette) with hexane–acetone (99:1, v/v). The purity of each derivative was checked by TLC (Alufolio silica gel 60, Merck, Darmstadt, Germany) with a mobile phase of hexane–acetone (100:8, v/v) and detection with iodine vapour. Fatty acids were converted into the aromatic derivatives as described by Wood and Lee [11]. Derivatives were purified by elution from a BondElut NH2 column (Analytichem International, Cambridge, UK) with hexane–diethyl ether (9:1, v/v).

2.3. High-performance liquid chromatography

A Gynkotek Model 480 pump (Severn Analytical, Macclesfield, UK) was used with a Varex evaporative

light-scattering detector Model Mk III (PS Instruments, Sevenoaks, UK). A column (250×4.6 mm) of Nucleosil 100-5SA (Hichrom, Reading, UK) was converted to the silver form as described earlier [1]. The temperature of the column was maintained at 20±1.0°C by fitting it into a water jacket through which water was pumped from a temperature control unit. Samples of approximately 100 μg of each fatty acid derivative were injected as a solution in dichloroethane (5 μl). A mixture of dichloroethane–dichloromethane–acetonitrile was used as mobile phase at a flow-rate of 1.5 ml/min. The dichloroethane–dichloromethane ratio was kept constant (1:1, v/v) and the proportion of acetonitrile was varied depending on the derivatives (see tables). The dead volume of the column was determined by repeated injection of docosane. The mean retention time for six injections was 1.99±0.01 min. Retention (capacity) factors (k') were determined as the mean of three parallel measurements with relative standard deviations not exceeding 3.2%.

3. Results

As in previous work [4], 0.01 to 0.025% of acetonitrile in a 1:1 mixture of dichloromethane–dichloroethane was used as mobile phase. The precise role of the acetonitrile has not been determined, but we believe that acetonitrile competes strongly with unsaturated analytes for binding sites on silver ions (it is a powerful solvent for silver nitrate). Otherwise, such low concentrations would not be expected to alter the selectivity of the mobile phase.

3.1. Retention characteristics of aromatic ester derivatives

Fig. 1 illustrates the formulae of the benzyl and the phenacyl species studied. When examined under the same experimental conditions, species eluted in the order: pentafluorobenzyl<*p*-bromobenzyl<*p*-bromophenacyl < *p*-methoxybenzyl < phenacyl < *p*-methoxyphenacyl esters. As is evident from the retention data listed in Table 1, benzyl derivatives were held much less strongly than the corresponding phenacyl derivatives. Substituents in the benzene ring influenced the retention clearly. *p*-Methoxyphenacyl derivatives were held much stronger than were *p*-bromophenacyl compounds. The substituents affected the phenacyl derivatives most: *p*-bromophenacyl esters were held about 2.5 times less strongly than *p*-methoxyphenacyl esters, while *p*-methoxybenzyl derivatives were held about 1.7 times more strongly than *p*-bromobenzyl derivatives. It had earlier been shown that 2,4-dinitrophenylmethyl esters were rather poor substrates for silver ion chromatography [4]. Pentafluorobenzyl derivatives had the lowest k' values.

Selectivity of resolution did not correspond directly to the increase in k' value, however; all benzyl derivatives were resolved only partially, while all phenacyl derivatives were resolved to the baseline. This is clearly seen by comparing the magnitude of k' and R_s values of the *p*-methoxybenzyl and *p*-bromophenacyl derivatives in Table 1, for example. The *p*-bromophenacyl derivatives of 6-, 9- and 11-18:1 were held less strongly than the corresponding *p*-methoxybenzyl derivatives but were completely resolved (R_s>1 for 6-/9- and 9-/11-pairs), while the *p*-methoxybenzyl derivatives (the highest k' values among the benzyl derivatives) were resolved only partially (R_s<1). *p*-Methoxyphenacyl esters provide the best resolution of 6-, 9- and 11-18:1 observed so far (cf. the R_s values listed in Table 1), and a sample chromatogram is illustrated in Fig. 2.

3.2. Retention characteristics of esters with short normal- and branched-chain fatty alcohols

The formulae of the aliphatic derivatives examined are also shown in Fig. 1, and the retention and resolution data for the isomers are listed in Table 2. The alkyl esters eluted in the order: *tert.*-amyl=2-methyl-1-butyl = isoamyl- = *tert.*-butyl < *n*-butyl- = *n*-propyl<isopropyl-<methyl-=ethyl-, i.e. retention decreased with elongation of the carbon chain of the fatty alcohol. Based on the range of the k' values, the derivatives could be divided into three more or less distinct groups of species with equal or very close k'; methyl, ethyl and isopropyl esters were held most strongly, *n*-propyl and *n*-butyl esters had medium k' values and the branched-chain butyl esters were held

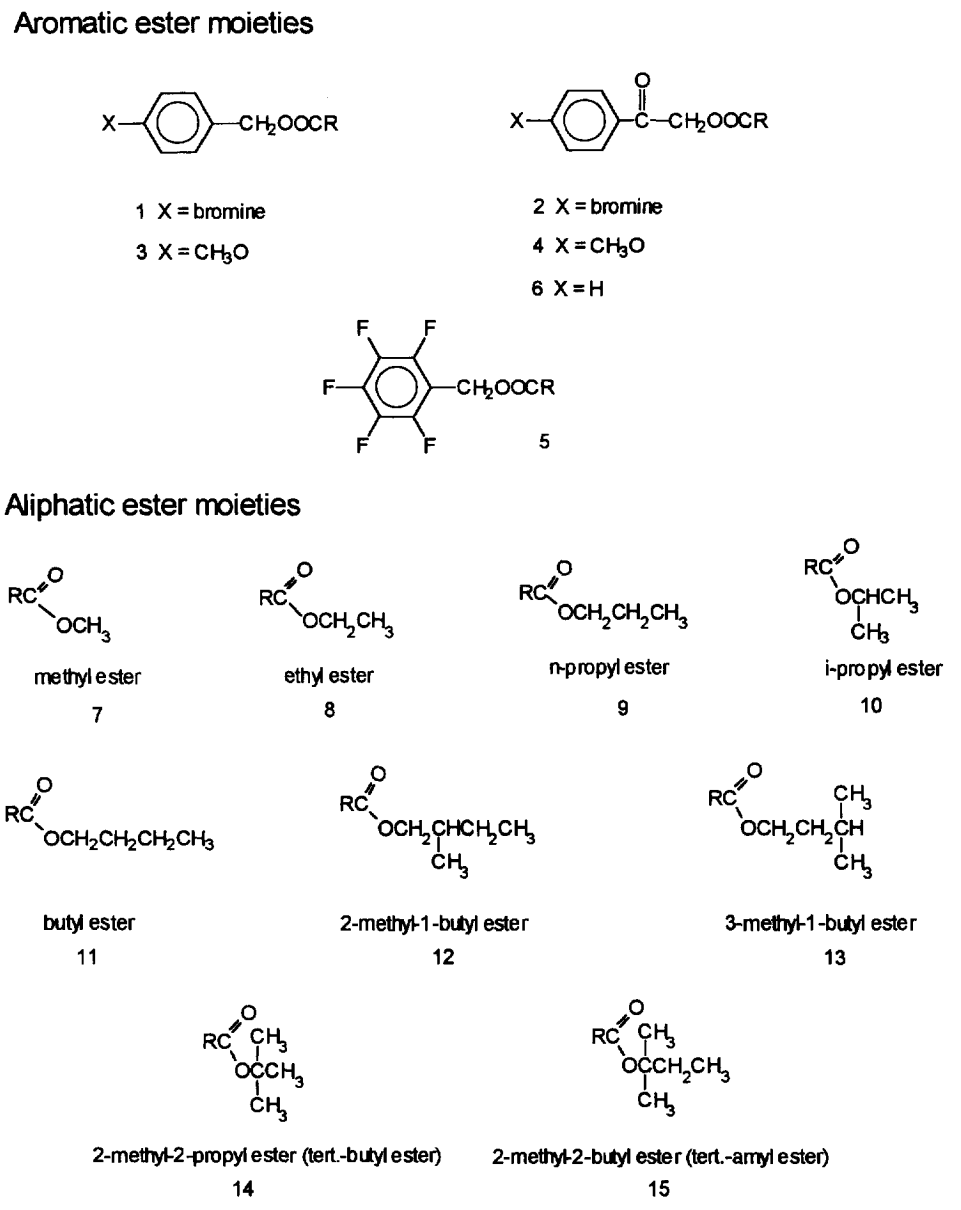

Fig. 1. Structures of the aromatic and aliphatic esters used in this study.

least strongly. The arrangement of the carbon atoms in the alkyl chain of the ester has some effect, and isopropyl esters, for example, were held more strongly than the *n*-propyl esters, but branched-chain butyl esters were held less strongly than *n*-butyl esters.

However, once more the resolution of the three positionally isomeric fatty acids were not related to the k' values and depended on the type of the alkyl chain in the alcohol moiety. All derivatives provided baseline separation of 9-18:1 and 11-18:1 fatty acids ($R_s > 1.0$) with resolution increasing in the order butyl- = 2-methyl-1-butyl < isoamyl- < isopropyl- < methyl = ethyl = propyl < *tert.*-butyl- = *tert.*-amyl- < isopropyl. Resolution of 6- and 9-18:1 isomers was less clear and varied with the structure of the alcohol moiety; 2-methyl-1-butyl derivatives were not re-

Table 1
Retention (k') and resolution (R_s) values of petroselinic (6-18:1), oleic (9-18:1) and *cis*-vaccenic (11-18:1) acids as aromatic derivatives on silver ion HPLC

Derivative	k'			R_s	
	6-18:1	9-18:1	11-18:1	6-/9-	9-/11-
p-Bromobenzyl	3.9	3.9	3.7	–[a]	–
p-Methoxybenzyl	6.9	6.5	5.9	–	–
Pentafluorobenzyl	2.3	2.5	2.4	–	–
p-Bromophenacyl	6.2	5.2	4.1	1.6	1.8
p-Methoxyphenacyl	15.8	12.4	9.8	3.4	3.4
Phenacyl	8.4	6.7	5.6	1.5	2.4

Nucleosil 100-5SA column in silver ion form, mobile phase=dichloromethane–dichloroethane–acetonitrile (50:50:0.025, v/v).
[a] not resolved.

solved, isoamyl-<methyl=ethyl<butyl=*tert.*-amyl derivatives were resolved partially, and *n*-propyl-< isopropyl=*tert.*-butyl derivatives (R_s values increased in this order) were resolved completely. On the other hand, even the best resolved alkyl esters did not have R_s values exceeding 1.7, i.e. about half that of the best-resolved aromatic esters, *p*-methoxyphenacyl.

4. Discussion

Lipid retention in silver ion HPLC is a complex process in which complexation between double bonds and silver ions is the main but not the only interaction in the column. Polar interactions of lipids with the silica base of the stationary phase, interactions between the mobile and stationary phases, and interactions between silver ions and components of the mobile phase are possible also, and may affect retention and especially the resolution.

Complexation is undoubtedly the main interaction that governs the retention and, therefore, the magnitude of the retention factor, k', responds to the strength of the complex; the stronger the complex the higher should be the k'. By comparing k' values of fatty acids in the form of various derivatives, it was possible to elucidate specific effects of the structure

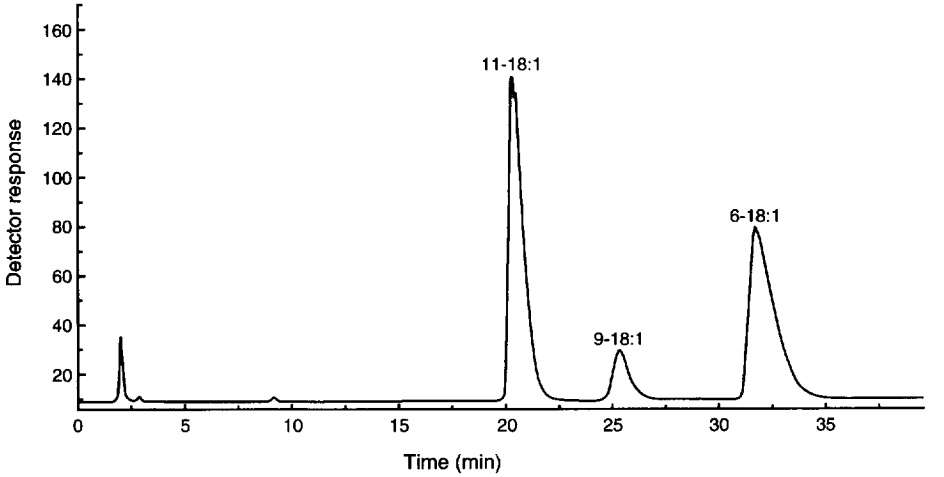

Fig. 2. Silver ion HPLC separation of *p*-methoxyphenacyl esters of *cis*-11-, 9- and 6-18:1 fatty acids. The Nucleosil 100-5SA column in the silver ion form was utilized with a mobile phase of dichloromethane–dichloroethane–acetonitrile (50:50:0.025, v/v) at a flow-rate of 1.5 ml/min.

Table 2
Retention (k') and resolution (R_s) of derivatives of petroselinic (6-18:1), oleic (9-18:1) and *cis*-vaccenic (11-18:1) acids with normal- and branched-chain alcohols on silver ion HPLC

Derivative	k'			R_s	
	6-18:1	9-18:1	11-18:1	6-/9-	9-/11-
Methyl	9.0	8.4	7.5	0.6	1.3
Ethyl	8.9	8.4	7.4	0.7	1.3
n-Propyl	7.3	6.4	5.3	0.7	1.3
Isopropyl	8.4	7.8	6.6	1.3	1.7
n-Butyl	7.2	6.0	5.5	0.8	1.0
2-Methyl-1-butyl	6.0	5.7	4.8	–[a]	1.0
3-Methyl-1-butyl	5.7	5.6	4.8	0.3	1.1
2-Methyl-2-propyl	6.0	5.4	4.8	1.3	1.6
2-Methyl-2-butyl	5.6	5.1	4.6	0.9	1.6

Nucleosil 100-5SA column in silver ion form, mobile phase=dichloromethane–dichloroethane–acetonitrile (50:50:0.01, v/v).
[a] not resolved.

of the ester moiety on complexation with silver ions. The following discussion is based on the experimentally confirmed ability of silver ions to complex simultaneously with two electron-releasing centres [12–14].

The comparison between the phenacyl and benzyl derivatives was intended to confirm the participation of the carbonyl oxygen in the phenacyl moiety in the complexation with silver ions, with the weak interactions of the benzene ring balancing out [12,15]. Retention of aromatic esters was indeed influenced very strongly by the structure of the alcohol moiety. Phenacyl derivatives (a carbonyl oxygen in the molecule) were held much more strongly than the corresponding benzyl derivatives. Retention order was determined by substitution in the benzene ring. Electron-donating groups (like *p*-methoxy) increased and electron-withdrawing groups (bromine) decreased the k' values significantly. Substitution effects phenacyl and benzyl derivatives in a similar way, but phenacyl derivatives were more strongly affected and monoenoic *p*-methoxyphenacyl esters had the highest k' values for monoenes measured so far in our studies. The much stronger response of the phenacyl derivatives to substitution confirms our earlier suggestion [4] that the free electron pair at the carbonyl oxygen in the phenacyl moiety co-ordinates with silver ion as a second interaction site in the fatty acid molecule. The formation of more stable chelate-type complex appear to be highly probable. In this instance, the carbonyl oxygen of the ester moiety must have a lesser role.

It is not easy to decide whether there is a second reaction site in the molecules of the benzyl derivatives (either the carbonyl oxygen of the ester group or the benzene ring). *p*-Substituents in the benzyl moiety also affect retention, but the k' values of these derivatives are much lower and the values of the three fatty acid isomers with the same derivative do not differ significantly. This suggests that formation of a chelate type complex with the benzyl derivatives is unlikely.

In the case of alkyl esters, the second reaction site must be the free electron pair on the carbonyl oxygen in the ester moiety. Electron-donors which could increase the electron density on the oxygen (alkyl groups are weak electron donors) were expected to increase k'. When energies of complexation were calculated [16] it appeared that a methyl ester such as methyl acrylate forms a more stable complex with silver ion than does acrylic acid and this was assigned to the weak electron-donating properties of the methoxyl group. The electronic effects on k' values of simple alkyl normal- and branched-chains were, however, not so clear as to allow unambiguous conclusions to be made, presumably because these effects were weak, and hydrophobic interactions and steric hindrance must be taken into account. Hydrophobic effects on elution order were relatively important as k' decreased with the elongation of the carbon chain of the derivative moiety (cf. the k' of normal-chain esters which decreased in the order *n*-butyl=*n*-propyl-<ethyl-=methyl-). Branching of the chain generally led to lower k' values (steric

hindrance or increased affinity to the mobile phase or both) with one important exception, isopropyl esters, which were retained more strongly than were the *n*-propyl esters (differences are statistically significant). The result can be explained by the higher positive inductive effect of the isopropyl moiety compared to that of the *n*-propyl moiety. 2-Methyl-1-butyl, *tert.*-butyl, isoamyl, and *tert.*-amyl esters have similar k' values generally, and lower than all the rest. Substitution on carbon 1 of the *tert.*-butyl group lead again to higher k' values for 6-18:1 derivative and improved resolution of the isomers (R_s values were equal to those of isopropyl esters).

Interactions between oxygenated functions in organic molecules and silver ion have been described (see [12], for example, and the literature cited therein). Recently, the formation of a monolayer of short-chain saturated fatty acids on silver has been explained by the ability of the carboxyl group to bind as a bidentate or bridging ligand [17]. In an earlier paper [4], we found that monoenoic alcohols were retained almost as strongly as were dienoic fatty acid derivatives on Ag-HPLC. A dual interaction of hydroxyl oxygen and double bond with silver ions was suggested for some terpenes in a recent paper [15]. Participation of a carbonyl group in the correct position was also assumed in this study [15]. Indeed, when energies of complexation were calculated ab initio for simple model compounds [16], it appeared that formaldehyde (a free electron pair at the carbonyl oxygen), for example, formed a more stable complex than ethylene (single double bond) while acrolein (single double bond and carbonyl oxygen) formed a stronger complex than formaldehyde, because of the dual interaction of silver ion with double bond and carbonyl oxygen. Preliminary results have shown the same is true for fatty acids.

The effects of the ester moieties were most strong on 6-18:1 and least strong on 11-18:1 derivatives. The probability of a dual interaction should decrease with the increasing distance between the double bond and ester group. On the other hand, a very close distance between the double bond in the carbon chain and the oxygenated function (or another reaction site in the ester moiety (cf. our results with the allyl esters of positionally isomeric monoenes [7] and the k' values for the 3-18:1 fatty acid esters [4]) will cause a delocalization of the electrons and decrease the stability of the complexes with silver ions. The same effect was observed for the retention of model monounsaturated aliphatic esters, terpenes and pheromones on a silver ion column of this type [15]. This assumption was confirmed when complexation energies of a series of model unsaturated fatty acids were calculated [16]. Energy decreases when the distance between the double bond and ester moiety increases up to a certain number of methylene groups. Further increase of the distance causes a decrease in the stability of the complexes. Indeed, it was found experimentally that retention of methyl and phenacyl fatty acid esters passed through a maximum (at 7 and 5 methylene groups, respectively) as a double bond moved away from the carboxylic group [4].

The effects of the dual interaction and the requirements for an optimal distance between the two reaction sites in the fatty acid molecule have a direct relationship to the quality of the resolution. R_s values do not necessarily follow the order of the k' values, and less retained derivatives often were better separated. It is evident that ester moieties affect most strongly the retention of 6-18:1. Some important practical conclusions can be made on this basis. First, by choosing an appropriate derivative separation of positionally isomeric monoenoic fatty acids can be improved significantly. Secondly, even when the derivatives ensure the formation of stronger complexes with silver ion, the effect will be most clear for species with a double bond close to the carboxyl group. Stronger complexation does not mean necessarily better resolution, however. Thus, although *p*-methoxybenzyl derivatives are held more strongly than *p*-bromophenacyl derivatives (electron-donor versus electron-withdrawing substituents), the latter are much better resolved (Table 1). It is possible to speculate that in the case of benzyl derivatives, a chelate type complex is not formed, double bond position has no measurable effect on the complex stability and hence the resolution is poor. Vice versa, the carbonyl oxygen in the *p*-bromophenacyl moiety has the correct position in the molecule and participates in a three centre complex with double bond and silver ion. General retention could be weaker because of the electron-withdrawing effect of the bromine in the *para*-position, but stability of complexation is influenced by the posi-

tion of the double bond and decreases in the order 6-18:1, 9-18:1 and 11-18:1.

Alkyl esters behave in a similar way. However, isopropyl, *tert.*-butyl and *tert.*-amyl esters provide as clear resolution of the fatty acid isomers as isopropyl esters despite the weaker retention. Compared to the other alkyl esters studied here these branched alkyl chains should have higher electron-donating effects, thus increasing the electron density on the carbonyl oxygen. When the double bond and carbonyl oxygen are at the correct distance (as in 6-18:1, for example) a dual interaction with silver could be expected and hence, stabilisation of the complex. As double bond is shifted away, stability should decrease. The result is that despite the relatively low k' values, fatty acid isomers are clearly differentiated.

Other interactions may play a significant part in the quality of resolution, and the nature of the mobile phase must influence the strength of complexation. Early work [4] showed that phases containing acetonitrile in chlorinated solvents are highly selective for the resolution of unsaturated fatty acids, and we normally use 0.01 to 0.025% of acetonitrile in a 1:1 mixture of dichloromethane–dichloroethane. Recently, Adlof and co-workers [18] were able to obtain distinctive separations of *cis* and *trans* positionally isomeric monoenes (as methyl esters) with 0.08% acetonitrile in hexane as mobile phase. Thus, selectivity of resolution in the analysis of isomeric fatty acid can be influenced by choosing the optimum derivative and a suitable composition of the mobile phase.

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References

[1] W.W. Christie, J. High Resolut. Chromatogr. Chromatogr. Commun., 10 (1987) 148.
[2] B. Nikolova-Damyanova, in Advances in Lipid Methodology-One, The Oily Press, Ayr, Scotland, 1992, p. 181.
[3] G. Dobson, W.W. Christie and B. Nikolova-Damyanova, J. Chromatogr. B, 671 (1995) 197.
[4] B. Nikolova-Damyanova, B.G. Herslof and W.W. Christie, J. Chromatogr., 609 (1992) 133.
[5] B. Nikolova-Damyanova, W.W. Christie and B.G. Herslof, J. Chromatogr. A, 653 (1993) 15.
[6] B. Nikolova-Damyanova, W.W. Christie and B. Herslof, J. Planar Chromatogr., 7 (1994) 382.
[7] B. Nikolova-Damyanova, W.W. Christie and B. Herslof, J. Chromatogr. A, 693 (1995) 235.
[8] B. Nikolova-Damyanova, Sv. Momchilova and W.W. Christie, Phytochem. Anal., 1 (1996) 136.
[9] F.D. Gunstone, I.A. Ismail and M. Lie Ken Jie, Chem. Phys. Lipids, 1 (1967) 376.
[10] W.W. Christie, Gas Chromatography and Lipids, A Practical Guide, The Oily Press, Ayr, Scotland, 1989.
[11] R. Wood and T. Lee, J. Chromatogr., 254 (1983) 237.
[12] Gmelin Handbuch der Anorganishen Chemie, Vol. 61, Springer, Berlin, 1975.
[13] A.J. Canty and R. Colton, Inorg. Chim. Acta, 220 (1994) 99.
[14] M. Ignaczak and A. Grzejdziak, Monatsh. Chem., 125 (1994) 15.
[15] T.A. Van Beek and D. Subrtova, Phytochem. Anal., 6 (1995) 1.
[16] J. Kaneti, B. Nikolova-Damyanova and W.W. Christie, in preparation.
[17] E.L. Smith and M.D. Porter, J. Phys. Chem., 97 (1993) 8032.
[18] R.O. Adlof, L.C. Copes and E.A. Emken, J. Am. Oil Chem. Soc., 72 (1995) 571.