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Identification of Furan Fatty Acids as Minor Components of Human Lipids by Multidimensional GC-MSD

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KEYWORDS

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Furan Fatty Acids

ABSTRACT

The fatty acid composition of plasma, red blood cells and platelets were investigated. Human milk was analyzed and the fatty acid composition compared to cow's milk. After lipid extraction and transesterification the methyl esters were analyzed without any further pre-analytical separation steps involved. 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 were directly identified by means of their mass spectra.

From the ten samples of human milk analyzed only one contained the furan fatty acid 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F-acid 10, **Figure 1**), which was also present in the cow's milk sample. Furan fatty acids were found in all blood samples in differing relative amounts. F-acids 5,8 and 10 were detected in red blood cells, F-acids 8 and 10 in plasma and in platelets only F-acid 10 was found so far.

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 propyl- and pentyl-side chain 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]. Most of these tri- and tetrasubstituted furan fatty acids belonged to the propyl- and pentyl-side chain group and only a few were found with butyl-, hexyl- or even heptyl-side chains in the 5-position. The only 2,5-disubstituted F-acid so far reported, is the one found in *exocarpus cupressiformis*.

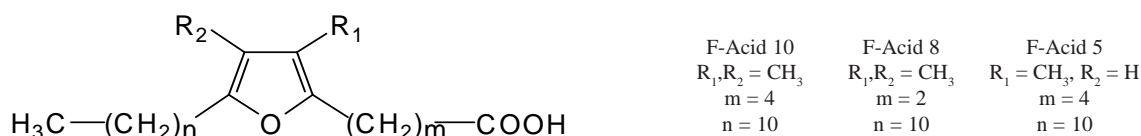


Figure 1. Furan fatty acids.

The presence of dibasic furanpropionic acids (**Figure 10** and **Figure 11**) in human urine [17] and blood [18] caused further metabolic studies in which the F-acids were regarded as possible precursors [19-22]. Supporting this theory is the fact, that hyperlipidaemic patients treated fish oil showed an increase in the serum concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (**Figure 10**) soon after treatment had started [20]. Later it was shown that the fish oil used contained 14 different furan fatty acids [23].

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid as one of the two major furanpropionic acids proved to be a metabolite with several adverse biochemical and physiological effects [24,25], especially in connection with chronic renal failure, where it is found at highly elevated levels in plasma [26].

This report describes a method for the analysis of minor component fatty acids in complex sample matrices. After lipid extraction and transesterification furan fatty acid methyl esters can directly be identified by the use of a multidimensional GC-MSD-system without any further pre-analytical separations. Direct transesterification of the serum permitted the simultaneous analysis of both the furan fatty acids and the furanpropionic acids.

EXPERIMENTAL

Sample preparation. Red blood cells, platelets and plasma were obtained from the local blood bank in a very high purity separation. Lipids from blood samples and milk (5 ml) were extracted by the method of Folch et al. [27]. In case of red blood cells both total cells and isolated membranes were used. The lipid extracts or 5 ml of untreated sample were transesterified by sequential saponification and esterification [28]. The reaction was carried out under a nitrogen atmosphere to avoid oxidation of polyunsaturated fatty acids.

GC-MS-System. Electron-impact ionization was performed with a Hewlett-Packard (Hewlett Packard, Avondale PA, USA) HP 5890/5971 GC-MS system equipped with a HP 7673 automatic sampler and a 25 m x 0.2 mm i.d. HP-1 (dimethylpolysiloxane) column. The column head pressure was set to 60 kPa and the injection volume was 1 μ l with a split ratio of 1:10. The injector and the transfer line temperatures were 280 and 300°C, respectively; after injection the column temperature was programmed at 2°C/min from 130 to 300°C, held for 20 min.

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 5972 A, Hewlett Packard, Avondale PA, USA).

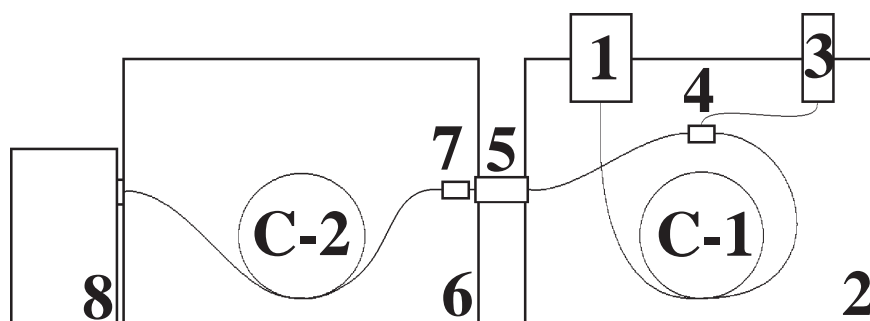


Figure 2. Schematic diagram of the applied system which consists of a temperature programmable cold injection system with a septumless sampling head (1), a GC (2) configured with a monitor FID (3), column switching device (4) and pneumatics, connected via a heated transfer line incorporating a cryotrap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

For column switching and transfer of cuts the cryotrap is cooled with liquid nitrogen from its normal temperature of 200 °C to -150 °C two minutes before the cut, followed by heating at 12 °C/second to 280 °C for reinjection of the focused cut to the second column and to the MSD.

Analysis conditions.

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

After transesterification of the lipid extracts the samples were analyzed by single column GC-MSD in order to determine the retention times later used in multidimensional GC-MSD together with the FID monitor chromatogram. It was also used to characterize the different fatty acid compositions of red blood cells, platelets, serum and human milk (**Figure 3-6**).

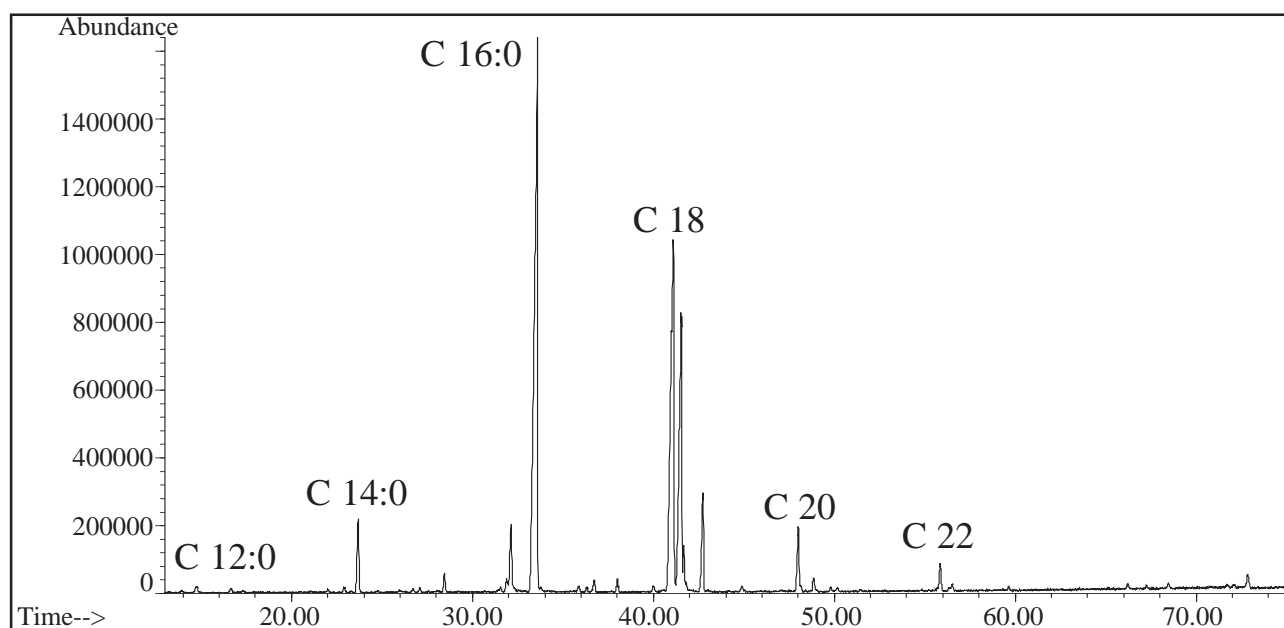


Figure 3. Total ion chromatogram of serum.

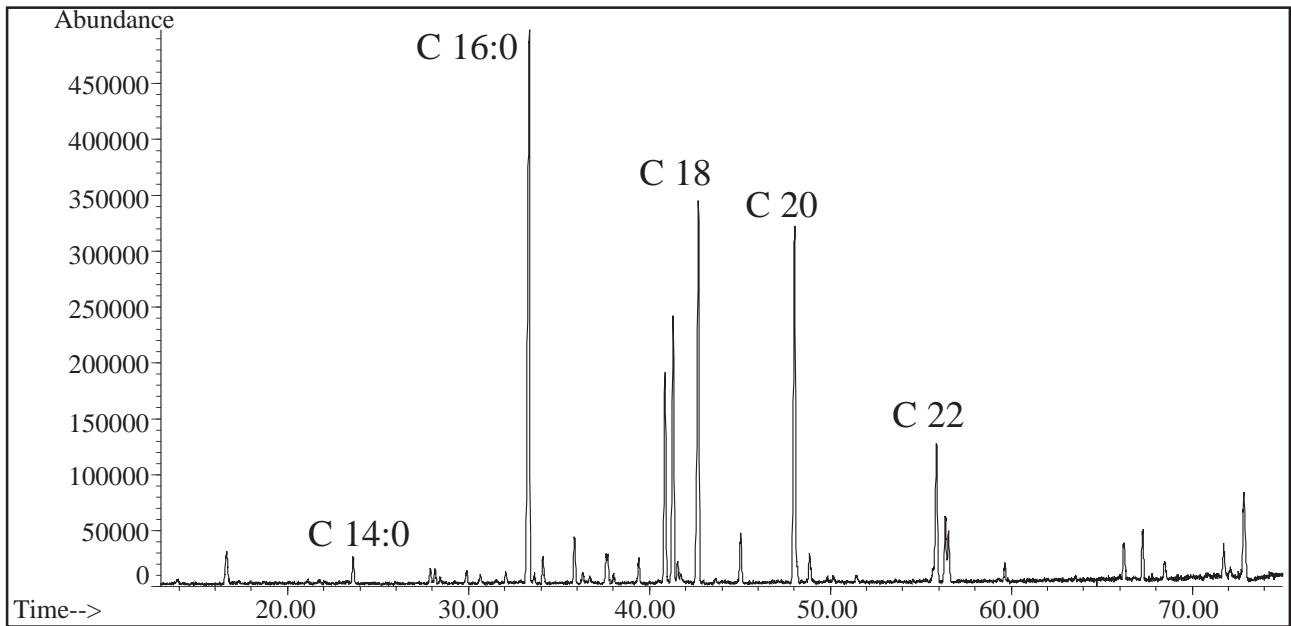


Figure 4. Total ion chromatogram of red blood cells.

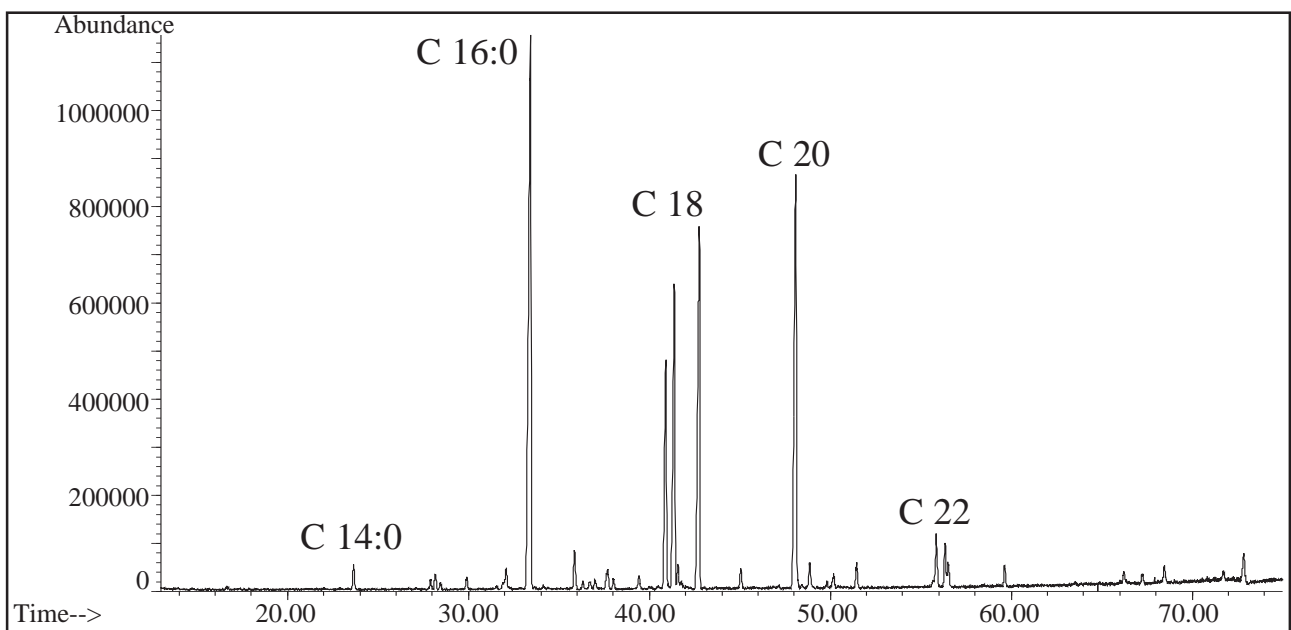


Figure 5. Total ion chromatogram of platelets.

The major fatty acid components found in the blood samples were palmitic (C 16:0), linoleic (C 18:2, ω 6), oleic (C 18:1 ω 9), stearic (C 18:0) and arachidonic (C 20:4, ω 6) acid. **Table I** lists the average relative amounts found in serum, red blood cells, platelets and human milk.

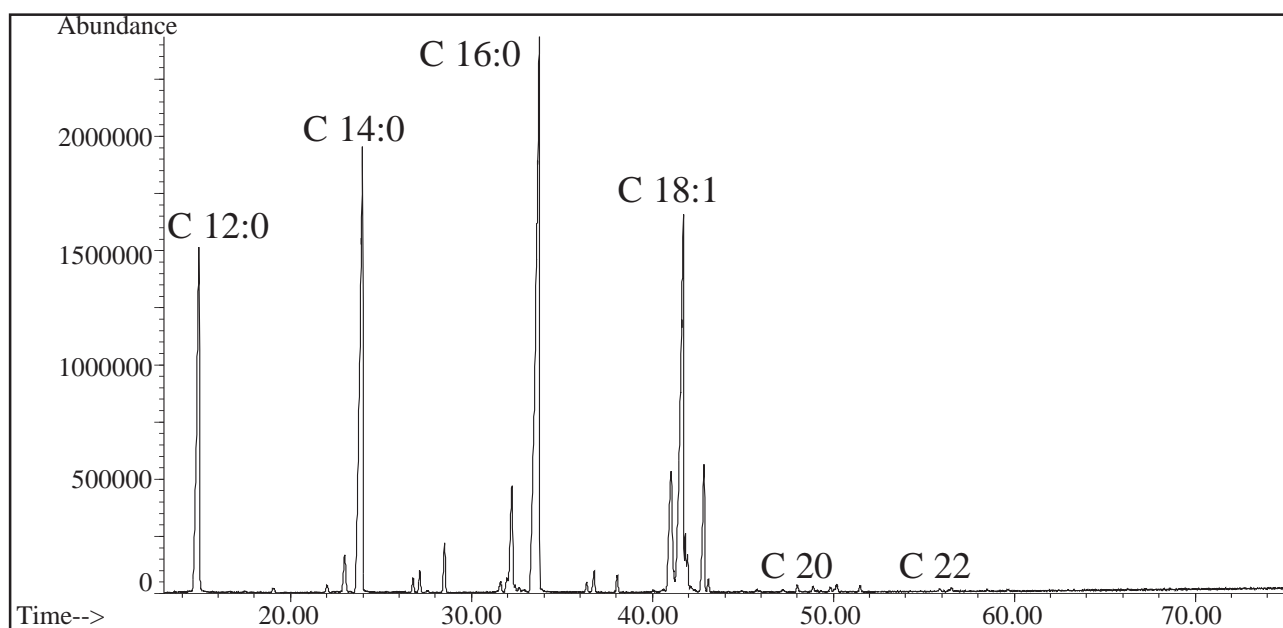


Figure 6. Total ion chromatogram of human milk.

	Serum	red blood cells	platelets	human milk
C 12:0	0,2 ± 0,1	< 0,05	< 0,05	11,5 ± 5,2
C 14:0	2,5 ± 1,2	0,4 ± 0,1	0,5 ± 0,1	13,7 ± 10,3
C 16:0	23,7 ± 2,3	21,4 ± 4,9	18,9 ± 3,0	20,8 ± 9,9
C 18:2, ω6	29,6 ± 4,6	10,8 ± 1,4	12,2 ± 3,1	10,5 ± 6,8
C 18:1, ω9	20,8 ± 3,2	11,6 ± 2,1	13,2 ± 2,8	28,5 ± 13,4
C 18:0	7,4 ± 1,9	16,5 ± 2,1	15,1 ± 1,4	9,8 ± 4,9
C 20:4, ω6	5,5 ± 0,4	13,4 ± 1,1	22,3 ± 3,2	0,4 ± 0,2

Table I. Average fatty acid composition (%).

From the ten samples of human milk analyzed only one contained the furan fatty acid 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F-acid 10) which was also present in the cow's milk sample.

By choosing the right cut times in the total ion chromatogram of the precolumn for the described configuration of the multidimensional GC-MSD-System the methyl esters of furan fatty acids become well separated on the second column as shown for the analysis of F-acid 10 in red blood cells (**Figure 7**).

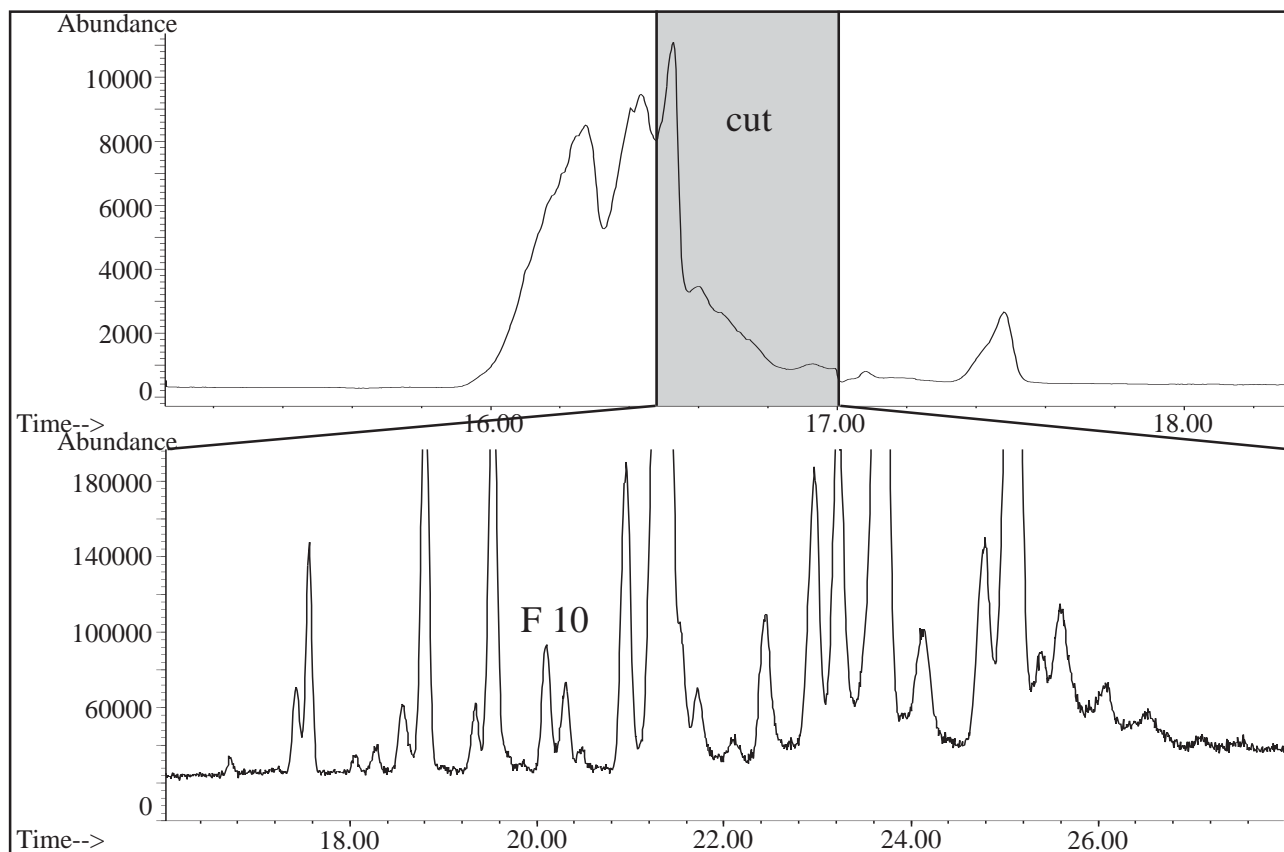


Figure 7. Multidimensional analysis of red blood cells, pre- (top) and main column chromatogram.

The mass spectrum of F-acid 10 (**Figure 8**) now is free of any ions due to interfering substances.

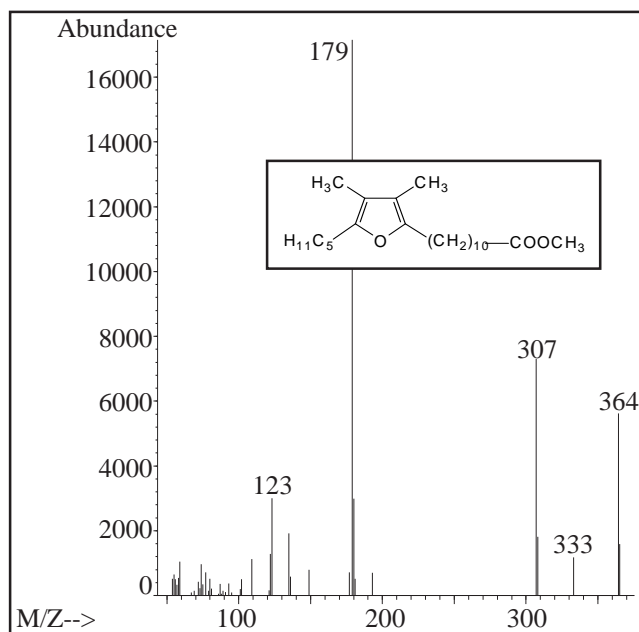


Figure 8. Mass spectrum of F10 by multidimensional GC-MSD.

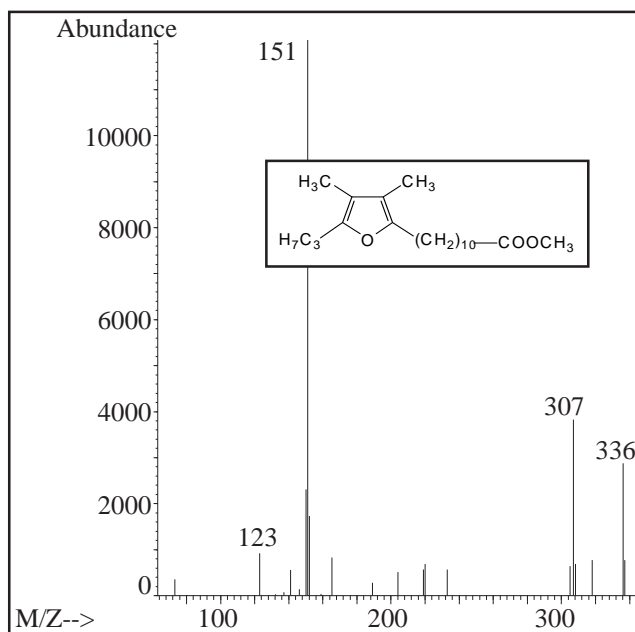


Figure 9. Mass spectrum of F8 by multidimensional GC-MSD.

Next to furan fatty acid F-acid 10, which was found in all blood components, F-acid 8 (**Figure 9**) was found in red blood cells and plasma. In the case of red blood cells there was no significant difference observed when whole red blood cells were used instead of the isolated membranes. The analysis of serum samples lead to variable results: in most cases furan fatty acids could be detected, but not in all. Since furan fatty acids have also been found in red blood cells and platelets there might be an exchange taking place depending on the time the serum stays in contact with. In serum furan fatty acids were detected only in the phospholipid fraction, but not in cholesterol esters nor in triglycerides. This is in agreement with an earlier report by Puchta et al. [15].

Direct transesterification of the serum samples made the simultaneous detection of furan propionic acids and furan fatty acids possible. Ion chromatograms with ions m/z 236 and m/z 208 were used to locate 3-carboxy-4-methyl-5-pentyl-2-furanpropionic acid in single column GC analysis and the background subtracted mass spectrum (**Figure 10**) confirmed identity.

Figure 8 shows the mass spectrum of the F-acid 10 found in the same serum sample and identified by multidimensional GC-MSD. The homologous propylfuranpropionic acid was located using ions m/z 179 and m/z 208 in the ion chromatogram and was also confirmed by its mass spectrum (**Figure 11**). The corresponding furan fatty acid (F-acid 8, **Figure 9**) was again identified in the same sample .

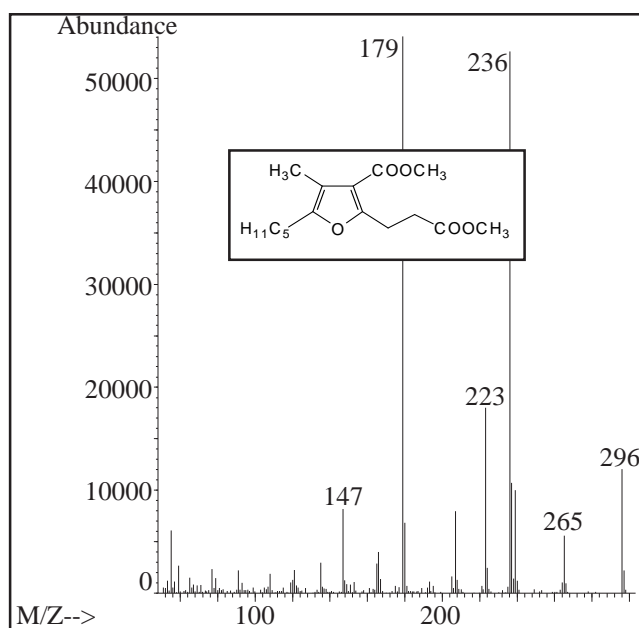


Figure 10. Mass spectrum of 3-carboxy-4-methyl-5-pentyl-2-furanpropionic acid.

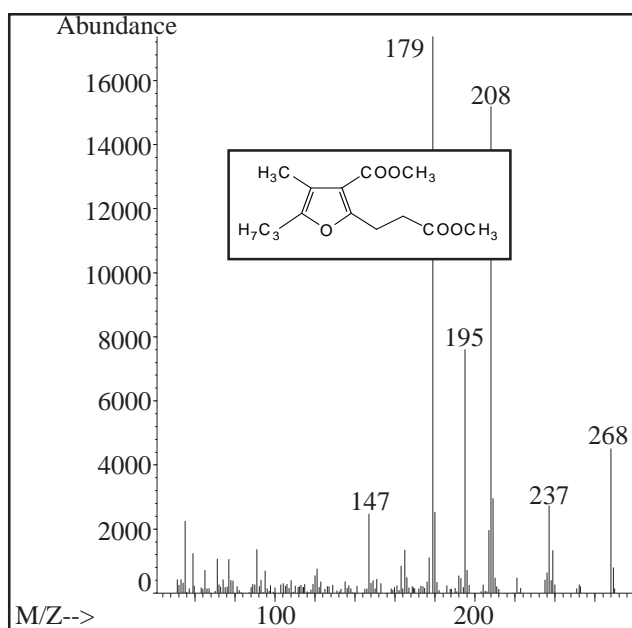


Figure 11. Mass spectrum of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid.

CONCLUSION

The identification of minor component furan fatty acids in blood lipids and human milk has been achieved by use of a multidimensional GC-MSD system. After transesterification there is no need for any further pre-analytical separation or concentration steps. This method should also apply for the analysis of F-acids in human body tissues. The presence of both furanpropionic acids and furan fatty acids and furan fatty acids in serum is further support for the precursor role of furan fatty acids.

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