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Identification of furan fatty acids in human blood cells and plasma by multi-dimensional gas chromatography–mass spectrometry

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Abstract

The separation and determination of low-concentration furan fatty acids in complex sample matrices, such as oils, tissue lipids or blood, previously required time-consuming pre-analytical separation steps in order to obtain sufficient resolution in single-column gas chromatography. By using a multi-dimensional GC–MS system, it is now possible to identify directly the methyl esters of furan fatty acids without any further pre-analytical separations. The Folch method was used to isolate the lipids from the different blood components and transesterification of total lipids was carried out by sequential saponification and esterification. Individual lipid classes were isolated by preparative TLC separation and transesterified into their methyl esters by a one-step reaction using acetyl chloride as catalyst. Furan fatty acids were found in all blood samples in differing relative amounts. 12,15-Expoxy-13,14-dimethyleicosa-12,14-dienoic acid (F6) and 12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoic acid (F4) were detected in both red blood cells and in plasma; in platelets, only F6 was found. In serum, furan fatty acids were detected only in the phospholipid fraction, and not in cholesterol esters or triglycerides.

1. Introduction

As one of the first furan fatty acids (F-acids, Fig. 1), disubstituted 9,12-epoxyoctadeca-9,11-dienoic acid was found in the 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], soft corals [9], different plants [10,11], vegetable oils [12,13], amphibians [14], reptilians [14] and mammals [15,16], including man [17]. Elaborate studies on hepatopancreatic lipids of the crayfish *Procambarus clarkii* revealed a total of 30 F-acids [14].

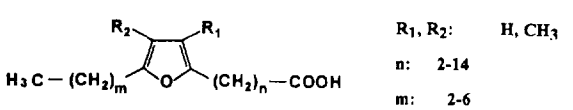

Fig. 1. Structure of furan fatty acids.

The presence of dibasic furanpropionic acids in human urine [18] and blood [19] led to further metabolic studies in which the F-acids were regarded as possible precursors [20–23]. Supporting this theory is the fact that hyperlipidaemic patients treated with fish oil showed an increase in the serum concentration of 3-

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carboxy-4-methyl-5-propyl-2-furanpropionic acid soon after treatment had stated [22]. Later it was shown that the fish oil used contained fourteen different furan fatty acids [24]. 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 [25,26], especially in connection with chronic renal failure, where it is found at highly elevated levels in plasma [27–30]. The origin, metabolism and possible physiological role of furan acids are not yet clearly understood and the success of further clinical studies will also depend strongly on the quality of the analytical techniques applied.

In order to obtain sufficient resolution in single-column gas chromatography, several pre-analytical steps were applied in the past for the analysis of furan fatty acids in complex matrices. After extraction and transesterification, various conditions for hydrogenation were used, which affected only the unsaturated fatty acids and left the furan ring unchanged [2,3,14,16,31]. F-acids have also been separated from polyunsaturated fatty acids by argentation TLC [1,3–7,11–13], in which the methyl esters of furan fatty acids migrate closely behind the saturated and monoene acids. Following hydrogenation or argentation TLC, F-acids were separated from saturated straight-chain fatty acids by urea fractionation, as the F-acid methyl esters do not form urea inclusion complexes [2,3,5–7,14,16,31].

Yet another method is hydrogenation with 5% rhodium on aluminium oxide in hexane under a 150 kPa hydrogen pressure for 2 h, which will attack the furan ring, thus yielding isomeric tetrahydrofuran derivatives [10,17]. According to the *syn*-addition of hydrogen, the all-*cis* configurations in amounts up to 88% of the four isomers were formed [17]. After separation by TLC the tetrahydrofuran derivatives were identified and quantified by single-column GC–MS [10,17].

This paper describes a method for the identification of furan fatty acids in different blood components. After lipid extraction and transesterification, the methyl esters can directly be identified by the use of a multi-dimensional GC–MS system without any further pre-analytical separations. This technique should also be applicable to any other minor component fatty acid in complex sample matrices.

2. Experimental

2.1. Chemicals

All solvents and reagents used were of the highest grade available. The reference lipids for the TLC separation were purchased from Sigma Chemie (Deisenhofen, Germany). TLC plates (20 cm × 20 cm glass plates precoated with a 0.25-mm layer of silica gel 60) were obtained from Merck (Darmstadt, Germany).

2.2. 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 (20 ml) were extracted by the method of Folch et al. [32]. For red blood cells, both total cells and membranes [33] were used. The lipid extracts were transesterified by sequential saponification and esterification [34,35]. The reaction was carried out under a nitrogen atmosphere to avoid oxidation of polyunsaturated fatty acids. Individual lipid classes of cholesterol esters, triglycerides and phospholipids were isolated by preparative TLC separation [36] and transesterified by a modified one-step reaction [36,37] as reported by Lepage and Roy [38,39].

2.3. GC–MS system

Electron impact ionization was performed with a Hewlett-Packard (Avondale, PA, USA) HP 5890/5971 GC–MS system equipped with an HP 7673 automatic sampler and a 25 m × 0.2 mm I.D. × 0.33 μm HP-1 (dimethylpolysiloxane) column. The column head pressure was set to 60 kPa and the injection volume was 1 μl with a splitting 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

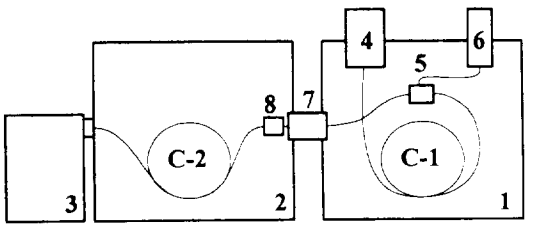

Fig. 2. Schematic diagram of the multi-dimensional system applied. 1 = GC oven with precolumn C-1; 2 = GC oven with analytical column C-2; 3 = mass-selective detector; 4 = cold injection system CIS-3; 5 = column-switching device; 6 = flame ionization detection (FID) monitor; 7 = cryotrap; 8 = second switching device.

to 300°C, the final temperature being held for 20 min.

2.4. Multi-dimensional GC–MS

Fig. 2 shows schematically 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, 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, Mülheim an der Ruhr, Germany). The second oven is equipped with an HP 5971 A mass-selective detector (Hewlett Packard).

For column switching and transfer of cuts, the cryotrap is cooled with liquid nitrogen from its normal temperature of 200°C to −150°C 2 min before the cut, followed by heating at 12°C/s to 280°C for "reinjection" of the focused cut to the second column and to the MS detector.

2.5. Analytical conditions

The following conditions were used:

Columns:	
Precolumn C-1	HP 1 (Hewlett-Packard), 25 m × 0.32 mm I.D. × 1.05 μm
Analytical column C-2	Stabilwax (Restek) 30 m × 0.25 mm I.D. × 0.25 μm
Pneumatics:	
Carrier gas (He)	p_i = 130 kPa, split 1:20 p_c = 40 kPa, 10 ml/min, p_{c1} = 35 kPa
FID	H_2, 30 ml/min; air, 300 ml/min; N_2, 30 ml/min
Temperatures:	
CIS	$80°C \xrightarrow{12°C/s} 300°C$
GC oven 1	$200°C \xrightarrow{5°C/min} 300°C$
GC oven 2	$180°C \xrightarrow{5°C/min} 240°C$
CTS	$280°C \xrightarrow{12°C/s} -150°C$ $-150°C \xrightarrow{12°C/s} 280°C$
FID	320°C
MS	280°C

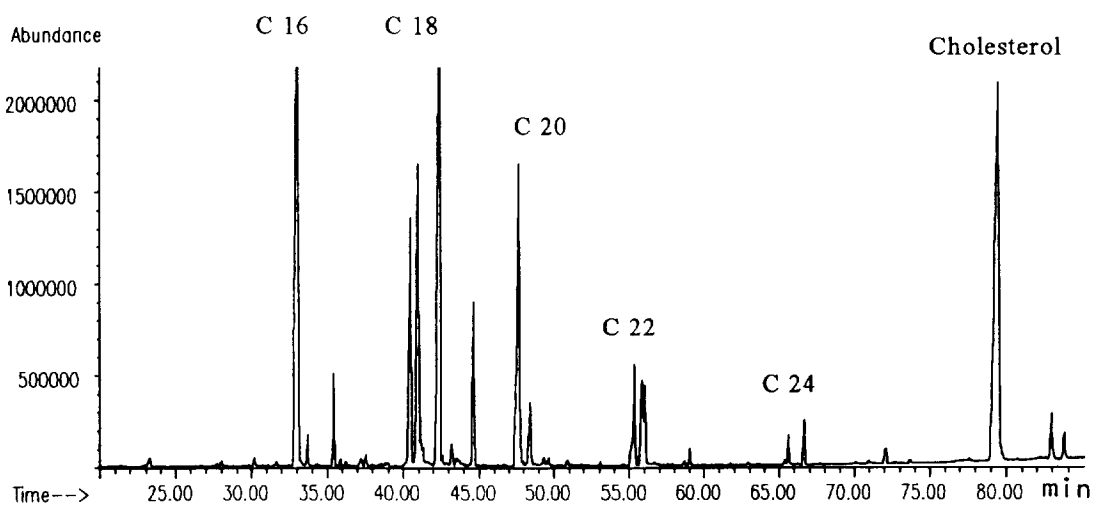

Fig. 3. Total ion chromatogram of red blood cells.

Detectors:	
Monitor detector in GC 1	FID
Main detector in GC 2	MSD, scan 50–450 u

3. Results and discussion

After transesterification of the lipid extracts, the samples were analysed by single-column GC–MS (see Fig. 5a) in order to determine the retention times used later for the cuts in multi-dimensional GC–MS (see Fig. 5b) together with the FID monitor's chromatogram. It was also used to characterize the fatty acid compositions of red blood cells, platelets and plasma.

3.1. Single-column chromatography

The major fatty acid components found in the blood samples were palmitic (C16:0), linoleic (C18:2, ω6), oleic (C18:1, ω9), stearic (C18:0) and arachidonic acid (C20:4, ω6). In plasma linoleic acid showed the highest relative concentration (26% of total fatty acids), followed by palmitic and oleic (21–24%), stearic (8%) and arachidonic acid (5%). The five major fatty acid

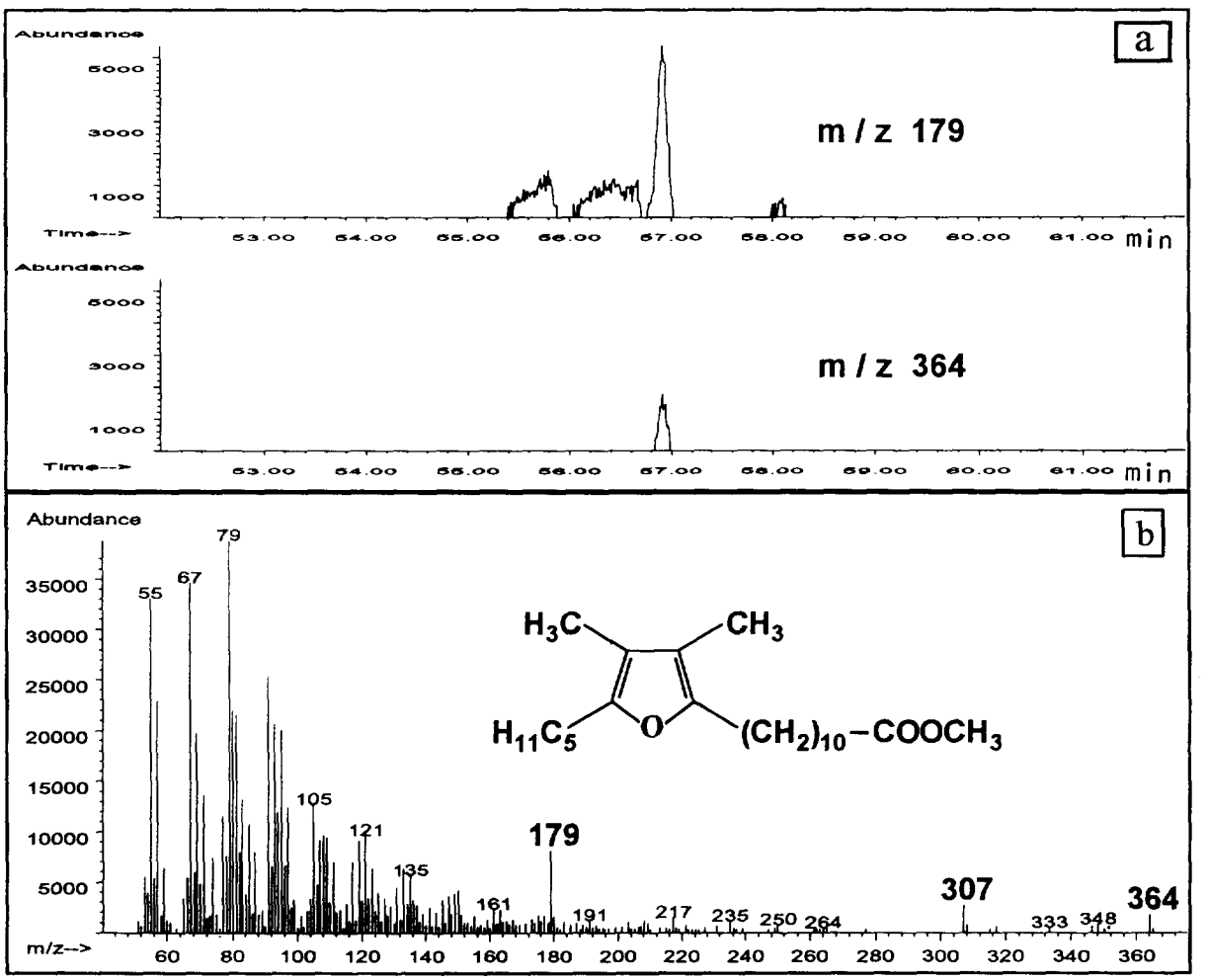

Fig. 4. (a) Ion chromatogram and (b) mass spectrum of furan fatty acid F6 obtained by single-column GC–MS (TIC Fig. 5a).

constituents in red blood cells were palmitic and stearic (17–20%), followed by arachidonic, oleic and linoleic acid with a similar distribution range of 10–14%. Finally, platelets revealed the highest relative amount of arachidonic acid (24% of total fatty acids), followed by palmitic (17%), stearic (14%), linoleic (14%) and oleic acid (10%). These relative amounts are in agreement with those reported in the literature [40,41]. Fig. 3 shows a typical chromatogram of a red blood cell sample.

Only furan fatty acid F6 (Fig. 4b) could be detected in all blood samples by single-column GC–MS when using single-ion monitoring. After injection of a larger volume of concentrated samples with the mass spectrometer operating in full-scan mode, it was possible to identify F6 in the ion chromatogram (Fig. 4a) by its mass spectrum. However, even after background subtraction there are many ions left in the mass spectrum of F6 due to unseparated substances, especially in the lower mass region (Fig. 4b).

3.2. Multi-dimensional GC–MS

By choosing the right cut times in the total ion chromatogram of the precolumn (Fig. 5) for the described configuration of the multi-dimensional

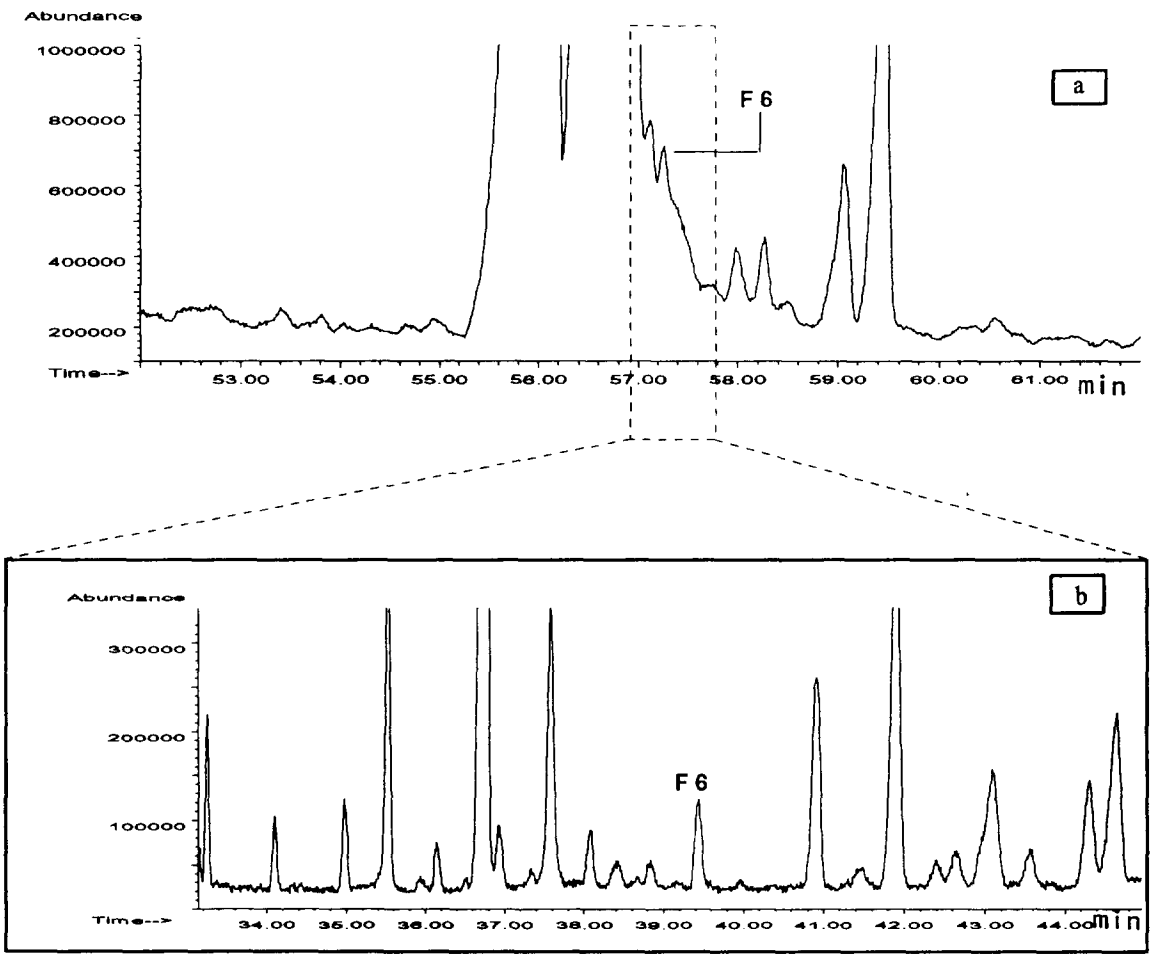

Fig. 5. Multi-dimensional GC–MS: TIC of (a) precolumn and (b) analytical column (red blood cells).

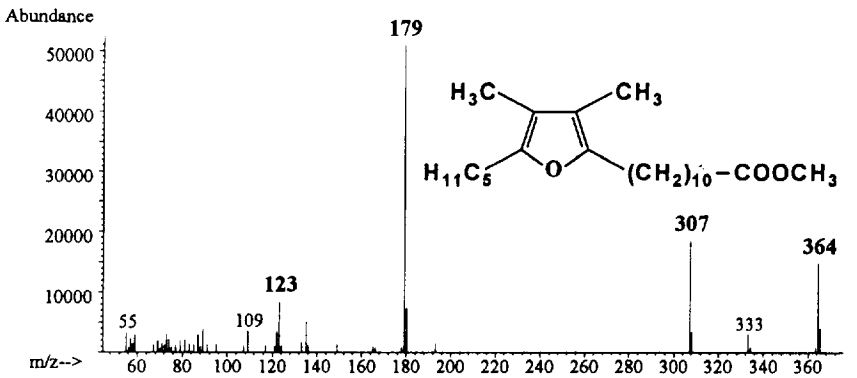

Fig. 6. Mass spectrum of furan fatty acid F6 obtained by multi-dimensional GC–MS.

GC–MS system, the methyl esters of furan fatty acids become well separated on the second column, as shown for the identification of F6 in red blood cells (Fig. 5). The mass spectrum of F6 (Fig. 6) is now free of any ions due to interfering substances. After furan fatty acid F6, which was found in all blood components, F4 (Fig. 7) was found in red blood cells and plasma. For 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 led to variable results: in most instances furan fatty acids could be detected, but not in all. As 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. In serum furan fatty acids were detected only in the phospholipid fraction, and not in cholesterol esters or triglycerides. This is in agreement with an earlier report by Puchta et al. [17].

4. Conclusions

The identification of minor component furan fatty acids in blood lipids has been achieved by using a multi-dimensional GC–MS system. Except for transesterification there is no need for any further pre-analytical separation or concentration steps. This method should also be applicable to the identification of any other minor component fatty acid in complex sample matrices.

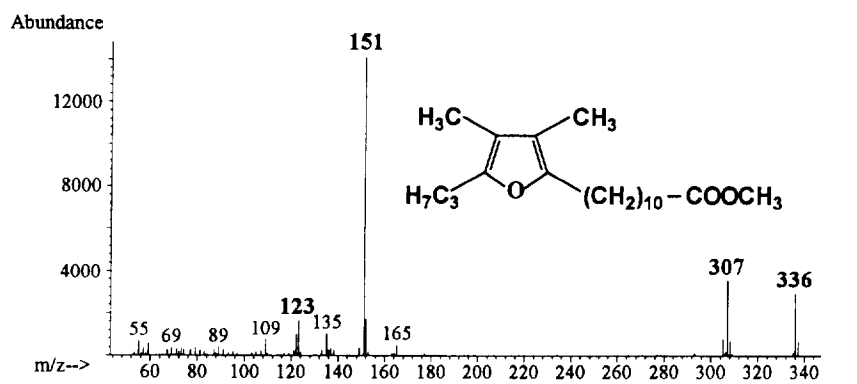

Fig. 7. Mass spectrum of furan fatty acid F4 obtained by multi-dimensional GC–MS.

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