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Trans Fatty Acid Analyses in Samples of Marine Origin: The Risk of False Positives

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ABSTRACT: At conditions commonly applied for *trans* fatty analyses by gas chromatography, fatty acids naturally occurring in marine lipids may overlap chromatographically with C16 and C18 *trans* fatty acids and lead to false positives. Elution patterns were studied by tracking retention indices at shifting temperature conditions on two cyanopropyl-coated capillary columns. Most overlaps can be avoided by selecting the right chromatographic conditions, but it was not possible to find a single condition that eliminates the risk of overlap between *trans* fatty acids and interferents. In total, 17 compounds were identified as potential interferents, and the amounts of these compounds were quantified in various samples of marine origin. The interferents that will most likely contribute to incorrect assessments of *trans* fatty acids in marine lipids are probably 18:3 *n*−4 and 18:1 *n*−11.

KEYWORDS: *trans* fatty acids, marine lipids, interferents

INTRODUCTION

Trans fatty acids (TFA) can be defined as all fatty acids carrying at least one C−C double bond with *trans* configuration, also referred to as the *E*-configuration. There are three important sources for TFA in food: partially hydrogenated fats,[1,2] high-temperature processing of edible oils,[3−5] and the natural occurrence of TFA in ruminant meat and dairy products.[6,7] High intake of TFA has basically been associated with increased risk of coronary heart disease, but there are also other possible negative health effects, such as altered lipid and prostaglandin metabolism and increased risk of insulin resistance.[8−10]

Because of the negative health effects, maximum allowable amounts and labeling of TFA are regulated in several countries. Danish and Swiss regulations set a maximum allowable limit for industrially produced TFA to 2% of the fat content in food products.[11,12] The United States has regulations stating that TFA should be declared for products containing >0.5 g of TFA per serving.[12] Similar rules for labeling also exist in Malaysia[12] and Canada.[13]

These regulations require adequate methods for estimating the TFA content in a broad range of food matrices. A good overview of methodology for TFA in foods is given in a recent review.[14] TFA are usually analyzed by gas chromatography (GC) with flame ionization detection (GC-FID), infrared spectroscopy (IR), liquid chromatography (LC), or combinations of these techniques. Because GC is the only routine method that is able to quantify TFA levels below 5%, it is usually the preferred choice when foods are assessed in light of the regulations.

There are several official methods and recommendations for analyses of TFA on GC-FID; the most common are the AOCS methods Ce 1h-05,[15] Ce 1f-96,[15] and Ce 1c-89[15] and ISO 15304.[16] In addition, examples of chromatographic resolution and recommended conditions are given in AOAC 994.15,[17] which is a combined GC and IR method. Recommended retention patterns are identical in AOCS Ce 1f-96 and ISO 15304. For simplicity, these two methods will therefore be referred to as Ce 1f-96.

Except for Ce 1h-05, the official methods state specifically that they are intended for analyses of hydrogenated or refined vegetable oils or, in the case of AOAC 994.15, fats from terrestrial animals with >5% TFA. Ce 1h-05 has broader application than the other methods, but it is stated in the scope that it is not suitable for ruminant or marine fats. There exists no official methodology that claims to be suitable for a broader range of sample types. Because of the requirements to state the amount of TFA in all varieties of food samples intended for human consumption, the recommendations given in these methods are applied on samples with different and more complex fatty acid profiles than the sample types for which they were originally intended. The intention of this work has been to identify and describe the chromatographic properties of potentially false positives when samples containing lipids of marine origin are analyzed for TFA at conditions similar to those specified by the official methods and recommendations.

All of the above-mentioned methods specify the use of highly polar GC columns with cyanopropyl (CP)-coated stationary phases. Assessments of TFA in the scientific literature are also based almost exclusively on these phases, and no suitable alternative has been proposed. The polarity of the phases varies with the ratio of cyanopropyl to methyl or other groups linked to the polysiloxane backbone. In the lower end of the polarity scale used for TFA analysis are columns such as BPX-70 (SGE) and DB-23 (Agilent), whereas SP-2330 (Supelco), SP-2560 (Supelco), and CP-Sil 88 (Varian) are popular columns for TFA analyses in the upper range of the polarity scale. A special property of the CP phases is that the polarity is far more temperature dependent than for other commercial stationary phases.[18,19] The temperature-dependent polarity offers great flexibility in tuning of the retention patterns, and overlapping

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Table 1. Summary of Chromatographic Conditions Applied in the ECL Tracking Experiments

program	column[a]	start temp (°C)	temp gradient (°C/min)	max temp (°C)	carrier velocity (cm/s)	total program time (min)	t_R 18:3 $n-3$	est SN	ECL 16:1 $n-7$	ECL 18:1 $n-9$	ECL 18:2 $n-6$	ECL 18:3 $n-3$
1	BPX-70	140	1.5	220	26.0	60.0	36.05	23.4	16.43	18.35	18.99	19.77
2	BPX-70	160	1.5	220	26.0	47.3	26.38	21.6	16.47	18.39	19.05	19.85
3	BPX-70	180	1.5	220	26.0	34.7	19.69	17.9	16.52	18.44	19.13	19.95
4	BPX-70	200	1.5	230	26.0	28.7	15.93	12.7	16.55	18.49	19.22	20.07
5	BPX-70	220	1.5	240	26.0	22.7	14.11	9.9	16.57	18.52	19.29	20.18
6	BPX-70	240	1.5	260	26.0	23.3	13.30	7.0	16.58	18.55	19.33	20.26
7	SP-2560	120	1.5	230	24.0	79.3	55.68	23.0	16.64	18.53	19.43	20.50
8	SP-2560	140	1.5	230	24.0	66.7	43.77	23.4	16.68	18.58	19.48	20.55
9	SP-2560	160	1.5	235	24.0	57.3	33.50	21.6	16.73	18.63	19.56	20.64
10	SP-2560	180	1.5	235	24.0	44.7	25.69	18.8	16.79	18.69	19.66	20.77
11	SP-2560	200	1.5	240	24.0	35.3	20.80	15.0	16.84	18.76	19.79	20.95
12	BP-20	160	2.0	260	26.0	57.3	32.59	18.0	16.27	18.20	18.66	19.30

[a] Dimensions and additional details given under Experiemental Procedures.

peaks can often be resolved by minor adjustments of temperature or column flow. The shifts in retention patterns can also be utilized for identification purposes.[20,21] A drawback of the strong temperature dependence is that retention patterns achieved on one system may not be reproduced on another system unless the conditions are carefully optimized in each specific case. Systematic studies of how temperature shifts affect elution patterns of C18-*trans* monoenes,[22,23] *trans* isomers of EPA and DHA,[24] and interference between 18:3 $n-3$ *trans* isomers and 20:1 isomers[25] have been performed previously.

The purpose of this work was to check for possible interferents with TFA, by tracking the elution patterns of fatty acids in marine lipids under shifting chromatographic conditions. This was done by selecting two CP columns in each end of the polarity scale commonly used for analysis of TFA and varying the temperature conditions to achieve a broad range of polarities. The elution patterns were described by equivalent chain lengths (ECL).[26] The levels of the interferents were also quantified in a selection of fish filets and marine oils.

EXPERIMENTAL PROCEDURES

Samples. The GLC-409 and GLC-461 FAME reference mixtures and 16:1 $n-7$ *trans*, 18:1 $n-9$ *trans*, 18:2 $n-6$ *cis*, and 18:3 $n-3$ *cis* FAMEs were acquired from Nu-Chek Prep (Elysian, MN). *Trans* isomers of 18:2 $n-6$ and 18:3 $n-3$ FAME were prepared and isolated as described in ref 27.

Partially hydrogenated fish oils and partially hydrogenated soybean oils were from Denofa, Fredrikstad, Norway. Fish oil of herring and capelin were provided by the Nofima fish feed production facility, Bergen, Norway. Salmon oil was acquired from Biomega AS, Storebø, Norway. *Calanus* oil was acquired from Calanus AS, Tromsø, Norway. Cod liver oil, 2 different brands of krill oils, and 11 different brands of fish filets were bought on the local market. *Tetraselmis* was acquired from Microalgae AS, Vigra, Norway. *Skeletonema* was acquired from Institute of Marine Research, Bergen, Norway.

Sample Preparation. Fish filets were extracted according to the Bligh and Dyer extraction.[28] Fatty acids in the *Calanus* oil were isolated by alkaline hydrolysis of the oil followed by hexane extraction of the fatty alcohols. Fatty acid methyl esters (FAME) were prepared from approximately 20 mg of fat in methanolic NaOH followed by methanolic BF_3 according to AOCS Ce 1b-89.[15] FAME was extracted by 2 × 1 mL isooctane and diluted to appropriate concentrations for GC.

Monounsaturated fatty acids in hydrogenated fats were fractionated into *cis* and *trans* isomers by silver ion LC on a Chromspher 5 Lipids column, 4.6 mm × 250 mm (Varian, Middelburg, The Netherlands). Gradients of hexane and acetone was applied on an LC system controlled by Trilution 1.4 software and consisting of 331/332 pumps, a 302 fraction collector, and a PrepELS detector (all parts Gilson, Middleton, WI).

Tracking Experiments. All GC-MS analyses were performed on a 5890 GC equipped with split/splitless injector, electronic pressure control (EPC), 7673A autosampler, and 5972 MS detector (all parts HP/Agilent). The system was controlled by G1034C MS Chemstation software (Agilent). BPX-70, L = 70 m, i.d. = 0.25 mm, df = 0.25 μm (SGE, Ringwood Australia), SP-2560, L = 100 m, i.d. = 0.25 mm, df = 0.20 μm (Supelco, Bellefonte, PA), and BP-20 (L = 60 m, i.d. = 0.22 mm, df = 0.25 μm (SGE) were used as analytical columns. Helium, 99.996%, was used as carrier gas.

The sample (1 μL) was injected splitless, and the split valve was opened after 4 min. The following temperature and flow conditions were applied. Injection was made at 60 °C, at which the oven temperature was held for 4 min; thereafter, a temperature gradient of 30 °C/min to A°C followed by a temperature gradient of B°C/min was used until the last compound had eluted. Different polarities of the CP columns were achieved by varying A in 20 °C increments from 140 to 240 °C on BPX-70 and from 120 to 200 °C on SP-2560. Further details about the different programs are given in Table 1. The carrier gas pressure was adjusted with temperature to give an estimated average carrier gas velocity of 24−26 cm/s. Injector temperature was 250 °C, and MS transfer line temperature was 260 °C. The MS detector was used in selected ion monitoring (SIM) mode where ions of m/z 55, 74, 79, 80, 91, and 93 were scanned with a sampling frequency of 3.5 scans/s. The selection of ions was based on previous results.[29]

On the basis of an initial screening of fatty acid composition, one sample from each of the following was selected for tracking of ECL values under different conditions: fractionated and unfractionated partially hydrogenated fish oil and vegetable oil, *Tetraselmis*, *Skeletonema*, cod liver oil, capelin oil, salmon oil, and *Calanus* oil, and filets of trout, cod, and halibut. In addition, all *trans* reference compounds and GLC-461 were analyzed with the same conditions. GLC-409, which contains only saturated FAMEs, was used to establish relationships between retention times and retention indices and was run for every 10th sample.

Peaks were identified by analyzing the samples by GC-MS in full-scan mode and a fingerprint scan mode as described in ref 29. GC conditions were identical to program 2 in Table 1, except that the temperature gradient was 2 °C/min. Spectra and ECL values were matched with an

in-house database of marine fatty acids and *trans* fatty acids acquired under the same conditions. These conditions have also been used to identify FAME in previous works on *trans* fatty acids and marine fatty acids.[20,21,24,30,31] Calculation of ECL values and compound identification was performed in an in-house written program, Q (05−10), running under Matlab 6.5 (Mathworks, Natick, MA).

Quantitative Analyses. Quantitative analyses were performed by GC-FID on a Thermo Trace gas chromatograph at conditions described as program 1 (BPX-70) and program 12 (BP-20) in Table 1. Injector and detector temperatures were 250 and 260 °C, respectively. The detector response was frequently monitored using GLC-793 FAME mixture. Response factors (sensitivity of single FAMEs divided by sensitivity of 18:0 FAME) varied from 0.87 to 1.01, and relative standard deviation of response factors varied from 0.1 to 2.1%. All samples were analyzed in two replicates.

Nomenclature. In general, the shorthand notation *a*:*b* *n*−*c* described in ref 32 is used to designate the number of carbons (*a*), the number of double bonds (*b*), and the position of the first double bond relative to the methyl end of the carbon chain (*c*). This is followed by signification of the double-bond geometry. When there are more than one double bond, the geometry are given by letters *c* and *t* for *cis* and *trans*, respectively, where the first letter describes the geometry of the double bond closest to the carbonyl group. If double-bond geometry is not specified, the fatty acid is the *all-cis* isomer.

RESULTS AND DISCUSSION

Chromatographic Conditions. The ECL values for 18:3 *n*−3 achieved by the 11 programs on the CP phases are given in Table 1. It can be seen that the ECL for this fatty acid varies over 1.18 units and that there is only 0.24 unit between the most polar program on BPX-70 and the least polar program on SP-2560. The range spanned by each column is 0.49 for BPX-70 and 0.45 for SP-2560.

The resolution, R_s, between two chromatographic peaks (1 and 2) is given by eq 1

$$R_s = \frac{1.78(t_{R(2)} - t_{R(1)})}{w_{h(1)} + w_{h(2)}} \approx \frac{0.888(t_{R(2)} - t_{R(1)})}{w_h} \quad (1)$$

where $t_{R(1)}$ and $t_{R(2)}$ are the retention times of the two peaks and $w_{h(1)}$ and $w_{h(2)}$ are the corresponding peak widths at half peak height. Peak widths of closely eluting peaks are usually similar, and it is therefore common to use the expression to the right in eq 1, where w_h can be $w_{h(1)}$, $w_{h(2)}$, or the average peak width.[33]

In temperature-programmed GC overall separation efficiency in a region of the chromatogram can be expressed as the separation number, SN, which is roughly equal to the number of peaks that can be separated between two consecutive members of a homologous series. SN is defined according to eq 2

$$SN = \frac{t_{R(z+1)} - t_{R(z)}}{w_{h(z+1)} + w_{h(z)}} - 1 \quad (2)$$

where $t_{R(z)}$ and $t_{R(z+1)}$ are the retention times of two homologues differing in mass by one methylene unit and $w_{h(z)}$ and $w_{h(z+1)}$ are the corresponding peak widths measured at half height.

If retention and peak widths are expressed in retention index units defined according to a homologous series, eq 2 is simplified because the numerator is given by definition. It will be 1 in the case of ECL values and 100 if the Kovats scale is used. With linear temperature programming, peak widths are usually approximately constant over a wide range of homologues. One can

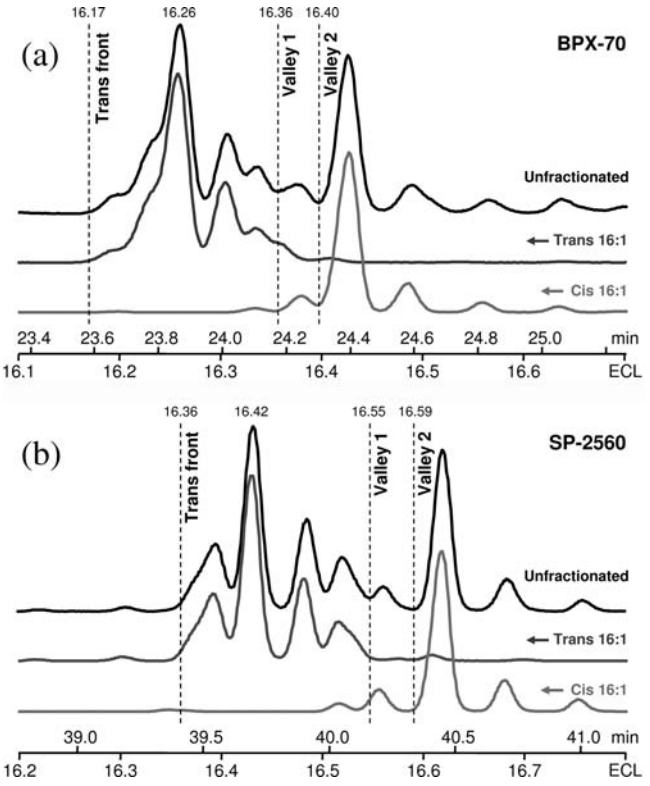

Figure 1. Elution patterns of C16 monoenes in partially hydrogenated fish oil on BPX-70, program 1 (a), and on SP-2560, program 7 (b). Upper trace shows an unfractionated sample. Middle and lower traces show, respectively, *trans* and *cis* isomers of 16:1 that were isolated by silver ion chromatography from the sample shown in the upper trace.

therefore use any peak width in the region as an estimate for w_h, and an estimate for SN can be calculated by eq 3.

$$SN \approx \frac{1}{2w_{h,ECL}} - 1 \quad (3)$$

By combining eqs 1 and 3, an expression for the difference separation in ECL units to acquire a certain chromatographic resolution can be found by

$$\Delta ECL = \frac{0.849R_s}{SN + 1} \quad (4)$$

Although peaks with R_s of 1 are not completely resolved, this resolution is usually sufficient for the quantification of two isomers of similar size. From Table 1 it can be seen that the highest separation number was slightly above 20, which is in accordance with previously reported values for similar programs on BPX-70 and BP-20.[34] Although higher separation efficiencies can be achieved using conditions that give very high elution times,[35,36] an SN of 20 is reasonably good separation efficiency for the conditions applied under routine analysis. With this separation efficiency it can be calculated by eq 4 that a difference in ECL units of 0.04 is required to achieve R_s of 1. With the poorest separation (SN = 7) a difference in ECL of around 0.11 is required to achieve R_s of 1. However, *trans* fatty acids are usually small peaks that elute near the base of the *all-cis* isomers or other major peaks, and it may therefore be necessary to have some margins on these figures.

From Table 1 it can be seen that the highest SN was achieved only with programs with the lowest polarities. A significant

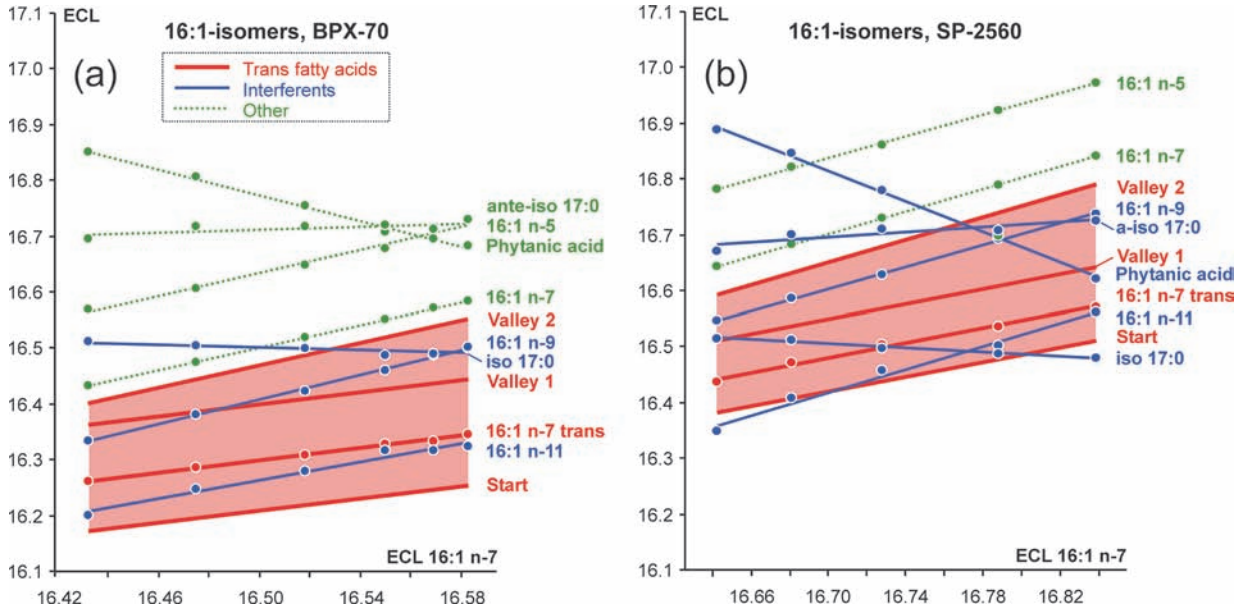

Figure 2. Overlap patterns between the 16:1 *trans* band and possible interferents on BPX-70 (a) and SP-2560 (b).

increase in the polarity can be achieved by increasing the temperature or the temperature gradient,[20,21] but both of these will decrease separation efficiency. It can be argued that the conditions leading to the lowest SNs in Table 1 will not be used in a practical analysis of *trans* fatty acids because the conditions are far from optimal.

On the basis of the above calculations, peaks separated by <0.05 ECL unit are regarded as overlapping, whereas peaks separated by 0.05−0.10 ECL unit are regarded as potentially overlapping. Unfortunately, SN or R_s values are rarely reported in method papers on *trans* fatty acids, but a rule of thumb that the difference in ECL values should be at least 0.05 unit, and ideally >0.10 unit, for sufficient peak separation seems to fit well with observations in cases when chromatograms are shown together with ECL values.[16,37−40]

Overlap Patterns for 16:1 Isomers. Chromatograms of the 16:1 region of fractionated and unfractionated partially hydrogenated fish oil on BPX-70 and SP-2560 are shown in Figure 1, panels a and b, respectively. It can be seen that there is a partial overlap between the *trans* isomers and the *cis* isomers. To quantify *trans* isomers in an unfractionated sample, one usually regards the entire region from the start of the *trans* peak to the deepest valley between the *trans* and the *cis* peak as *trans* isomers. An alternative is to use the valley in front of the major *cis* peak (16:1 *n*−7) as a reference point for quantification of the *trans* isomers. These two reference points are referred to as valley 1 and valley 2, respectively.

The chromatograms shown are acquired under the conditions that gave highest SN for the respective columns. Because the valleys vanish as separation efficiency is decreased, and because the overlap pattern between *cis* and *trans* isomers changes with temperature, it was not possible to track the ECL values of the valleys accurately under changing chromatographic conditions. It has been shown previously that shifts in polarity gives shifts in ECL values that are almost identical for isomers with the same number of *cis* and *trans* double bonds;[22−24] that is, the distances in ECL units between the isomers will remain constant. For the other programs the start and the end of the *trans* bands were therefore defined with reference to the ECL of the 16:1 *n*−7 isomers using the values given below.

The ECL of the peak maximum in the *trans* region corresponds with the ECL of the 16:1 *n*−7 *trans* isomer, which is used as reference point for the start of the *trans* region and valley 1. The distance between *trans* 16:1 *n*−7 and the start of the *trans* region was 0.09 unit on BPX-70 and 0.06 unit on SP-2560. Corresponding values for the distance to valley 1 were 0.10 and 0.07 unit. Valley 2 is defined relative to the *cis* isomer of 16:1 *n*−7. The distance between this isomer and valley 2 was 0.03 ECL unit on BPX 70 and 0.05 unit on SP-2560.

There are no strict rules for setting the border between the *trans* and *cis* isomers, and the actual definition of the end of the *trans* band will vary between methods, sometimes also with sample types. However, it is likely that a position intermediate between valleys 1 and 2 will be chosen in most cases.

Trans isomers of 16:1 are basically an issue in products containing ruminant fat[36,41] or in partially hydrogenated oils.[41] Five fatty acids were found to be potential interferents with the *trans* 16:1 region. These were three branched saturated fatty acids, together with the *cis* isomers 16:1 *n*−9 and 16:1 *n*−11.

The three branched fatty acids, *iso* 17:0 (15-methylhexadecanoic acid), *ante-iso* 17:0 (14-methylhexadecanoic acid), and phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), are common constituents in marine lipids.[42,43] The *iso* and *ante-iso* 17:0 may also be interferents in the assesment of 16:1 *trans* isomers in milk fat, in which they are abundant.[41,44]

Marine lipids also contain a broader range of positional isomers of monounsaturated fatty acids than most other sources. In addition to the main *cis* isomer in most organisms, 16:1 *n*−7, small amounts of other *cis* isomers with the double bonds in odd-numbered positions (e.g., *n*−5, *n*−9, *n*−11) can also be found. Among these, 16:1 *n*−9 and 16:1 *n*−11 elute before the *n*−7 isomer and may interfere with the *trans* band.

The ECL values of the 16:1 *trans* isomers, possible interferents, and other closely eluting peaks are plotted against the ECL of 16:1 *n*−7 in Figure 2. The linear regression equations for these relationships are given in Table 2. The results show similar

Table 2. ECL Values on the BP-20 Stationary Phase, Coefficients for Calculating ECL for Selected Fatty Acids in the 16:1 Region on BPX-70 and SP2560 Phases Using the Equation $ECL_X = a \times ECL_{16:1\ n-7} + b$, and Corresponding Correlation Coefficients, R^2

	BPX-70				SP-2560				BP-20
fatty acid	*a*	*b*	R^2	notes[a]	*a*	*b*	R^2	notes[a]	ECL
16:1 *n*−7 *trans*	0.5494	7.23	0.9967		0.6596	5.46	0.9910		16.26
16:1 *trans* front	0.5494	7.14	0.9967		0.6596	5.40	0.9910		16.21
16:1 *trans* valley 1	0.5494	7.33	0.9967		0.6596	5.53	0.9910		
16:1 *trans* valley 2	1.0000	−0.03	1.0000		1.0000	−0.05	1.0000		
iso 17:0	−0.1219	18.51	0.6133	a	−0.1884	19.65	0.9719	b	16.52
ante-iso 17:0	0.1188	14.75	0.4584		0.2273	12.90	0.7706	b	16.67
phytanic acid	−1.1508	35.76	0.9946		−1.3698	39.69	0.9982	b	16.93
16:1 *n*−11	0.8312	2.55	0.9766	a	0.9546	0.48	0.9835	b	16.13
16:1 *n*−9	1.0969	−1.69	0.9981	a	0.9499	0.74	0.9983	b	16.20
16:1 *n*−7	1.0000	0.00	1.0000	c	1.0000	0.00	1.0000	c	16.27
16:1 *n*−5	1.0421	−0.56	0.9900		0.9563	0.87	0.9994		16.39

[a] a, potential interferent on BPX-70; b, potential interferent on SP-2560; c, ECL or regression line given by definition.

patterns as reported elsewhere.[22−24] Fatty acids with the same number of *cis* and *trans* double bonds have parallel slopes, and *trans* double bonds contribute to lower shifts in ECL than *cis* double bonds. The highly branched phytanic acid has negative slopes, whereas the *iso* and *ante-iso* have slopes close to 0.

It can be seen that 16:1 *n*−9, 16:1 *n*−11, and *iso* 17:0 are possible interferents on the BPX-70 column. *Iso* 17:0 is only a problem with the highest temperatures, whereas 16:1 *n*−9 and *n*−11 are always within the *trans* region. On SP-2560, phytanic acid and *ante-iso* 17:0 are also possible interferents. The most problematic peaks seems to be 16:1 *n*−11 on BPX-70 and *iso* 17:0 on SP-2560, because these are <0.1 ECL unit from 16:1 *n*−7 *trans*, which is the main 16:1 *trans* isomer in ruminant fats.[36]

Overlap Patterns for 18:1 Isomers. Chromatograms of the 18:1 region of the fractionated and unfractionated partially hydrogenated fish oil on BPX-70 and SP-2560 are shown in Figure 3, panels a and b, respectively. The overlap between *cis* and *trans* isomers is more severe for the 18:1 isomers than for the 16:1 isomers. Also for 18:1 isomers, there is no general rule on where to separate between the *trans* and *cis* isomers, and the patterns vary between columns and with temperature conditions.[15,22,23] However, it seems to be generally recommended to use the valley immediately before the main 18:1 *cis* peak, which is basically 18:1 *n*−9.[15−17,45] This approach is marked as valley 2 in Figure 3. An alternative approach would be to use the deepest valley between the main *trans* peak and the main *cis* peak, which is marked as valley 1 in Figure 3. However, this would lead to a substantial loss of late-eluting *trans* isomers. Also for the 18:1 isomers, valleys 1 and 2 were defined relative to the main *trans* isomer (18:1 *n*−9 *trans*) and the main *cis* isomer (18:1 *n*−9 *cis*), respectively. On both columns the start of the *trans* band was 0.05 ECL unit before 18:1 *n*−9 *trans*, valley 1 was 0.08 unit after 18:1 *n*−9 *trans*, and valley 2 was 0.03 unit before 18:1 *n*−9.

The overlap patterns for the 18:1 region are shown in Figure 4. On BPX-70 (Figure 4a) it can be seen that 18:1 *n*−11, 16:4 *n*−3, and 16:4 *n*−1 are the potential interferents. 18:1 *n*−11, which is a common minor fatty acid in marine lipids, will always elute just in front of 18:1 *n*−9. Thus, this fatty acid will always be an interferent as long as the border between *trans* and *cis* isomers is defined according to valley 2. The intersection between 16:4 *n*−3 and the *trans* band occurs only at medium to high polarities.

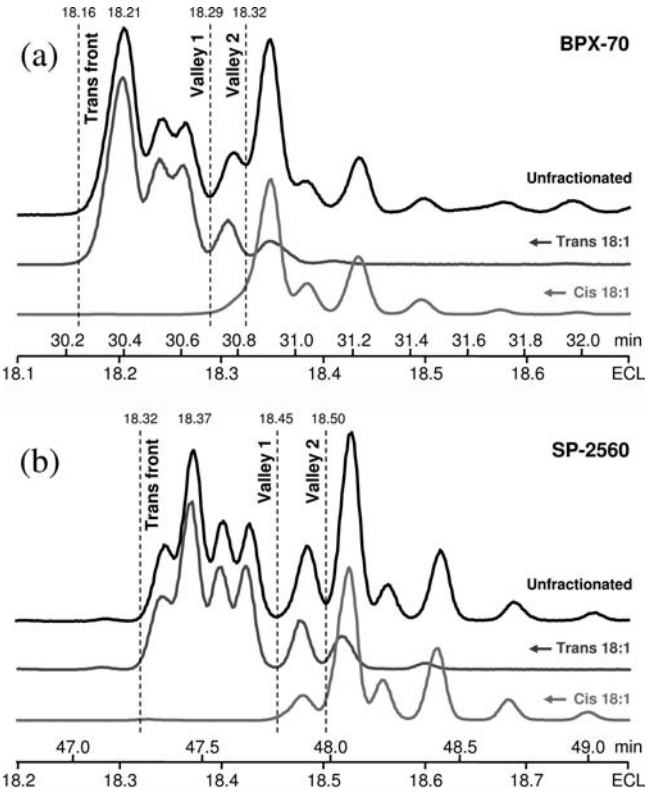

Figure 3. Elution patterns of C18 monoenes in partially hydrogenated fish oil on BPX-70, prorgram 1 (a), and SP-2560, program 7 (b). Upper trace shows an unfractionated sample. Middle and lower traces show, respectively, *trans* and *cis* isomers of 18:1 that were isolated by silver ion chromatography from the sample shown in the upper trace.

It should therefore be possible to avoid this overlap by using low column temperatures. However, at too low temperatures, there may be interference between 16:4 *n*−1 and the upper range of the *trans* band, and 16:4 *n*−1 is usually more abundant in marine lipids than 16:4 *n*−3 (see Quantitative Analyses). Still, it may be possible to avoid both of these overlaps using the conditions from program 2 or using slightly higher temperatures. Following the recommendations in AOCS Ce 1f-96[15] and ISO 15304[16] 18:1

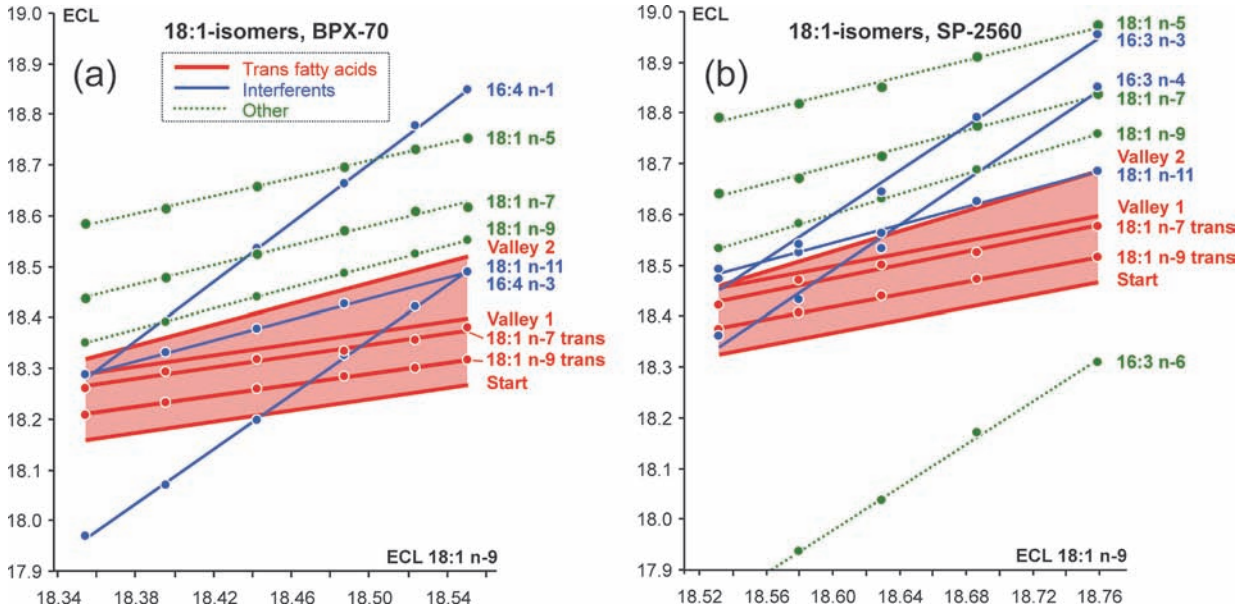

Figure 4. Overlap patterns between the 18:1 *trans* band and possible interferents on BPX-70 (a) and SP-2560 (b).

Table 3. ECL Values on the BP-20 Stationary Phase, Coefficients for Calculating ECL for Selected Fatty Acids in the 18:1 Region on BPX-70 and SP2560 Phases Using the Equation $ECL_X = a \times ECL_{18:1\ n-9} + b$, and Corresponding Correlation Coefficients, R^2

	BPX-70				SP-2560				BP-20
fatty acid	*a*	*b*	R^2	notes[a]	*a*	*b*	R^2	note[a]	ECL
18:1 *n*−9 *trans*	0.5368	8.36	0.9987		0.6204	6.88	0.9993		18.22
18:1 *n*−7 *trans*	0.5500	8.17	0.9870		0.6494	6.40	0.9828		18.27
18:1 *trans* front	0.5368	8.31	0.9987		0.6204	6.83	0.9993		18.18
18:1 *trans* valley 1	0.5368	8.44	0.9987		0.6204	6.96	0.9993		
18:1 *trans* valley 2	1.0000	−0.03	1.0000		1.0000	−0.08	1.0000		
18:1 *n*−11	1.0001	0.06	0.9998	a	0.8789	2.20	0.9943	b	18.16
18:1 *n*−9	1.0000	0.00	1.0000	c	1.0000	0.00	1.0000	c	18.20
18:1 *n*−7	0.9219	1.52	0.9946		0.8838	2.26	0.9944		18.27
18:1 *n*−5	0.8439	3.10	0.9990		0.8158	3.66	0.9884		18.40
16:3 *n*−6	1.5103	−10.75	0.9981		2.1158	−21.38	0.9982		16.94
16:3 *n*−4	2.2033	−22.80	0.9994		2.2173	−22.75	0.9910	b	17.18
16:3 *n*−3	2.2210	−23.03	0.9994		2.1820	−21.99	0.9918	b	17.29
16:4 *n*−3	2.6120	−29.96	0.9994	a	2.4753	−26.96	0.9893		17.58
16:4 *n*−1	2.8197	−33.46	0.9991	a	2.6262	−29.40	0.9879		17.74

[a] a, potential interferent on BPX-70; b, potential interferent on SP-2560; c, regression line given by definition.

n−9 should have an ECL of approximately 18.46. Under these conditions 16:4 *n*−3 will coelute with the main 18:1 *trans* peak. Although variations can be found, the recommendations given in the reference methods seem to be followed quite closely, with column temperatures around 190 °C.[46−48]

On SP-2560 (Figure 4b) 16:3 *n*−4 and 16:3 *n*−3 were found to be possible interferents, in addition to 18:1 *n*−11. Of the two 16:3 fatty acids, the *n*−4 isomer is probably the largest problem. The 16:3 *n*−3 interferes only at low temperatures, whereas 16:3 *n*−4 interferes at low to moderate temperatures. SP-2560 and CP-Sil 88 are highly similar phases in the upper end of the polarity scale for cyanopropyl columns.[45,49] When the equation given in Table 3 was applied on ECL data given in AOCS Ce 1f-96,[15] the errors for 18:1 *n*−9 *trans* and 18:1 *n*−7 *trans* were 0.01 ECL unit or less. It can therefore be expected that the pattern shown in Figure 4b is also valid for CP-Sil 88 and similar phases. The recommended conditions for the highly polar columns in Ce 1f-96 have ECL values of 18.66 (CP-Sil 88) and 18.68 (SP-2340) for 18:1 *n*−9. Under these conditions 16:3 *n*−4 and 18:1 *n*−11 are interferents in the region around valley 2.

Overlap Patterns for 18:2 *n*−6 Isomers. *Trans* isomers of 18:2 *n*−6 are formed by high-temperature refining of edible oils.[5,50,51] In these processes there is minimal double-bond migration, and the number of isomers formed is much lower than by partial hydrogenation. One will therefore usually quantify individual peaks, instead of unresolved clusters as with the *trans* monoenes. The overlap pattern of *trans* isomers of 18:2 *n*−6 is shown in Figure 5.

On BPX-70 19:0 is a possible interferent at moderate temperatures, whereas it coelutes with 18:2 *n*−6 at low temperatures.

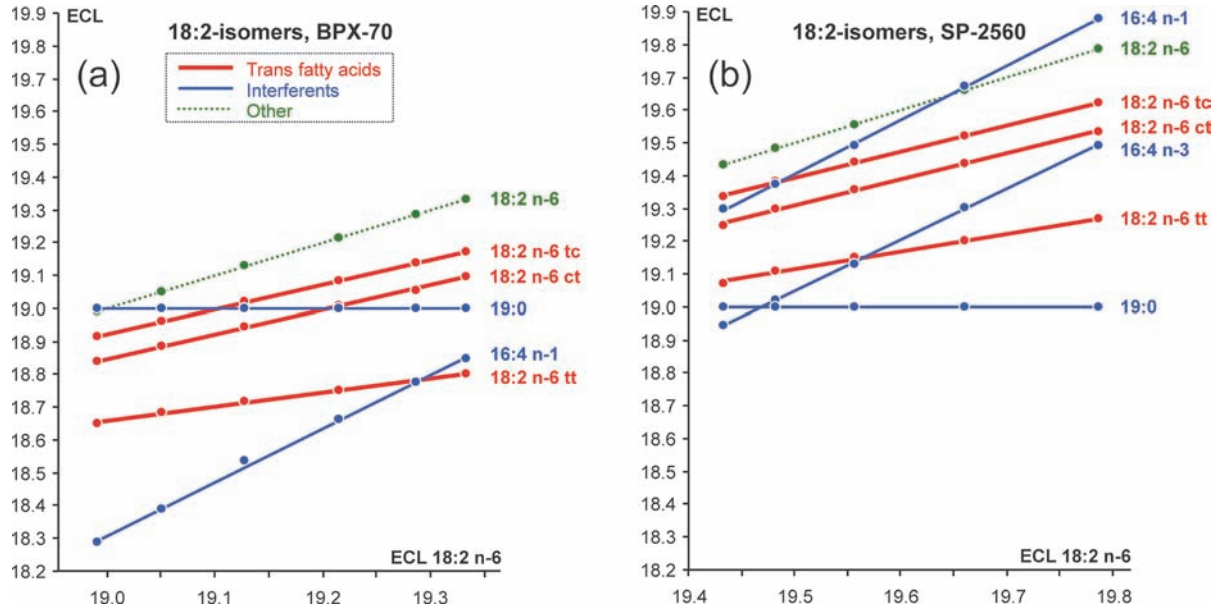

Figure 5. Overlap patterns between the 18:2 *n*−6 isomers and possible interferents on BPX-70 (a) and SP-2560 (b).

Table 4. ECL Values on the BP-20 Stationary Phase, Coefficients for Calculating ECL for Selected Fatty Acids in the 18:2 Region on BPX-70 and SP2560 Phases Using the Equation $ECL_X = a \times ECL_{18:2\ n-6} + b$, and Corresponding Correlation Coefficients, R^2

	BPX-70				SP-2560				BP-20
fatty acid	*a*	*b*	R^2	notes[a]	*a*	*b*	R^2	notes[a]	ECL
18:2 *n*−6 *tt*	0.4230	10.62	0.9969		0.5432	8.52	0.9958		18.71
18:2 *n*−6 *ct*	0.7433	4.72	0.9993		0.8011	3.69	0.9991		18.71
18:2 *n*−6 *tc*	0.7497	4.68	0.9998		0.8032	3.73	0.9993		18.79
18:2 *n*−6	1.0000	0.00	1.0000	a	1.0000	0.00	1.0000	a	18.66
19:0	0.0000	19.00		a, b	0.0000	19.00		a, c	19.00
16:4 *n*−3	1.5133	−10.76	0.9982	b	1.5610	−11.39	0.9995	c	17.58
16:4 *n*−1	1.6341	−12.74	0.9985		1.6574	−12.92	0.9996	c	17.74

[a] a, ECL or regression line given by definition; b, potential interferent on BPX-70; c, potential interferent on SP-2560.

This fatty acid may be present in trace amounts in marine lipids. 16:4 *n*−1 is a possible interferent with 18:2 *n*−6 *tt*, but only at the highest temperatures. Thus, interferents can probably be avoided by using BPX-70 at low temperatures. When the equation given in Table 4 was used for calculating the ECL values for the 18:2 isomers given in Ce 1f-96, the deviations between the calculated values and values reported in the methods varied from 0.01 to 0.03 ECL unit. These methods specify an ECL of the *all-cis* isomer of 19.14, which will lead to coelution between 19:0 and the 18:2 *n*−6 *ct* and *tc* isomers if present.

Three fatty acids, 19:0, 16:4 *n*−3, and 16:4 *n*−1, were found to be possible interferents on SP-2560. On this column it seems that the best conditions may be to select a polarity that is slightly above the one achieved with program 10, with which the ECL of 18:2 *n*−6 is 19.70. Under these conditions 16:4 *n*−3 and 18:2 *n*−6 *tt* may be resolved with an ECL of 0.10 or more, and 16:4 *n*−1 is well resolved from 18:2 *n*−6 *tc*. The recommended conditions for CP-Sil 88 in AOCS Ce 1f-96 has an ECL of 18:2 *n*−6 of 19.63, which is close to this region. When the equation given for SP-2560 in Table 4 was used for calculating the ECL values for the 18:2 isomers given for CP-Sil-88 in Ce 1f-96, the errors varied from 0.01 to 0.02 ECL unit.

Overlap Patterns for 18:3 Isomers. Similar to the 18:2 *n*−6 isomers, *trans* isomers of 18:3 *n*−3 are formed by geometrical isomerization in high-temperature processing of edible oils.[3−5,50−52] The *cct* and *tcc* isomers are usually formed in largest amounts, followed by the *tct* and *ctc* isomers; other isomers are usually absent or seen only in trace amounts.[4,45,50]

The overlap patterns of 18:3 *n*−3 *trans* isomers are shown in Figure 6. On BPX-70 there are three possible interferents, 20:0, 18:3 *n*−6, and 18:3 *n*−4. Whereas 20:0 is only a problem at moderate and high polarities, the two latter show overlaps in the entire region. Of these 18:3 *n*−4 is the most problematic compound because it will always elute just in front of 18:3 *n*−3 and overlap with the important 18:3 *n*−3 *tcc* isomer. Conditions that give an ECL for 18:3 *n*−3 of 19.93 are recommended in Ce 1f-96. At these conditions both 18:3 *n*−6 and 18:3 *n*−4 overlap with *trans* isomers of 18:3 *n*−3.

Also, on SP-2560 (Figure 6b) the overlaps between 18:3 *n*−6 and 18:3 *n*−4 are problematic, but 20:0 interferes only at the lowest temperatures. However, the polarity of this column is so high that 20:1 fatty acids may interfere with *trans* isomers of 18:3 *n*−3, except at the lowest polarities. The possible overlap between 20:1 *n*−9 and 18:3 *n*−3 *tcc* in vegetable oils is well-

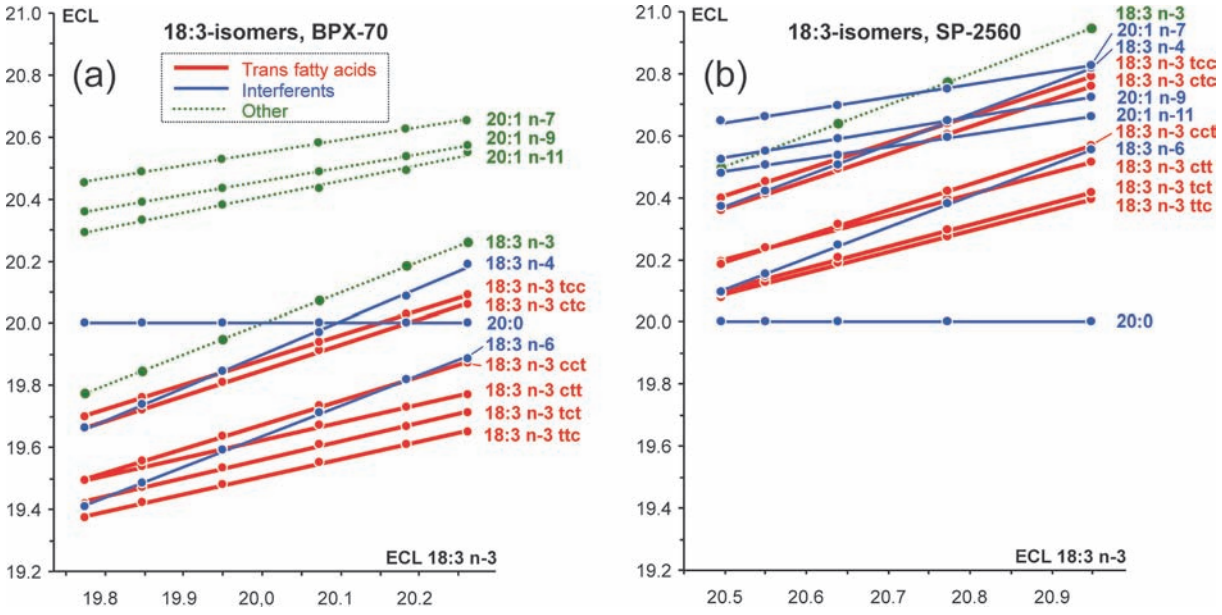

Figure 6. Overlap patterns between the 18:3 *n*−3 isomers and possible interferents on BPX-70 (a) and SP-2560 (b).

Table 5. ECL Values on the BP-20 Stationary Phase, Coefficients for Calculating ECL for Selected Fatty Acids in the 18:3 Region on BPX-70 and SP2560 Phases Using the Equation $ECL_X = a \times ECL_{18:3\ n-3} + b$, and Corresponding Correlation Coefficients, R^2

	BPX-70				SP-2560				BP-20
fatty acid	*a*	*b*	R^2	notes[a]	*a*	*b*	R^2	notes[a]	ECL
18:3 *n*−3 *ctt*	0.5638	8.23	0.9988		0.6841	6.06	0.9970		19.33
18:3 *n*−3 *tct*	0.5977	7.61	0.9989		0.6999	5.76	0.9976		19.37
18:3 *n*−3 *ttc*	0.5789	8.05	0.9987		0.6953	5.95	0.9982		19.45
18:3 *n*−3 *cct*	0.7769	4.14	0.9995		0.8414	2.94	0.9994		19.25
18:3 *n*−3 *ctc*	0.8220	3.41	0.9995		0.8802	2.32	0.9996		19.44
18:3 *n*−3 *tcc*	0.7982	3.92	0.9998		0.8545	0.89	0.9996		19.44
18:3 *n*−6	0.9820	0.00	0.9992	a	1.0098	−0.60	0.9999	b	18.96
18:3 *n*−4	1.0623	−1.34	0.9989	a	0.9916	0.05	0.9996	b	19.17
18:3 *n*−3	1.0000	0.00	1.0000	c	1.0000	0.00	1.0000	c	19.30
20:0	0.0000	20.00		a, c	0.0000	20.00		c	20.00
20:1 *n*−11	0.5132	10.14	0.9946		0.4001	12.28	0.9979	b	20.16
20:1 *n*−9	0.4365	11.73	0.9997		0.4376	11.56	0.9999	b	20.20
20:1 *n*−7	0.4120	12.31	0.9985		0.4049	12.34	0.9971	b	20.28

[a] a, potential interferent on BPX-70. b, potential interferent on SP-2560; c, ECL or regression line given by definition.

known and discussed elsewhere.[25,45] In marine lipids 20:1 *n*−11, which elutes immediately before 20:1 *n*−9, is usually also abundant. Interference between 20:1 and 18:3 *n*−3 *trans* isomers will therefore occur over a wider range of polarities in samples containing marine lipids than in vegetable oils. At the highest polarities, where 18:3 *n*−3 *tcc* elutes after 20:1 *n*−9, interference with 20:1 *n*−7 may also occur. The 20:1 fatty acids common in marine lipids will therefore interfere with mono *trans* isomers of 18:3 *n*−3, except with the lowest polarities. However, at low polarities, both 18:3 *n*−4 and 18:3 *n*−6 interfere with important isomers of 18:3 *n*−3 (*tcc*, *ctc*, and *tct*).

Several sources recommend using conditions for CP-Sil 88 that position 20:1 *n*−9 between 18:3 *n*−3 *tcc* and *all-cis* 18:3 *n*−3.[46,47,49] This resolution pattern is also shown in the Ce 1f-96 and Ce 1h-05 methods. This pattern was achieved when the ECL value of 18:3 *n*−3 reported in Ce 1f-96 was 20.67, and from Figure 5b it can be seen that a similar polarity gave the same pattern on SP-2560. This can be an optimal solution for a vegetable oil, because it also leads to optimal resolution between 18:3 *n*−6 and the *trans* isomers of 18:3 *n*−3. However, if 20:1 *n*−11 is present, it is obvious from both Figure 6b and other sources[15,46,47,49] that it will overlap with 18:3 *n*−3 *tcc*. At the same conditions, overlap between 18:3 *n*−3 *tcc* and *ctc* isomers with 18:4 *n*−3 should also be expected.

Two alternative approaches to the resolution of *trans* 18:3 *n*−3 isomers have also been applied on CP-Sil 88 and SP-2560. One approach is to use a higher polarity, where *tcc* and *ctc* isomers elute after 20:1 *n*−9.[49] With marine lipids, this would lead to interference with 20:1 *n*−7, and it may also be difficult to achieve high column efficiency with the temperatures that are necessary for this polarity. The other approach is to use a very low polarity, where 20:0 is positioned in the space between 18:3 *cct* and *ctc*.[4,53]

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Table 6. Amounts of Interferents Found in Various Samples of Marine Origin[a]

	16:1 *n*−11	16:1 *n*−9	16:3 *n*−4	16:3 *n*−3	16:4 *n*−3	16:4 *n*−1	i-17:0	*ai*-17:0	phytanic	18:1 *n*−11	18:3 *n*−6	18:3 *n*−4	19:0	20:0	20:1 *n*−11	20:1 *n*−9	20:1 *n*−7
filets																	
cod	0.253	0.256	0.168	0.011	0.028	0.188	0.079	0.062	0.143	0.705	0.102	0.152	0.028	0.099	0.400	3.520	0.136
cod	0.205	0.228	0.066	nd[b]	nd	0.073	0.073	0.095	0.186	0.524	0.081	0.061	0.029	0.054	0.268	1.927	0.107
halibut	0.505	0.418	0.136	0.024	0.034	0.098	0.178	0.103	<0.010	2.308	0.137	0.086	0.051	0.086	1.632	7.712	0.785
halibut	0.461	0.319	0.169	0.016	<0.010	0.176	0.120	0.117	0.120	1.573	0.114	0.079	0.023	0.078	0.956	6.942	0.272
saithe	0.253	0.130	0.050	nd	nd	0.052	0.146	0.097	0.193	0.945	0.089	0.042	0.042	0.036	0.342	1.932	0.062
salmon	0.240	0.184	0.280	0.028	0.027	0.281	0.095	0.047	0.023	0.650	0.137	0.292	0.037	0.211	0.606	5.033	0.158
salmon	0.379	0.200	0.409	0.022	0.052	0.480	0.142	0.072	0.024	0.958	0.154	0.352	0.049	0.111	1.001	6.559	0.250
salmon	0.252	0.181	0.294	0.030	0.034	0.383	0.080	0.042	0.022	0.620	0.122	0.256	0.037	0.184	0.516	4.807	0.185
salmon	0.241	0.185	0.202	0.037	0.031	0.249	0.074	0.037	<0.010	0.646	0.116	0.164	0.030	0.251	0.559	4.906	0.145
salmon	0.404	0.209	0.290	nd	0.079	0.265	0.137	0.071	<0.010	1.033	0.155	0.253	0.042	0.131	0.827	6.790	0.193
trout	0.280	0.246	0.400	0.033	0.044	0.370	0.102	0.055	<0.010	0.527	0.147	0.350	0.037	0.147	0.401	3.068	0.155
oils																	
caplin	0.191	0.169	0.285	0.023	0.041	0.353	0.079	0.080	0.497	0.387	0.149	0.049	<0.01	0.097	0.619	16.841	0.455
cod liver	0.365	0.377	0.229	0.024	0.052	0.214	0.185	0.145	0.264	2.127	0.185	0.066	0.013	0.048	1.563	10.984	0.338
herring	0.428	0.172	0.210	0.026	0.092	0.496	0.115	0.067	0.019	0.504	0.125	0.035	0.031	0.151	1.273	11.146	0.181
herring	0.394	0.162	0.140	0.018	0.033	0.309	0.085	0.059	0.041	0.721	0.090	0.028	0.038	0.168	1.885	12.701	0.225
krill	0.517	0.127	0.141	0.063	0.170	0.606	0.205	0.059	1.235	<0.100	0.218	0.069	0.017	0.032	nd	0.316	0.308
krill	0.439	0.121	0.154	0.050	0.110	0.616	0.209	0.062	2.215	<0.100	0.196	0.051	0.016	0.046	0.062	0.744	0.325
salmon	0.215	0.196	0.274	0.036	0.034	0.391	0.078	0.034	0.021	0.596	0.131	0.249	0.027	0.232	0.409	4.843	0.160
note[c]	a	a	b	a	b	b	a	b	b	c	a	a	b	a	a	a	a

[a] Values are reported as weight percent relative to total fatty acids. [b] nd, not detected (<0.002%). [c] a, quantified on BPX-70; b, quantified on BP-20; c, calculated by difference: (18:1 *n*−11 + 16:4 *n*−1 on BPX-70) − (16:4 *n*−1 on BP-20).

However, it should be emphasized that this strategy was used for *trans* isomers of 18:3 *n*−3 only, and it may not be suitable for *trans* fatty acids in general. The conditions are outside the region investigated for SP-2560 in this study, but the ECL values for the mono- and di-*trans* isomers of 18:3 *n*−3 presented in ref 53 could be calculated from the equations presented in Table 5 with errors ≤0.01 ECL unit. Assuming that the other regression lines can be extrapolated with similar accuracy, it can be expected that both 18:3 *n*−4 and 20:0 will interfere with 18:3 *n*−3 *ctc*, but the risk of other overlaps should be low.

Polyethylene Glycol (PEG) Phase. PEG columns are not suitable for analysis of *trans* fatty acids because *cis* and *trans* isomers are poorly separated. However, because it has selectivity that is different from the CP phases, it can be used as a complementary column to confirm the presence of and to quantify the various interferents. PEG shows a polarity that is almost unaltered by temperature,[18] and a single temperature program was therefore used for analyses on the PEG phase. The conditions used gave an SN of 18.0, which is in accordance with previously reported values.[34]

The ECL values on the BP-20 PEG phase are reported in Tables 2−5. With regard to the interferents in the 16:1 area it can be seen from Table 2 that the branched interferents (*iso* 17:0, *ante-iso* 17:0, and phytanic acid all elute after the band of 16:1 isomers that range from 16.13 to 16.29. 16:1 *n*−7 *trans* will coelute with the *cis* isomer, and 16:1 *n*−9 and 16:1 *n*−11, which elutes in front of 16:1 *n*−7, will therefore not overlap with the *trans* isomers on this column.

With regard to interferents in the 18:1 region, the 16:3 and 16:4 isomers that interfered on the CP columns elute much earlier than the 18:1 isomers on the PEG phase. However, on this phase there is a risk that some of these isomers may coelute with C17 monoenes. Similar to the C16 MUFA, 18:1 *n*−9 and 18:1 *n*−7 *trans* both coelute with the corresponding *cis* isomers. 18:1 *n*−11, which elutes just before 18:1 *n*−9, will therefore not be identified as a *trans* isomer on this column.

The *trans* isomers of 18:2 *n*−6 elute after the *cis* isomers, and all of the possible interferents on the CP column elute far from this region on the PEG column. The majority of the 18:3 *n*−3 *trans* isomers elute after the *all-cis* isomer. However, 18:3 *n*−3 *cct* elutes before 18:3 *n*−3 and only 0.08 ECL unit from 18:3 *n*−4. Therefore, care must be taken to avoid confusion of these two fatty acids also on a PEG column. Other possible interferents on the CP columns are well resolved from 18:3 *n*−3 *trans* isomers on the PEG columns.

Quantitative Analyses. The levels of the interferents in 11 samples of pure fish filets and in 7 oils of marine origin are reported in Table 6. All products are from different species, brands, or qualities. The cod liver and krill oils are products intended as dietary supplements. The herring and capelin oils are feed grade oils typically used for salmon feed production, whereas the salmon oil is a food grade oil. Additional details are given under Samples.

Because of the many chromatographic overlaps, the samples had to be analyzed on both BPX-70 (program 1) and BP-20 (program 12) for resolution of all compounds of interest. The applied methods have a relative standard deviation of 5% or better for peaks above 1% of total fatty acids, but noise increases below this level. The limit of quantification (LOQ) was set to 0.010%, where the average difference between two replicates had increased to 20%. Only peaks above LOQ in both replicates were quantified. Compounds with levels below 0.002% in any replicate

were regarded as not detected. In the case of 18:1 $n-11$, which was calculated by subtracting the area percent of 16:4 $n-1$ on BP-20 from the area percent of the combined 16:4 $n-1$ and 18:1 $n-11$ peak on BPX-70, the relative errors can be expected to be higher than for other peaks if the fraction of 18:1 $n-11$ to 16:4 $n-1$ is low. The limit of quantification for this fatty acid was therefore set to 0.1%.

20:1 $n-9$ is by far the interferent that is present in largest amounts. However, it may not represent the largest problem for identification because this is a common fatty acid present in many FAME reference mixtures, and the overlap with 18:3 $n-3$ *trans* isomers will occur only under certain conditions. The potential overlap of these isomers has also been discussed previously.[25] If the risk of overlap is taken into consideration, 18:1 $n-11$ and 18:3 $n-4$ are probably the fatty acids that may contribute to the largest errors, because it is not possible to find conditions that will lead to proper resolution of these peaks from *trans* isomers of 18:1 and 18:3 $n-3$, respectively. These fatty acids are also present in levels that will lead to significant errors in the estimation of TFA. The sum of these two peaks was found to be above 0.5% in all samples except the krill oils, and the levels were above 2% in two samples. If *trans* isomers of 16:1 are reported, both 16:1 $n-9$ and $n-11$ will contribute to significant errors because they coelute with the region of *trans* isomers at all investigated conditions and are present above 0.1% in all samples. The overlaps with the branched fatty acids (*iso* 17:0, *ante-iso* 17:0, and phytanic acid) can be avoided, but they may contribute to significant errors in some cases. The level of phytanic acid was, for instance, above 2% in one of the krill oils.

Small amounts of *mono-trans* isomers of 18:2 $n-6$ was found in the trout and in four of the salmon filets in the study. The sum of the *trans* isomers ranged from 0.05 to 0.11% of total fatty acids. All of these samples had very high levels of 18:1 $n-9$ (12−30%) and 18:2 $n-6$ (3−10%), indicating that the filets were from farmed fish fed diets rich in vegetable oils. The amount of *trans* isomers relative to *all-cis* 18:2 $n-6$ was from 0.5 to 2.9%. These levels are typically formed under refining of vegetable oils,[5,50,51] and it is likely that the sources of the *trans* isomers are the feed oils. No other TFA was found.

General Discussion and Advice. The results presented in the first five headings of this section, where a large range of column polarities were spanned, indicate that it is not possible to find conditions where *trans* fatty acids can be analyzed in a single chromatographic analysis without interference from minor fatty acids present in marine lipids. Before reporting TFA in samples of marine origin, a minimum requirement should be that one is aware of possible interferents, where they will elute, and how they may contribute to false positives.

The equations given in Tables 2−5 may be of some help for location of possible interferents. Values listed for CP-Sil 88 in Ce 1f-96 could be predicted from the equations for SP-2560 with a standard error of prediction (SEP) of 0.02, and values for BPX-70 were predicted with an SEP of 0.03. However, it should be emphasized that the transferability of the equations to other systems has not been evaluated, and errors for the interferents may also be higher because they are structurally more different from the predictors (the *all-cis* isomers of 18:1, 18:2, and 18:3) than the fatty acids listed in the reference methods.

If available, knowledge about the source and history of the sample may be of some help to evaluate whether a certain chromatographic peak is a TFA or not. In this respect it should be considered that there is no known route for formation of TFA in fish, and significant amounts of TFA in the marine food web have not been reported. It should also be emphasized that TFA formed in industrial processes such as deodorization of oils are not formed alone. *Trans* isomers of 18:2 $n-6$ and 18:3 $n-3$ will usually appear in pairs or triplicates,[3,4] and the presence or absence of other peaks may therefore indicate if a certain peak is a TFA. The reaction rates of thermal isomerization are also highly dependent on the number of double bonds.[5,31,50−52] If *trans* isomers of 18:2 $n-6$ and 18:3 $n-3$ are seen in a pure fish oil, substantial amounts of *trans* isomers of the abundant and highly unsaturated 20:5 $n-3$ and 22:6 $n-3$ should also be expected.[31]

Far more challenging than the analysis of pure fish products is the analysis of mixed products, where fish may occur together with refined vegetable oils or milk lipids. In these cases one may expect that C16 and C18 TFA are present together with typical interferents from marine lipids. Unambiguous identifications will in these cases require a higher specificity than what can be achieved with a single GC-FID analysis. There are no routine methods for this, but much can be gained by combining information from several methods.

The combined information from two or more GC analyses performed under different conditions may in many cases be used to distinguish between TFA and possible interferents. In some cases it may be enough to use different temperature programs on the same column,[44] but it will often be necessary to use different columns. CP columns and PEG columns are a particularly suitable combination in this respect. Both column types are suitable for FAME analysis but have highly different *cis*/*trans* selectivities. Interferents on a CP column will therefore be resolved on a PEG column.[40]

The use of a mass spectrometric (MS) detector will also help to some degree. FAMEs with different number of double bonds are generally easily distinguished by electron impact mass spectra of FAME. There are also distinct differences between the spectra of 18:3 $n-6$, 18:3 $n-4$, and *trans* isomers of 18:3 $n-3$,[27,29] but these differences may be too small for identifications based on weak signals from minor and partially overlapping peaks. The same applies for separation between *cis* and *trans* monoenes. *Cis* and *trans* isomers that overlap on CP columns will differ in position of the double bond, and the geometry can therefore be assigned if the position can be correctly identified. Positional isomerism in monounsaturated fatty acids can be identified by several means,[54,55] but a high-quality spectrum is usually required.

The influence of interferents can also be reduced by a pre-separation of FAME by silver ion LC prior to GC analysis,[14,56,57] but this is a labor intensive methodology. Better selectivity can also be achieved using GC with infrared detection,[58,59] but low sensitivity of GC-IR will usually limit the feasibility of this method. Two-dimensional gas chromatography seems to be a promising technique for the identification and quantification of TFA with minimal interference from other compounds. In the typical configuration used for FAME, in which a nonpolar column is combined with a CP column, *trans* fatty acids will appear in distinct bands in the two-dimensional chromatograms, and interferents that differ in the number of double bonds and other structural features can be separated in the second dimension.[60,61] However, this is not yet a routine technique.

The current work has focused on possible interferents that are common in marine lipids. It should be emphasized that the marine food web contains a very large variety of fatty acid structures that may be interferents in special cases. This includes

branched saturated and unsaturated fatty acids, polyunsaturated fatty acids, with non-methylene interrupted double bonds, and fatty acids with special functional groups. A review of the fatty acid diversity in marine organisms is given in ref 62. There is also a wide range of non-fatty acid structures that can be found in a typical fatty acid chromatogram from samples of marine origin. Plasmalogens are glycerolipids that are present in various amounts in tissues and organisms, in which one of the fatty acids is bound to the glycerol via a vinyl ether bond. In marine organisms plasmalogens are particularly abundant in bivalves.[63] With certain FAME derivatization methods they give rise to a series of dimethyl acetals and methyl enol ethers that may be possible interferents with TFA.[64] Other possible interferents that are naturally present in low levels in marine organisms are fatty alcohols[42,65] and hydrocarbons.[65]

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ABBREVIATIONS USED

CP, cyanopropyl; FAME, fatty acid methyl ester; FID, flame ionization detector; GC, gas chromatography; IR, infrared; LC, liquid chromatography; MS, mass spectrometry; PEG, polyethylene glycol; SN, separation number; TFA, *trans* fatty acid.

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