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# Properties of *trans* isomers of eicosapentaenoic acid and docosahexaenoic acid methyl esters on cyanopropyl stationary phases

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**Abstract**

The *trans* isomers of 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA) methyl esters were prepared by isomerisation with paratoluenesulfinic acid (PTSA) in dioxane. The isomers were fractionated by silver ion liquid chromatography with baseline resolution between the isomers with different number of *trans* double bonds. The fractions were analysed by GC–MS and the gas chromatographic properties of the EPA and DHA isomers with one and two *trans* double bonds were investigated on BPX-70 and SP-2560 cyanopropyl stationary phases. Different temperature and pressure programs were applied to introduce variations in retention indices of the isomers. The retention indices of all the *trans* isomers showed a strong linear correlation to the retention indices of the equivalent all-*cis* isomer, but the slopes for corresponding linear regression lines varied with the number of *trans* double bonds in the molecule. The regression lines were used to predict optimal conditions for the separation of *trans* isomers from the corresponding all-*cis* isomers. For DHA on BPX-70, and for EPA on both columns, it was possible to find windows where isomers with one *trans* double bond can be resolved from the corresponding all-*cis* isomers with $R_s > 1.0$. In general, BPX-70 seems to have a more suitable selectivity for the analysis of these isomers than SP-2560. Two-dimensional fatty acid retention indices (2D-FARI) were found to be suitable for identification of *trans* geometry in polyunsaturated fatty acids (PUFA). Although there were substantial overlaps in the range of retention times between the all-*cis* isomers and isomers with one and two *trans* double bonds, 2D-FARI separated the isomers into distinct groups according to the number of *trans* double bonds.


*Keywords:* Gas chromatography; *Trans* fatty acids; Cyanopropyl phases; Silver ion liquid chromatography; Marine fatty acids; Two-dimensional fatty acid retention indices; Equivalent chain lengths

## 1. Introduction

The presence of *trans* fatty acids (TFA) in food is believed to have negative health effects [1], and it is therefore of interest to be able to determine their levels both in food and biological tissues. The term '*trans* fatty acids' covers a wide range of fatty acids with large variations in structure and properties. TFA in food has three major sources, partial hydrogenation of fats, high-temperature processing of edible oils, and the natural occurrence of TFA in ruminant meat and diary products. TFA in ruminant and hydrogenated fats are mainly monoenes and dienes [2,3].

*Trans* geometry can also be introduced in polyunsaturated fatty acids (PUFA) by thermal isomerisation occurring in high-temperature processes, such as deodorization [4]. Thermal isomerisation is almost exclusively geometrical isomerisation, leading to isomers with the double bonds in the same position as in the original fatty acid [4]. Research in this field has mainly focused on isomerisation of alpha linolenic acid (ALA) 18:3 n-3, which has been extensively studied together with the spectroscopic and chromatographic properties of the isomers formed [4–10]. Although isomerisation has been discovered in heated fish oil esters [11], less work have been performed on the highly unsaturated fatty acids present in fish oil and other marine lipids, where the two most important PUFA are eicosapentaenoic acid (EPA), 20:5 n-3, and docosahexaenoic acid (DHA), 22:6 n-3. EPA and DHA are more unsaturated than ALA, and may therefore be more vulnerable to thermal isomerisation.

Highly polar cyanopropyl phases are today dominating in the GC analysis of *trans* fatty acids, basically because the *cis–trans* selectivity has been reported to be good [12–14]. For monoenes and dienes, *trans* isomers elute well ahead of the corresponding

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*cis* isomers [14], but the resolution pattern may be more complicated with isomers of PUFA, especially where the *trans* bonds are in positions far from the methyl end of the carbon chain [8,10,15].

Another feature with cyanopropyl columns is that the polarity of the column shows a stronger dependence on temperature than observed for other common stationary phases [13,16]. By combining information from several temperature programs, this shift in polarity can be utilised for identification of fatty acid structure, including the double bond geometry [17]. The polarity shifts also give large flexibility when optimal elution patterns are sought, a feature that is useful for solving difficult resolution problems [5].

The present work deals with the chromatographic properties of *trans* isomers of EPA and DHA analysed on cyanopropyl columns. The focus is mainly on the isomers with one *trans* double bond, which are the isomers most likely to be formed by thermal processing, but data for isomers with two *trans* double bonds are also given. EPA and DHA were isomerised and fractionated by silver ion LC (Ag-LC). The fractions were analysed by GC under varying chromatographic conditions and the retention data was applied to predict optimal resolution windows for the analysis of EPA and DHA *trans* isomers.

## 2. Methods

### 2.1. Materials

Fatty acid methyl esters (FAME) of EPA and DHA (>99% pure) were purchased from Nu-Chek Prep (Elysian, MN, USA). The fatty acid methyl esters were isomerised by heating 5 mg of the all-*cis* isomer with 5 mg paratoluenesulfinic acid (PTSA) in 1 mL dioxane for 1 h at 60 °C (10 mg PTSA in 1 mL dioxane was applied to produce the all-*trans* isomers). The isomerisation was terminated by the addition of 1 mL 1 M NaOH, and the isomers were extracted by 1 mL isooctane. Further details about the isomerisation procedure are given elsewhere [10,18].

### 2.2. Fractionation

Half the extract (500 μL) was injected onto an LC system equipped with a 4.6 mm × 250 mm silver ion column (ChromSpher 5 Lipids, Varian, Middelburg, The Netherlands), a fraction collector and a light scattering detector. The solvent flow was 1.5 mL/min and the following gradient program was applied: solvent A: hexane; solvent B: acetone; solvent C: 10% acetonitrile 90% acetone. 0–5 min: 100% solvent A, 5–9 min: gradient 100% A to 80% A/20% B, 9–10 min gradient 80% A/20% B to 100% B, 10–30 min: gradient 100% B to 100% C, 30–46 min: 100% C. Additional details about the chromatographic system can be found elsewhere [19]. The LC peaks with isomers containing 0–2 *trans* double bonds were divided into fractions with 0.5 min intervals. Fractions containing all isomers with the same number of double bonds were also collected manually (Fig. 1).

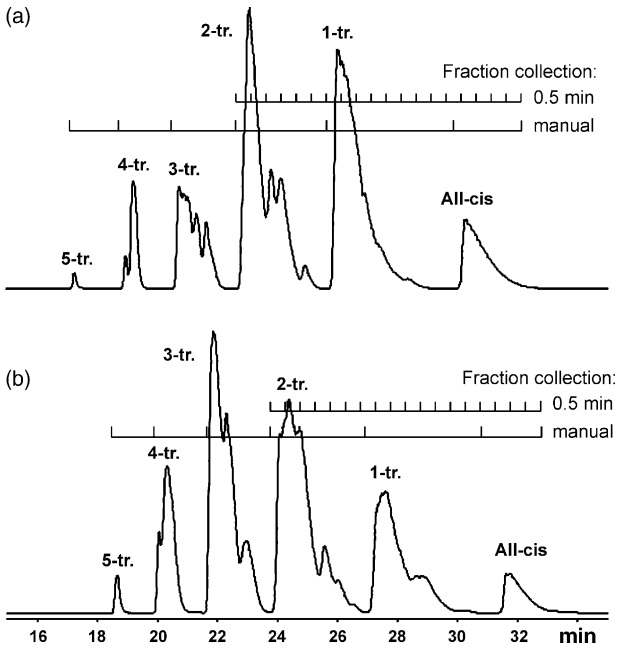


Fig. 1. Silver ion LC chromatogram and fractionation pattern of isomerised EPA (a) and DHA (b) on a ChromSpher 5 Lipids column. Linear gradients of acetone and acetonitrile in hexane were applied as mobile phase (1.5 mL/min). The fractions were collected manually or every 0.5 min. Further details are given in Section 2.2.

### 2.3. Gas chromatography

The LC fractions were analysed on an HP-5890 GC equipped with split/splitless injector, electronic pressure control, HP-7673A autosampler, HP-5972 MS detector, and G1034C MS Chemstation software. BPX-70, $L = 70$ m, I.D. = 0.25 mm, $d_f = 0.25$ μm (SGE, Ringwood Australia), and SP-2560, $L = 100$ m, I.D. = 0.25 mm, $d_f = 0.20$ μm (Supelco, Bellefonte, PA, USA) were used as analytical columns. Helium, 99.996% was used as carrier gas.

Different temperature and pressure programs were applied, five for BPX-70 and three for SP-2560. The samples (0.5 μL) were injected at an oven temperature of 60 °C that was held for 4 min. The temperature was increased by 30 °C/min to start temperature *A*, followed by a gradient of *B* °C/min until the last compound was eluted. The injector pressure was increased with the oven temperature to give a constant velocity of *C* cm/s. The levels of the parameters *A*, *B* and *C* are given in Table 1. The numbering in Table 1 is equal to the numbering used in Ref. [20], where further details can be found (Programs 6 and 7 were not used in this study). Injections were performed in splitless mode. The split valve was opened after 4 min. Injector temperature was 250 °C and MS transfer line temperature was 270 °C. The mass spectrometer was used in SIM mode where ions of 55, 74, 79, 80, 91 and 93 amu were recorded; additional details are given elsewhere [19,20].

### 2.4. Retention indices

For the analysis of FAME, equivalent chain length (ECL) is usually the preferred retention index system. The saturated