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Identification of Furan Fatty Acids in Nutritional Oils and Fats by Multi-dimensional GC-MSD

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Abstract

Identification of furan fatty acids as minor components in oils and fats require several pre-analytical separation steps in order to obtain sufficient resolution and sensitivity in single column gaschromatography. After extraction and transesterification hydrogenation, urea complex precipitation and silicagel column chromatography or Ag^{+}-TLC were applied prior to GC-analysis. By using a multidimensional GC-MSD System with cooled injection and flow controlled column switching with cold trapping in between, the methyl esters of furan fatty acids can be identified directly without any further pre-analytical separations.

Butter, milk and ten nutritional oils were investigated. Different transesterification methods were used for the characterization of the oils and compared with each other. Electron impact and chemical ionization were applied to identify the fatty acid methyl esters by GC-MS.

Fourteen furan fatty acids were identified in fish oil, two in butter and one in milk. The same furan fatty acid were found in peanut, thistle, sunflower, hazelnut and olive oil, whereas none were detected so far in sesame, corn, walnut or grape seed oil.

Introduction

As one of the first furan fatty acids (F-acids, **Figure 1**) the disubstituted 9,12-epoxy-octadeca-9,11-dienoic acid was found in seed oil of *exocarpus cupressiformis* in 1966 [1]. A series of tri- and tetrasubstituted F-acids were later demonstrated to be present in different species of fish [2-8], in soft corals [9], different plants [10,11], amphibians [12], reptilians [12] and in mammals [13,14], including man [15]. Elaborate studies on the hepatopancreatic lipids of the crayfish *Procambarus clarkii* revealed a total of 30 F-acids [12,16].

3-methyl-2,4-nonanedione, one of the major compounds that causes the light-induced off-flavour of soya-bean oil [17,18] was shown to be a photooxidation product of furan fatty acids [19]. Further studies on different vegetable oils revealed the presence of F-acids 5, 7 and 10 (**Table I**) in soya-bean, wheat germ, rapeseed and corn oil whereas none were detected in olive or sunflower oil. In butter and butter oil nine furan fatty acids were found [20].

R_2, R_1, $H_3C-(CH_2)_n$, O, $(CH_2)_m-COOH$

R_1, R_2: H,CH_3
m: 2,4,6-12,14
n: 2-6

Figure 1. *Furan fatty acids.*

This report describes a method for the analysis of minor components in complex sample matrices. After transesterification furan fatty acid methyl esters can directly be identified by the use of a multi-dimensional GC-MSD System with cooled injection and flow controlled column switching. Butter, milk and 10 nutritional oils were investigated and their fatty acid composition characterized by different methods of transesterification.

Experimental

Sample preparation. 500 mg of oil were transesterified to the corresponding methylesters by sequential saponification and esterification [21] under a nitrogen atmosphere to avoid oxidation of the polyunsaturated fatty acids. Transesterification were also achieved by use of Trimethylsulfonium Hydroxide (TMSH): 100 mg of oil were dissolved in 5 ml tert.-butylmethylether. 50 µl TMSH were added to 100 µl of the solution and injected into the single column GC [22].

GC-MS System. A Hewlett-Packard HP 5890/5971 GC-MS system equipped with a HP 7673 automatic sampler (Hewlett Packard, Avondale PA, USA) and a 25 m x 0.2 mm i.d. HP-1 (dimethylpolysiloxane) column was used for analysis with electron-impact ionization. The column head pressure was set to 60 kPa and the injection volume was 1 µl with a split ratio of 1:10. The injector and the transfer line temperatures were 280 and 290°C, respectively; after injection the column temperature was programmed from 130 to 260°C at 2°C/min and then at 40°C/min to 300°C, held for 20 min. A model TSQ 70 GC-MS system (Finnigan MAT, Bremen, Germany) was used for the chemical ionization analysis, methane being the reagent gas.

Multidimensional GC-MSD. **Figure 2** represents a scheme of the various components used to configure the system employed for this work. The apparatus consists of a temperature programmable cold injection system with a septumless sampling head (CIS-3, Gerstel GmbH, Mülheim an der Ruhr, Germany), two HP 5890 GC ovens (Hewlett Packard, Avondale PA, USA), connected via a heated transferline incorporating a cryotrap (CTS-1, Gerstel GmbH, Mülheim an der Ruhr, Germany). The second oven is equipped with a mass selective detector (HP 5971 A, Hewlett Packard, Avondale PA, USA).

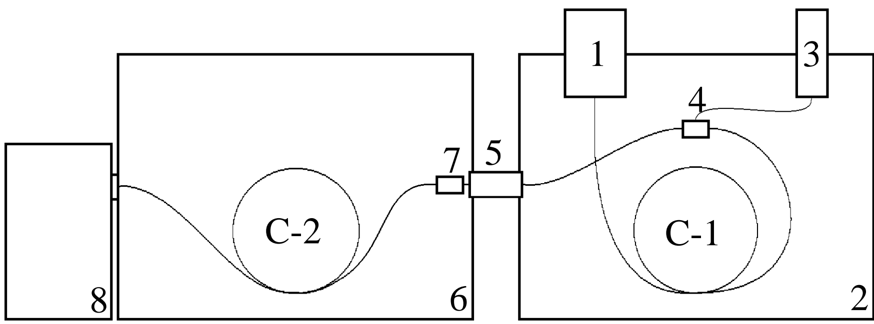


Figure 2. *Schematic diagram of the applied system which consists of a temperature programmable cold injection system with a septumless sampling head (1), a GC (2) configured with a monitor FID (3), column switching device (4) and pneumatics, connected via a heated transfer line incorporating a cryotrap (5) to a second GC (6) which has a second switching device (7) installed after the the transfer line with the main column to the msd (8).*

Analysis conditions.

Columns: Pre-column C-1: 25 m HP 1 (Hewlett Packard), d_i=0,32 mm, d_f=1,05 µm
Main column C-2: 30 m Stabilwax (Restek Corp.), d_i=0,25 mm, d_f=0,25 µm

Pneumatics: He, p_i = 130 kPa, split x:20, p_c = 40 kPa, 10 ml/min, p_{c1} = 35 kPa

Temperatures: CIS: 80°C to 300°C with 12°C/s
Oven 1: 200°C to 300°C with 5°C/min
Oven 2: 180°C to 240°C with 5°C/min
CTS: 280°C to -150°C, with 12°C/s, to 280°C with 12°C/s
FID: 320°C
MSD: 280°C

Detectors: Monitor detector in GC 1: FID
Main detector in GC 2: MSD, Scan 50 - 450 amu

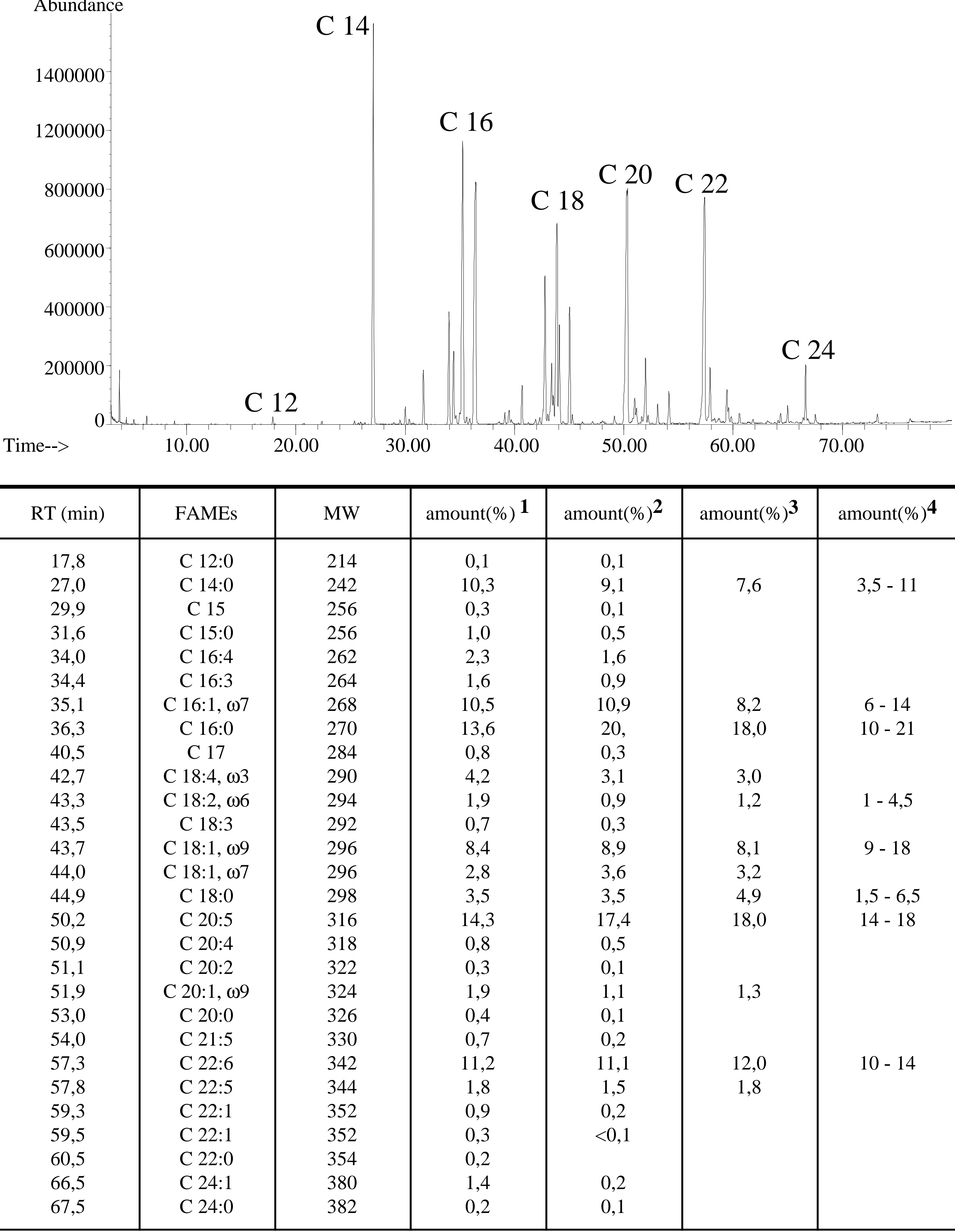
Results and Discussion

Fatty acid compostion of oils and fats. Identification of minor component fatty acids next to highly concentrated major components in complex sample matrices was achieved by multidimensional GC-MS analysis [21]. The fatty acids characterization of the different oils (**Table I**) was done by single column EI-GC-MS-analysis of the methylesters. In addition polyunsaturated fatty acids were identified by CI-GC-MS, as the molecular ion of longer chain highly unsaturated fatty acids often is missing in the EI mass spectra. TMSH was used as reagent for flash methylation by pyrolysis [22] and the results were compared to those of the sequential transesterification. As an example **Table II** shows the result for the fish oil sample, where the two methods were compared to each other and to the results cited in two references. Depending on the method used for transesterification there is a slight difference in the relative amounts of fatty acid composition.

	peanut	sesame	thistle	corn	walnut	grape seed	sunflower
C 16:0	8,7	8,9	5,9	9,9	7,3	6,4	6,1
C 18:2, ω6	39,9	45,9	77,5	57,2	64,2	69,8	61,6
C 18:1, ω9	42,1	37,9	12,4	29,0	16,0	18,1	25,5
C 18:0	2,2	5,0	2,1	1,8	2,5	3,9	4,6
C 20:1	1,3	0,1	0,2	0,1	0,8	0,2	0,1
C 20:0	0,9	0,4	0,3	0,4	0,3	0,1	0,2
C 22:0	3,0	<0,02	0,2	0,2	0,2	--	0,6
C 24:0	1,6	<0,02	--	0,2	--	--	0,2

Walnut: 7,6% linolenic acid (C 18:3).

Table I. *Fatty acid composition (%).*



RT (min)	FAMEs	MW	amount(%) [1]	amount(%) [2]	amount(%) [3]	amount(%) [4]
17,8	C 12:0	214	0,1	0,1		
27,0	C 14:0	242	10,3	9,1	7,6	3,5 - 11
29,9	C 15	256	0,3	0,1		
31,6	C 15:0	256	1,0	0,5		
34,0	C 16:4	262	2,3	1,6		
34,4	C 16:3	264	1,6	0,9		
35,1	C 16:1, ω7	268	10,5	10,9	8,2	6 - 14
36,3	C 16:0	270	13,6	20,	18,0	10 - 21
40,5	C 17	284	0,8	0,3		
42,7	C 18:4, ω3	290	4,2	3,1	3,0	
43,3	C 18:2, ω6	294	1,9	0,9	1,2	1 - 4,5
43,5	C 18:3	292	0,7	0,3		
43,7	C 18:1, ω9	296	8,4	8,9	8,1	9 - 18
44,0	C 18:1, ω7	296	2,8	3,6	3,2	
44,9	C 18:0	298	3,5	3,5	4,9	1,5 - 6,5
50,2	C 20:5	316	14,3	17,4	18,0	14 - 18
50,9	C 20:4	318	0,8	0,5		
51,1	C 20:2	322	0,3	0,1		
51,9	C 20:1, ω9	324	1,9	1,1	1,3	
53,0	C 20:0	326	0,4	0,1		
54,0	C 21:5	330	0,7	0,2		
57,3	C 22:6	342	11,2	11,1	12,0	10 - 14
57,8	C 22:5	344	1,8	1,5	1,8	
59,3	C 22:1	352	0,9	0,2		
59,5	C 22:1	352	0,3	<0,1		
60,5	C 22:0	354	0,2			
66,5	C 24:1	380	1,4	0,2		
67,5	C 24:0	382	0,2	0,1		

1: Transesterification (KOH/MEOH,MEOH/H_2SO_4/NH_4CL) Ref. 21
2: Transesterification TMSH, Ref. 22
3: Transesterification CH_3COCL/MEOH, Ref.24
4: Manufacturer's results (Eicosapen®, Hormon-Chemie München, Germany)

Table II. *Fatty acid composition of fish oil (EI-GC-MS).*

Identification of furan fatty acids. Considering the small amounts of F-acids encountered in most of the oils investigated one has to inject relatively large amounts of concentrated samples onto the precolumn. By means of the monitor FID and the retention times from the single column GC-analysis the cut times were set for the transfer onto the second column. Even different cuts within the same GC run can be transferred as the cryotrap causes focussing before "reinjecting" onto the second column. As an example **Figure 3** shows the pre-column chromatogram of the fish oil sample with two cuts and the chromatogram of the cryofocused, "reinjected" sample on the analytical column.

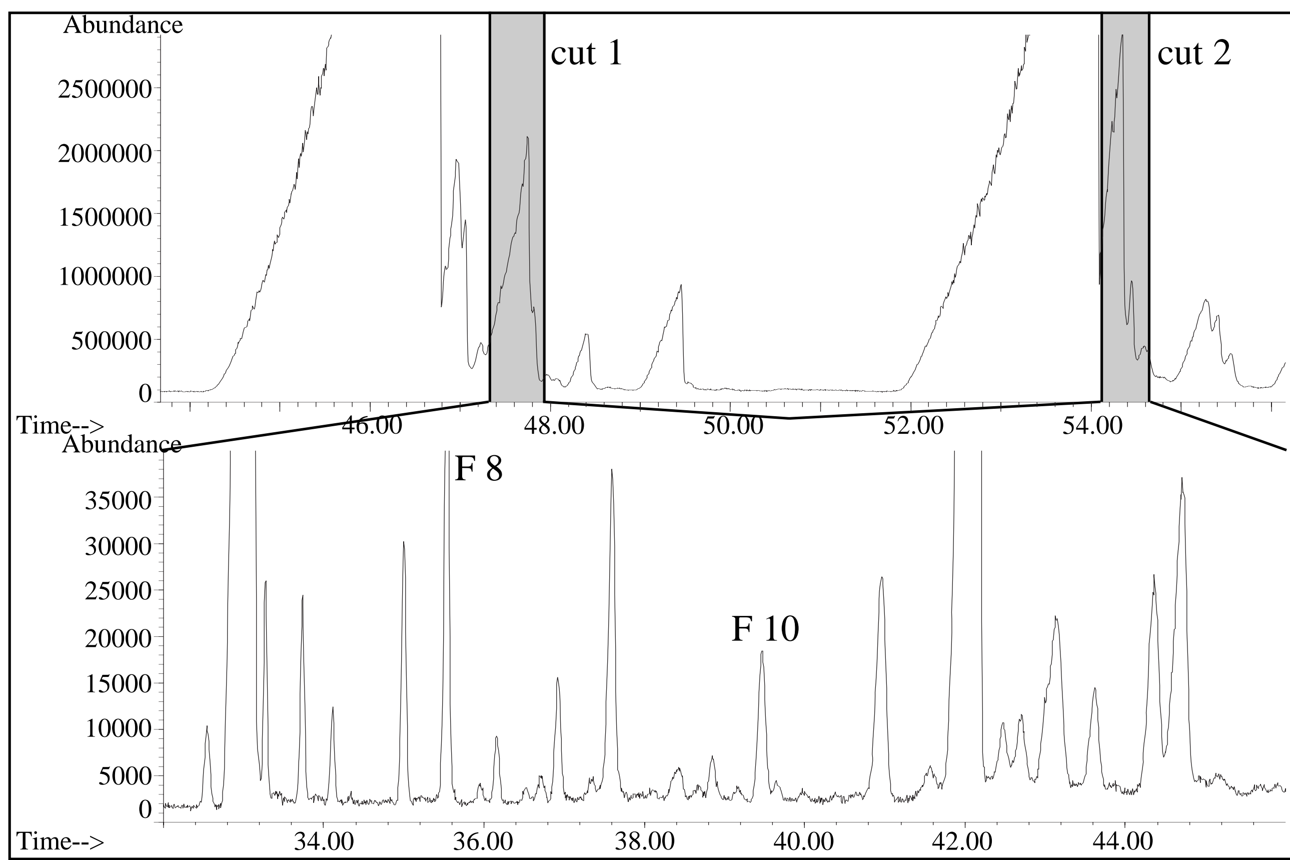


Figure 3. *Analysis of fish oil, pre- (top) and main column chromatogram.*

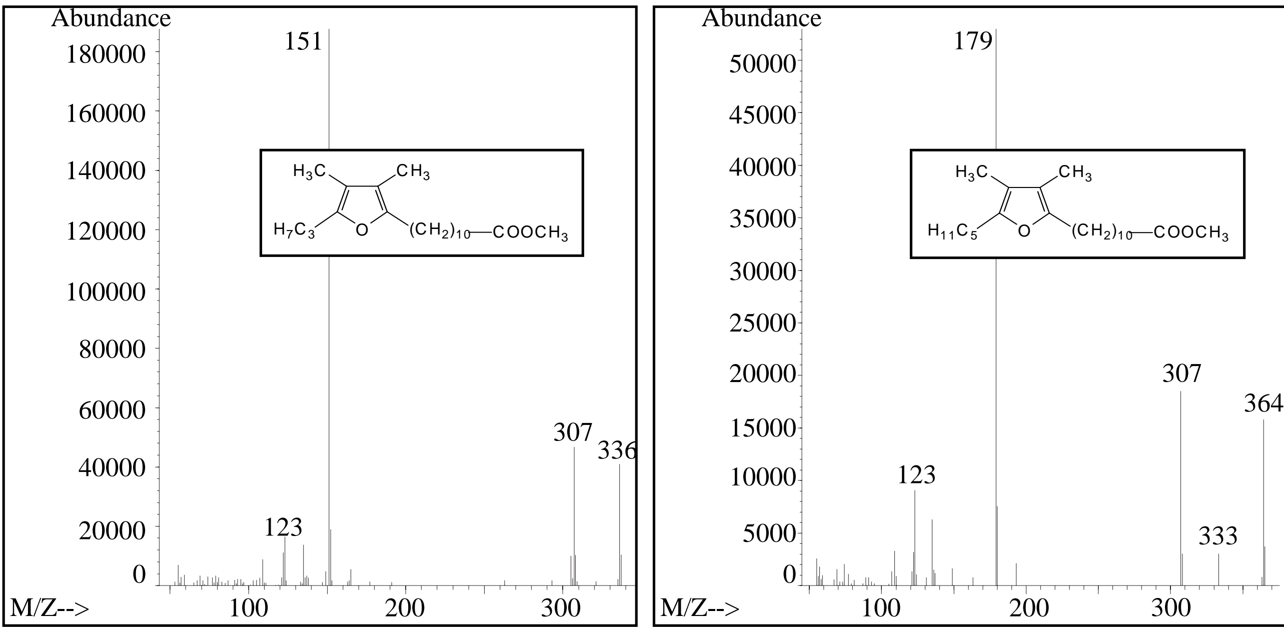


Figure 4. *Mass spectrum F-acid 8.*

Figure 5. *Mass spectrum F-acid 10.*

In single column analysis F-acids were detected only after multiple background subtraction or by selected ion monitoring. By using the multidimensional GC-MS system F-acids (here F-acid 8 and F-acid 10) were well separated and yielded well defined mass spectra (**Figure 4 and Figure 5**).

Furan fatty acids compostion of the investigated oils and fats. From the 14 furan fatty acids found in fish oil (F-acids 1-14, **Table III**) both F-acids 7 and 10 were detected in butter and F-acid 10 in cow's milk. F-acid 5 was found in peanut, thistle, sunflower, hazelnut and olive oil. In sesame, corn, walnut and grape seed oil no furan fatty acids have been detected so far.

H, CH_3
H_7C_3, O, $(CH_2)_n$—$COOCH_3$

n	Mol. peak (m/z)	M^+ - C_2H_5 (m/z)	Base peak (m/z)	Furan ring (m/z)	F-acids	RT (min)	Ref.
8	294 (13%)	265 (4%)	137	109 (7%)	3	34.3	[10,12,23]
10	322 (16%)	293 (5%)	137	109 (6%)	6	43.4	[10,12,23]

H_3C, CH_3
H_7C_3, O, $(CH_2)_n$—$COOCH_3$

n	Mol. peak (m/z)	M^+ - C_2H_5 (m/z)	Base peak (m/z)	Furan ring (m/z)	F-acids	RT (min)	Ref.
6	280	251	151	123	n.d.	-	[12]
8	308 (19%)	279 (25%)	151	123 (10%)	4	39.6	F1,[10,20]
10	336 (20%)	307 (24%)	151	123 (9%)	8	47.8	F4,[10,20]
12	364 (25%)	335 (25%)	151	123 (9%)	11	56.5	[6]
14	392 (34%)	363 (24%)	151	123 (13%)	14	62.5	[23]

F-acids: 1-14 detected in fishoil (n.d.: not detected), *Ref:* F1 - F8 listed in ref. 3,6, and 12; others as (cited), see references, *RT:* Retention times for single column GC-MSD

Table III a. *Propyl furan fatty acids.*

H CH_3

$H_{11}C_5$ O $(CH_2)_n$—$COOCH_3$

n	Mol. peak (m/z)	$M^+ - C_4H_9$ (m/z)	Base peak (m/z)	Furan ring (m/z)	F-acids	RT (min)	Ref.
2	238	181	165	109	n.d.	-	[12]
4	266 (16%)	209 (8%)	165	109 (8%)	1	24.0	[20,23]
6	294 (16%)	237 (7%)	165	109 (16%)	2	33.1	[12,20]
8	322 (15%)	265 (6%)	165	109 (11%)	5	42.2	F2,[20]
10	350 (18%)	293 (6%)	165	109 (12%)	9	50.6	F5,[20]
12	378 (16%)	321 n.d.	165	109 (11%)	12	58.3	F7
14	406	349	165	109	n.d.	-	[12]

H_3C CH_3

$H_{11}C_5$ O $(CH_2)_n$—$COOCH_3$

n	Mol. peak (m/z)	$M^+ - C_4H_9$ (m/z)	Base peak (m/z)	Furan ring (m/z)	F-acids	RT (min)	Ref.
2	252	195	179	123	n.d.	-	[12]
4	280	223	179	123	n.d.	-	[12,20]
6	308	251	179	123	n.d.	-	[12,20]
8	336 (28%)	279 (42%)	179	123 (16%)	7	46.8	F3,[20]
10	364 (29%)	307 (34%)	179	123 (17%)	10	54.4	F6,[20]
12	392 (30%)	335 (25%)	179	123 (19%)	13	61.1	F8

F-acids: 1-14 detected in fishoil (n.d.: not detected), *Ref:* F1 - F8 listed in ref. 3,6, and 12; others as (cited), see references, *RT:* Retention times for single column GC-MSD

Table III b. *Pentyl furan fatty acids.*

Mass spectra of furan fatty acids. The characteristic ions in the mass spectra of F-acid methyl esters are listed in **Table III**. Allylic cleavage of the alkylcarboxyl chain at the furan ring (A, **Figure 6**)produces the base peak (BP) m/z 179 (F-acids 7,10,13) and m/z 165 (F-acids 1,2,5,9,12) for the pentyl group, m/z 151 (F-acids 4,8,11,14) and m/z 137 (F-acids 3,6) for the propyl group F-acids. Allylic cleavage of the alkyl chain in 5-position (B, **Figure 6**) yields ions $M^+ - C_2H_5$ (R_3) and $M^+ - C_4H_9$ (R_3) , respectively. The furan ring itself gives rise to ions m/z 109 for the trisubstituted and m/z 123 for the

tetrasubstituted F-acids, produced by cleavage of both allylic positions (A and B, **Figure 6**) with hydrogen rearrangement.

$R_1, R_2 = H, CH_3$

Figure 6. *EI-fragmentation of furan fatty acids.*

Conclusions

The multidimensional GC-MSD-System described is a powerful tool for the analysis of low concentrated minor component fatty acids in complex sample matrices. It allows to separate small peaks adjacent to or covered by major component peaks just by choosing the right heart-cut time and the use of a focusing cryo-trap in between the two columns. On the other hand chromatographic separation can be improved by combination of different columns in separated GC-ovens with individual temperature programming.

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