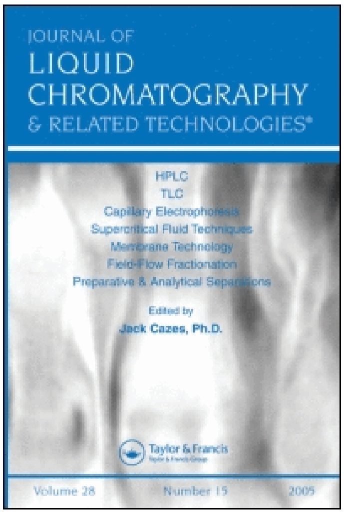

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SILVER ION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC DERIVATIVES OF POSITIONALLY ISOMERIC OCTADECENOIC FATTY ACIDS

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ABSTRACT

The chromatographic properties of positionally isomeric octadecenoic fatty acids in silver ion high performance liquid chromatography with hexane-based and dichloromethane-based mobile phases were studied after conversion in 2-naphthacyl, 2-naphthylmethyl and 9-anthrylmethyl derivatives. The retention decreased in the order 2-naphthacyl, 9-anthrylmethyl, 2-naphthylmethyl derivatives. Dichloromethane-acetonitrile mobile phases provided better resolution and 2-naphthacyl and 9-anthrylmethyl derivatives of 6-, 9-, and 11-18:1 were fully resolved with dichloromethane-acetonitrile 100:0.025 (v/v). The behavior of the aromatic derivatives, examined so far by Ag-HPLC, under comparable experimental conditions, was compared and a clear tendency of improving the resolution was observed when the number of aromatic nuclei in the aromatic fragment increased.

The effect was ascribed to the increased electron-donating effect of the aromatic rings in the ester moiety, which supposedly enhanced the formation of a three center complex between double bond, a carbonyl oxygen, and silver ion. The good resolution of 9-anthrylmethyl derivatives is of potential practical value since it allows for the determination of nanogram amounts of fatty acids by silver ion HPLC with fluorescent detection.

INTRODUCTION

The derivatization of fatty acids with different aromatic reagents prior to the chromatographic separation is very popular and widely used in reversed-phase high performance liquid chromatography. The aromatic esters ensure convenient and sensitive detection of the components in the UV region.[1] When some of these derivatives (phenacyl-, benzyl-, phenylethyl-, etc.) were subjected to silver ion high performance liquid chromatography (Ag-HPLC) substantial effect on the resolution of positionally isomeric octadecenoic fatty acids was observed. The separation was either much better, equal, or worse, than that obtained with the respective methyl esters.[2-5] Thus, it was found that besides the position and configuration of the double bond in fatty acid carbon chain, the retention and selectivity of resolution in Ag-HPLC depended on the type and structure of the derivatizing moiety. It was assumed that this effect was due to the formation of three center chelate-type complex between a fragment of the ester moiety, a suitably positioned double bond and a silver ion.[5-7]

Once the ester moiety was assumed to be a significant factor in the resolution of positionally isomeric fatty acids by Ag-HPLC, it appeared worthwhile to examine which structural element dominated in the interaction with the silver ion. Besides the scientific interest, the answer to this question was considered important in the search of approaches for further improvment of resolution. So, despite the fact that the benzene was known to complexate with silver ions through the aromatic π-electron system,[8] the results so far revealed that the phenyl moiety had either none or negligible effect on the resolution.[5] Good resolution of the three naturally occurring octadecenoic fatty acids: 6-18:1 (petroselinic), 9-18:1 (oleic), and 11-18:1 (*cis* vaccenic acid) was obtained after derivatization with acetophenone;[2-4] much better, with p-methoxyacetophenone; but none with p-methoxybenzene.[5,6] The effect was, therefore, attributed to the interaction of the carbonyl oxygen in the phenacyl moiety with the silver ion, with no, or minimal, effect of the phenyl fragment.[5] On the other hand, some preliminary experiments with naphthacyl- and anthrylmethyl derivatives provided promising results for the resolution of the same three isomeric octadecenoates.[2] Hence, it was assumed that these derivatives were suitable models to study the role of the aromatic fragment for the resolution of isomeric octadecenoic fatty acids by Ag-HPLC.

Here, the results on the retention and resolution in Ag-HPLC of 2-naphthylmethyl, 2-naphthacyl-, and 9-anthrylmethyl derivatives of a series of positional isomeric *cis*-octadecenoic fatty acids are reported with special emphasis on the three naturally occurring isomers. The effect of hexane- and dichloromethane-based mobile phases on the resolution of the isomers is also discussed.

EXPERIMENTAL

Materials

Dichloromethane and acetonitrile were HPLC/UV-grade and were used without further purification. All other solvents were analytical grade. Hexane, when used as a mobile phase component, was left for 24 h over potassium hydroxide and distilled. The reference fatty acids and the derivatizing reagents were purchased from Sigma-Aldrich (Poole, UK).

Derivatization

2-Naphthylmethyl, 2-naphthacyl, and 9-anthrylmethyl esters were prepared according to Wood and Lee.[9] Briefly, 2 mg free fatty acid were reacted with 1 mL solution (10 mg/mL in acetone) of2-(bromomethyl)naphthalene or 2-bromo-2-acetonaphthone, respectively, and with 0.5 mL solution (10 mg/mL in acetone) of triethylamine for 15 min in a boiling water bath. Acetic acid (70 µL) was added and the sample was heated for an additional 5 min. The preparation of 9-anthrylmethyl esters differed only in that free fatty acids were reacted with 1.5 mL solution of 9-(chloromethyl)anthracene in acetonitrile (10 mg/mL). The derivatives were purified by TLC on hand-made glass plates with 0.2 mm thick silica gel G layer by single development with mobile phase hexane-acetone; the solvent proportion was 100:12 (v/v) for the 2-naphthylmethyl and 9-anthrylmethyl esters (Rf of 0.60 and 0.55, respectively), and 100:15 (v/v) for the 2-naphthacyl esters (Rf=0.35).

Naphthacyl and 9-anthrylmethyl derivatives were detected as blue bands under UV light (366 nm). 2-naphthylmethyl esters were detected in the same way after spraying with fluorescent indicator (the edges of the plate only; the rest of the plate was carefully covered). The bands were scrapped, transferred to Pasteur pipettes and the derivatives were eluted with diethyl ether. The solvent was evaporated under nitrogen and the derivatives were redissolved in hexane to give 2 mg/mL solutions. All reference derivatives gave single peaks in Ag-HPLC.

Silver Ion HPLC (Ag-HPLC)

An ISCO (Lincoln, NE, USA) HPLC system equipped with model 2350 isocratic pump, Valco C6W injection valve with 10 µL sample loop and V4 UV/Vis detector was used. The column, Nucleosil 100-5SA (250 x 4.6 mm; Hichrom, Reading, UK) was converted to the silver ion form as described by Christie.[10] The injection volume was 10 µL and the sample size was 1-2 µg of each fatty acid derivative. The working wavelength was 250 nm for the anthrylmethyl and naphthacyl derivatives, and 275 nm for the naphthylmethyl derivatives. The absorption maxima were determined on silica gel TLC plate by spectrodensitometry using Shimadzu CS-930 densitometer (Shimadzu Corporation, Kyoto, Japan). Hexane, dichloromethane, and acetonitrile in different proportion were used as mobile phases. Data was integrated by ISCO Chemresearch version 2.3 software.

The void volume of the column was determined via an air bubble. The ratio k" of the retention factor of the analyte to that of the corresponding *trans* 9-18:1 derivative was used to improve the reproducibility of the measurements.

RESULTS AND DISCUSSION

Retention

The structures of the fatty acid derivatives are shown in Figure 1. In Figure 2 A-C, the plots of the relative retention factor k" of the octadecenoic fatty acid isomers against the position of the double bond are presented. Except for the instrument and the detection mode employed in the present work, the experimental conditions were close to those used previously for the phenacyl derivatives.[2] It was possible, therefore, to conclude that with a mobile phase of dichloromethane-dichloroethane-acetonitrile, 50:50:0.025 (by volume) the retention pattern of the phenacyl- and naphthacyl octadecenoates was generally the same. The graphs for naphthylmethyl- and anthrylmethyl derivatives were substantially flatter.

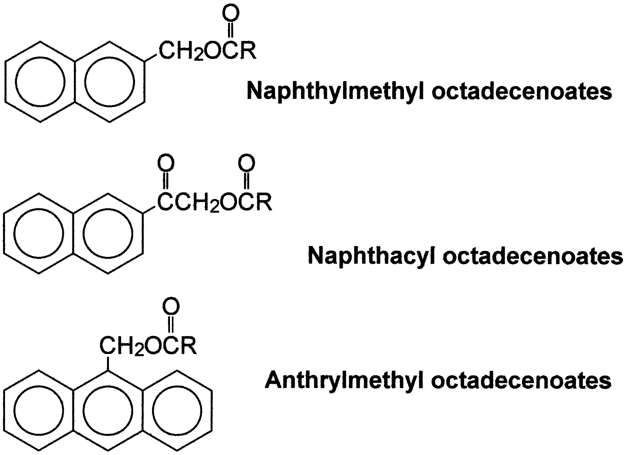

Figure 1. The structure of fatty acid aromatic derivatives. R, fatty acid carbon chain; double bonds are not indicated.

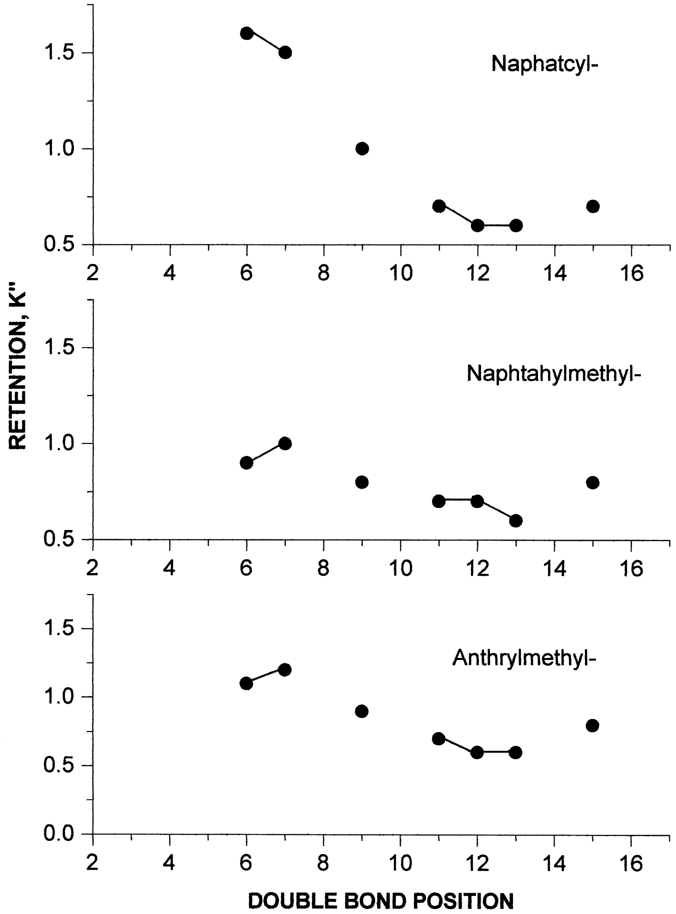

Figure 2. The k" values for Ag-HPLC of some positionally isomeric cis octadecenoates on Nucleosil 100-5SA at 20 ± 1°C column with mobile phase dichloromethane: dichloroethane:acetonitrile, 50:50:0.025 (v/v/v) at flow rate 1.5 mL/min.

The relatively high k" value for the naphthacyl derivatives could be predicted, since, it has been shown previously that the presence of a carbonyl oxygen in the derivatizing moiety substantially increases the retention.[5] Under identical experimental conditions, phenacyl-6-18:1 was retained weaker (k'=4.2) than was naphthacyl-6-18:1 (k'=5.9), i.e. the retention increased with increasing number of rings in the aromatic fragment. The same tendency was observed when comparing the retention of the 6-18:1 naphthylmethyl- and anthrylmethyl derivatives (2 and 3 aromatic rings, respectively): the k" differed with a small but statistically significant value in favor of the anthrylmethyl derivatives. Figure 2, also shows that the retention of isomers with a double bond position at 11-, 12-, and 13- carbon atom was not substantially effected by the nature of the derivatizing moiety, thus, confirming earlier observation with different kinds of derivatives.[2,6]

Resolution

The chromatographic resolution of the three naturally occurring 18:1 isomers, as the respective polynuclear aromatic derivatives in Ag-HPLC with mobile phase, dichloromethane-dichloroethane-acetonitrile, 50:50:0.025 (v/v/v) is presented in Figure 3, A-C. The mobile phase was used previously for the separation of the phenacyl and p-methoxyphenacyl derivatives of the same acids.[2,5] It was possible, therefore, to compare the respective Rs values (Table 1). It was clear that the resolution improved with the increased number of nuclei in the aromatic fragment. Thus, the resolution pattern of 6-, 9-, 11-18:1 naphthacyl derivatives was similar to that of the p-methoxyphenacyl derivatives. Similarly, the resolution increased from none for the benzyl-, to partial for the naphthylmethyl-, and to full for the anthrylmethyl derivatives. Assuming the hypothesis of the three center complex in which a fragment of the ester group provides a second reaction site in addition to the double bond, two possibilities could be considered: either the polynuclear aromatic moiety acted as a second reaction site *per se* or the increasing number of aromatic rings increased the electron density on the ester carbonyl oxygen which acted as such a site (as was supposed earlier for methyl esters and the esters with some branched-chain alcohols).[2.5] Despite the evidence that naphthalene and anthracene form stronger complexes with silver ion[8,11,12] than benzene, we assumed that the latter suggestion was more reliable: (i), because oxygen is a much stronger complexating agent for silver ion than are aromatic compounds and (ii), because aromatic moieties are also efficient electron-donating agents.

Mobile Phase Effects on the Resolution

Two tendencies exist at present, for the choice of mobile phases for Ag-HPLC of fatty acids. Christie and Breckenridge[13] introduced with very good results, the dichloromethane-dichloroethane-acetonintrile solvent system, while Adlof[14] successfully applied hexane-acetonitrile mixtures for the resolution of positional isomeric methyl octadecenoates. It has been shown in a previous paper, that hexane-acetonitrile phases are not suitable for use with aromatic esters because the higher overall polarity of the analyte required acetonitrile content above the solubility limit in hexane.[6] Two types of mobile phase were examined, therefore, for their effect on the resolution of 6-, 9-, and 11-18:1 fatty acids. In hexane-based phases the proportion of

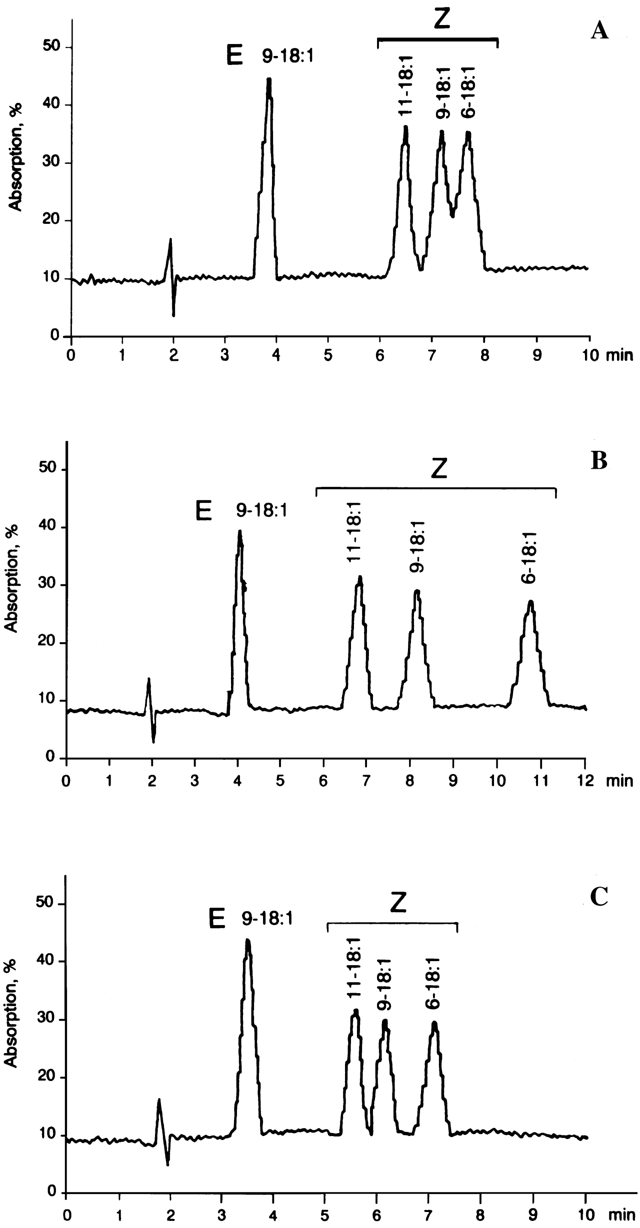

Figure 3. Separation of aromatic derivatives of cis- 11-, *cis*-9- and *cis*-6-18:1 fatty acids. A, Naphthylmethyl-; B, Naphthacyl-; C, Anthrylmethyl esters. Conditions as in Fig. 2.

Table 1

Resolution, Rs, of Aromatic Derivatives of Petroselinic (6-18:1), Oleic (9-18:1) and *cis*-Vaccenic (11-18:1) Acids in Ag-HPLC

Derivative	**Rs[d]** **6-/9-18:1**	 **9-/11-18:1**
Benzyl-[a]	---[c]	---
2-Naphthylmethyl-[a]	0.6	0.9
9-Anthrylmethyl-[a]	1.3	1.1
p-Methoxybenzyl-[b]	---	---
Phenacyl-[a,b]	1.5	2.4
p-Methoxy phenacyl-[a]	2.3	1.7
2-Naphthacyl-[a]	2.3	1.4

---

[a] Present work. [b] Reference No. 5. [c] Not resolved. [d] On Nucleosil 5 SA columns with mobile phase dichloromethane:dichloroethane:acetonitrile 50:50:0.025, (v/v/v).

acetonitrile was kept constant at 0.2 volume parts, and the elution power was increased by varying the dichloromethane proportion from 10 to 100 volume parts. In another set of experiments, the dichloromethane content was kept constant (at 100 volume parts) while the acetonitrile content was varied from 0.01 to 0.2 volume parts. Figures 4 and 5 show the plots of the resolution factors, Rs of the pairs 6-/9- and 9-/11-18:1, against the volume parts of the varied solvent.

In hexane-based phases, R_s remained practically constant over a quite large interval of dichloromethane proportions with almost equally poor resolution both at the lowest and at the highest contents of this solvent (Figure 4, A-C). Naphthacyl derivatives, only, were fully resolved with this solvent system. Maximal resolution ($Rs_{6\text{-}/9\text{-}18:1} = 1.7$ and $Rs_{9\text{-}/11\text{-}18:1}=1.3$) was achieved at 30 and 20 volume parts of dichloromethane, respectively. Naphthylmethyl and anthrylmethyl derivatives were only partially resolved with the pairs 9-/11-18:1 having higher Rs values than 6-/9-18:1.

Dichloromethane-based phases provided much better resolution of naphthacyl-, and also full resolution of anthrylmethyl derivatives while the resolution of naphthylmethyl derivatives only remained partial (Figure 5, A-C). All three graphs had similar patterns with the resolution decreasing when the content of acetonitrile in the mobile phase was increased. The naphthacyl and anthrylmethyl esters of 6-/9-18:1 were better resolved than the respective 9-/11-18:1 derivatives.

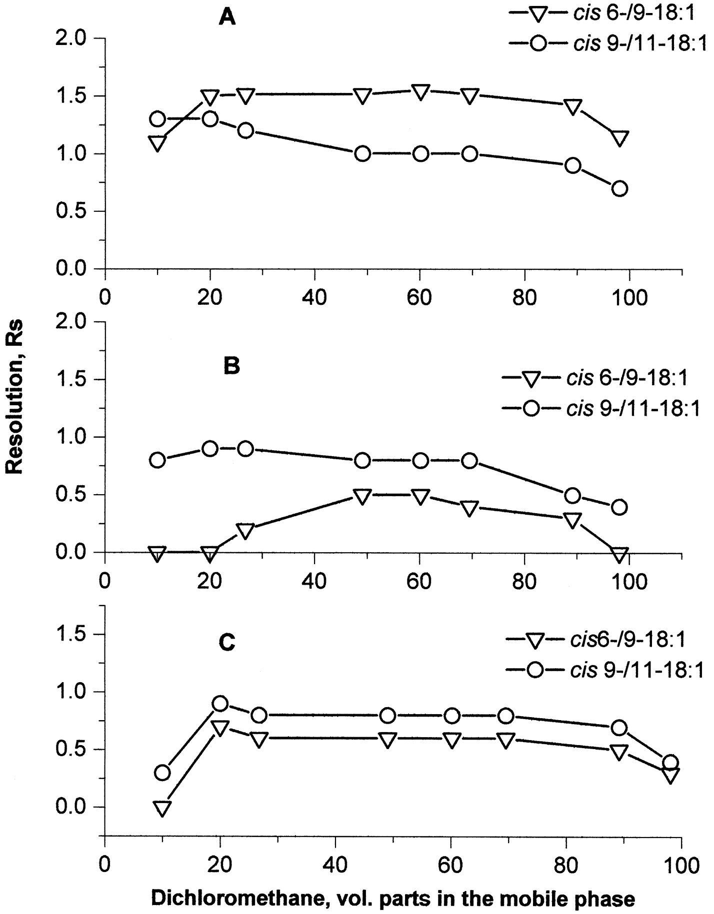

Figure 4. Effect of dichloromethane content on the resolution of *cis*-11-, *cis*-9- and *cis*-6-18:1with mobile phase hexane-dichloromethane:acetonitrile, (100-X):X:0.2, v/v/v. A, Naphthacyl-; B, Naphthylmethyl-; C, Anthrylmethyl esters. Column, flow rate and temperature as in Fig. 2.

So far, the mobile phase effects confirmed the observation made previously,[6] that the resolution of isomeric cis octadecenoates by Ag-HPLC is substantially improved when using dichloromethane-based mobile phases with low (less than 0.1 volume part) content of acetonitrile. Thus, the positive effect of derivatization with aromatic compounds is enhanced by the mobile phase composition.

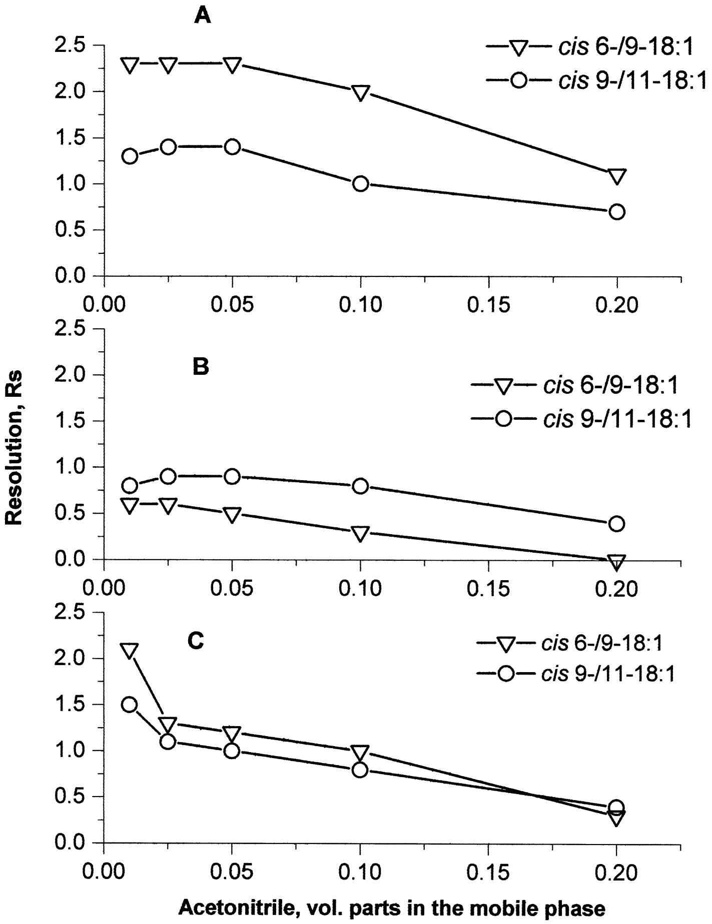

Figure 5. Effect of acetonitrile content on the resolution of *cis*-11-, *cis*-9- and *cis*-6-18:1with mobile phase dichloromethane-acetonitrile, 100:X, v/v. A, Naphthacyl-; B, Naphthylmethyl-; C, Anthrylmethyl esters. Column, flow rate and temperature as in Fig. 2.

CONCLUSION

The increased number of aromatic nuclei in the ester group enhanced the resolution of positionally isomeric *cis*-octadecenoic fatty acids. Napthacyl and anthrylmethyl *cis*- octadecenoates are fully resolved and may be of interest for the practice. Anthrylmethyl derivatives have the potential to allow for the determination of minute quantities of isomeric octadecenoates by using HPLC with fluorescent detection.

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