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Review

Chromatography of very long-chain fatty acids from animal and plant kingdoms

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Abstract

Use of chromatographic methods, high-performance liquid chromatography (HPLC) and capillary gas chromatography (GC) for separation, isolation and identification of very long-chain fatty acids (VLCFAs) containing more than 22 carbon atoms is described. Two tables and the text summarize the most important papers dealing with this topic.

Keywords: HPLC; GC; Very long-chain fatty acids (VLCFAs)

1. Introduction

The fatty acids (FAs) are ever-present constituent of each genus and species of living matter. Usually, they are bound as lipids in cells of most organisms with the exception of oldest bacteria (*Archaebacteriae* sp.) that contain isoprenoid chains bound to glycerol by the etheric bond. Mostly, FAs contain from 12 to 22 carbon atoms, nevertheless the number of 16–20 prevails [1–6]. In the nature the saturated or unsaturated FAs with a chain longer than 22 carbon atoms are rare. Also, in the year 1995, a special issue of *Journal of Chromatography* [7] was dedicated to the analysis of long-chain carboxylic acids.

Having analyzed very long-chain fatty acids (VLCFAs) we get into problems, when the chain of FA is longer than 22 carbon atoms. There are only a few papers dealing with their existence in microbes [8], plants and animals [9], insects [10], high animals [11], meibomian gland [12], wool wax [13], human beings suffering from pathobiochemical diseases (inability of α and β oxidation of FA) [14] and plant waxes [15].

As more than 270 citations were compiled and evaluated in this review, it is a fragment of current knowledge, when compared with the number of papers dedicated to FAs and quoted by Chemical Abstracts (CA-1967 to date, ∼143,300, CA-1987 to date, ∼78,300). Taking into account the last 14 years, the mean numbers of papers concerning FAs per year get near 5600. However, the majority of those papers are concerned on FAs with 12–22 carbon atoms.

In the following, we disregard such compound as Aikupikanyne F [16] or Aliclonyne [17] or Osirisynes A-F [18] that were described elsewhere in the literature. These substances were discovered as new natural compounds; nevertheless the method of their chromatographic separation was not described. In addition, these substances go beyond the classical definition of FA, even they consist of carboxyl groups and aliphatic chains. Unfortunately, the books and/or reviews treating the chromatography of FAs [19,20]

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almost completely avoid VLCFA. That is on of the reasons why this paper was written.

2. Standards

Very important sources about all areas of FAs, including the nomenclature are the following WWW page: http://www.lipid.co.uk/infores/lipids.html. In general, all the saturated straight-chain FAs up to 31:0 are commercially available; their price increases with the chain length and, also, the odd-number FAs are a bit more expensive. The problem exists with the acids higher than C_{31}, whose standards are not available on the market. Two approaches can be applied to overcome this situation. At first, a natural mixture possibly enriched with VLCFA can be used. Secondly, one has to synthesize those compounds in the laboratory.

Predominantly, rare and unusual FAs were synthesized [21] via boranes. With the help of organoborane chemistry, convenient and simple syntheses of long straight chain carboxylic acids were performed. First method entails the thermal isomerization of long-chain alkyldicyclohexylboranes, followed by oxidation. An alternative procedure involves the isomerization of internal alkynes to the terminal derivative, which can be oxidized directly to the carboxylic acid [22] or phosphoranes [23], i.e. alkyltriphenylphosphonium bromides with lithium hexamethyldisilazide, reacted with ω-oxo esters to give the corresponding methyl *cis*-alkenoates. By an alternative method, treatment of ω-iodo esters with the complexes formed from reactions of alkylcopper(I) and Grignard reagents gave methyl alkanoates, *cis*-alkenoates, and methylene-interrupted *cis*,*cis*-alka-dienoates and *cis*,*cis*,*cis*-trienoates [23]. Further paper [24] described stereoselective synthesis of unsaturated very long chain FA methyl esters based on the new transition metal-catalyzed coupling reaction between an organomagnesium reagent and a functionalized vinyl bromide.

If we strictly apply the definition of VLCFA as the acid higher than C_{22}then the only unsaturated acid available is nervonic acid (15–24:1). To get such compound, it is necessary, either to carry out many subsequent organic reactions or to perform a complex and very difficult isolation from the natural material. Sometimes, a very special and rare material must be used for the isolation like eyes retina, bull sperm, marine sponges, etc. In most cases, natural sources like herring were used for the isolation of VLCFA [25,26].

3. Separation of VLCFA

In this chapter we made an effort to cover up the papers dealing with an enrichment of original sample with VLCFA, nevertheless there are cited many papers, where high-performance liquid chromatography (HPLC) technique was in use.

As already mentioned in the introductory part, the content of VLCFA is rather variable and, therefore, it is suitable to employ the enriching methods in the first step. However, this fact is very often neglected. Few papers deal with the sample enrichment with VLCFA in a complex way. Principally, there are used two techniques of enrichment. The first one includes the separation of intact lipids containing VLCFA and subsequent evaluation of the present FA. The second approach derives benefit of a selective isolation of VLCFA from the total FA fraction. The former technique was used for identification of VLCFA in marine sponges that have represented a well-known source of VLCFA for last 40 years. The content of FA [27,28] in relationship to intact lipids in marine sponges was investigated. As an outstanding example of such study, the papers of Djerassi and coworkers [27] has to be cited herein [28]. The authors used the preparative HPLC for separation of the individual phospholipid classes phosphatidyl choline (PC), phosphatidyl ethanolamine and phosphatidyl serine and, subsequently, reversed phase high-performance liquid chromatography (RP-HPLC) for the isolation of molecular species (methanol–ethyl acetate–chloroform–water on C_{18}-phase column). The last of the 10 fractions eluted was repeatedly purified on chromatograph until the chemical individual was obtained. After a hydrolysis, the acid was identified by GC–MS of FAME, namely 5, 9–26:2 whose structure was confirmed by derivatization as *N*-acyl pyrrolidide. The structure of intact phospholipids after RP-HPLC was proven by fast atom bombardment mass spectrometry (FAB-MS). Such a way, the authors found that the marine sponges contained unusual symmetrical phospholipids (e.g. 1,2-di-5,9-hexacosadienoyl-glycero-3-phosphoethanol-amine).

Several authors reported the isolation of a very long-chain polyunsaturated fatty acid (VLCPUFA) from an animal eye's retina [29,30]. It is remarkable that the VLCPUFA was identified till 1987 as an animal eye's retina was investigated for few decades and all necessary information was available throughout. Because the content of FA longer than C_{24} is very low (tenths or hundredths per cent of total FA), it is necessary to enrich the total FA in VLCPUFA. Several methods of enrichment were used. Diglycerides were prepared from PC by the action of phospholipase C and, subsequently, separated by $AgNO_3$-impregnated thin layer chromatography (Ag^+-TLC). In the fraction of supraenes (more than six C=C), acids up to 36:3 were found, while the largest proportion of VLCPUFA was detected in undeca-, deca- and nonaenes. RP-HPLC with ODS column and acetonitrile as the mobile phase was used for the separation of individual fractions obtained by Ag-TLC. Such a way were obtained the molecular species of diglycerides with following FA: 36:6, 34:6 and 32:6 in dodecaenes and 36:5, 34:5 and 32:5 in undecaenes. Always, as the second acid was present 22:6.

If FA in the brain of patients suffering from Zellweger's syndrome were examined [31] most of VLCFA was present in a special PC fraction. By using a twice-repeated column chromatography, TLC and high performance thin layer chromatography (HPTLC), the previously mentioned PC could be separated from a PC having a "normal-lenght" FA. The high-molecular PC had a R_F (retardation factor) of 0.61, whereas the R_F value of normal PC was only 0.52. The structure of PC fraction containing VLCFA was determined by ^1H NMR (nuclear magnetic resonance) and FAB-MS. The amount of 2.8 μmol of the high-molecular PC was separated from 45 g of disordered brain mass sample. Fig. 1 compares a chromatogram of VLCFA methylesters that was prepared from healthy and disordered brain samples. It is remarkable that FAs with 36 carbon atoms containing 5 and 6 unsaturated bonds were detected in the healthy brain in many times lower concentrations.

As phosphatidylcholine [31] as sphingomyelin (SPH) [32] with VLCPUFA were described and separated. Simultaneously, the band exhibiting a higher R_F was enriched with VLCPUFA. Based on splitting technique, the structure of SPH and phosphatidylcholine (including the bound VLCPUFA) can be determined by FAB-MS. After hydrolysis, the VLCPUFA from the two polar lipids were estimated by GC–MS. Takayama and coworkers [33] published a good example of the technique that was described earlier. The authors applied the classic method of column chromatography by lipophilic Sephadex LH 20. An amount of 6 g of the saponifiable fraction of the sample was separated on a silica gel column. As a result of this operation was prepared 1.65 g of the VLCFA-enriched fraction. Another high yield separation [34] was achieved by using a 144 cm long Sephadex LH-20 column. There were obtained two fractions. The first one contained about 80 mg (C_{41}–C_{56}) and the second one 150 mg (C_{30}–C_{40}) of the material. After subsequent separation by chromatography techniques (TLC, RP-HPLC), the compounds were identified by using MS.

As van der Veen et al. [35] remarked that sodium salts of VLCFA could be selectively extracted into petroleum ether. So, the non-polar solvent phase is enriched with VLCFA. On contrary, the application of K^+, NH_4^+ and Ca^{2+} salts [36] does not support van der Veen finding [35]. Nevertheless, after a three-fold extraction with hexane, the total of 3.1 μg of sodium salts in the respective proportion of 1.00:1.01:1.01 was obtained from a mixture of sodium salts of the 18:0, 24:0 and 28:0 acids (a total of 6 mg at a respective mass proportion of 1:1:1). To conclude, none enrichment of the extract with VLCFA was observed. When TLC on silica gel was employed, it provided reliable results if the chromatography standards were applied, but failed when a real mixture has to be separated. Similar results were published by Qureshi et al. [37], which separated the VLCFA from mycobacteria as *p*-bromophenacyl esters into four fractions, by TLC technique. The following RP-HPLC demonstrates the low selectivity of the separation. All peaks of each four fractions obtained by TLC contain a different portion of corresponding VLCFA compounds.

When the reverse phase in combination with TLC or HPLC [36] was used, the better results were achieved. With help of RP-HPLC, the original model mixture (1:1:1) was enriched to the ratio 1:10:13. The enrichment ratio of VLCFA is then about 1 order. The disadvantage of the procedure is a small separation capacity of the layer with respect to the amount of the material that could be separated.

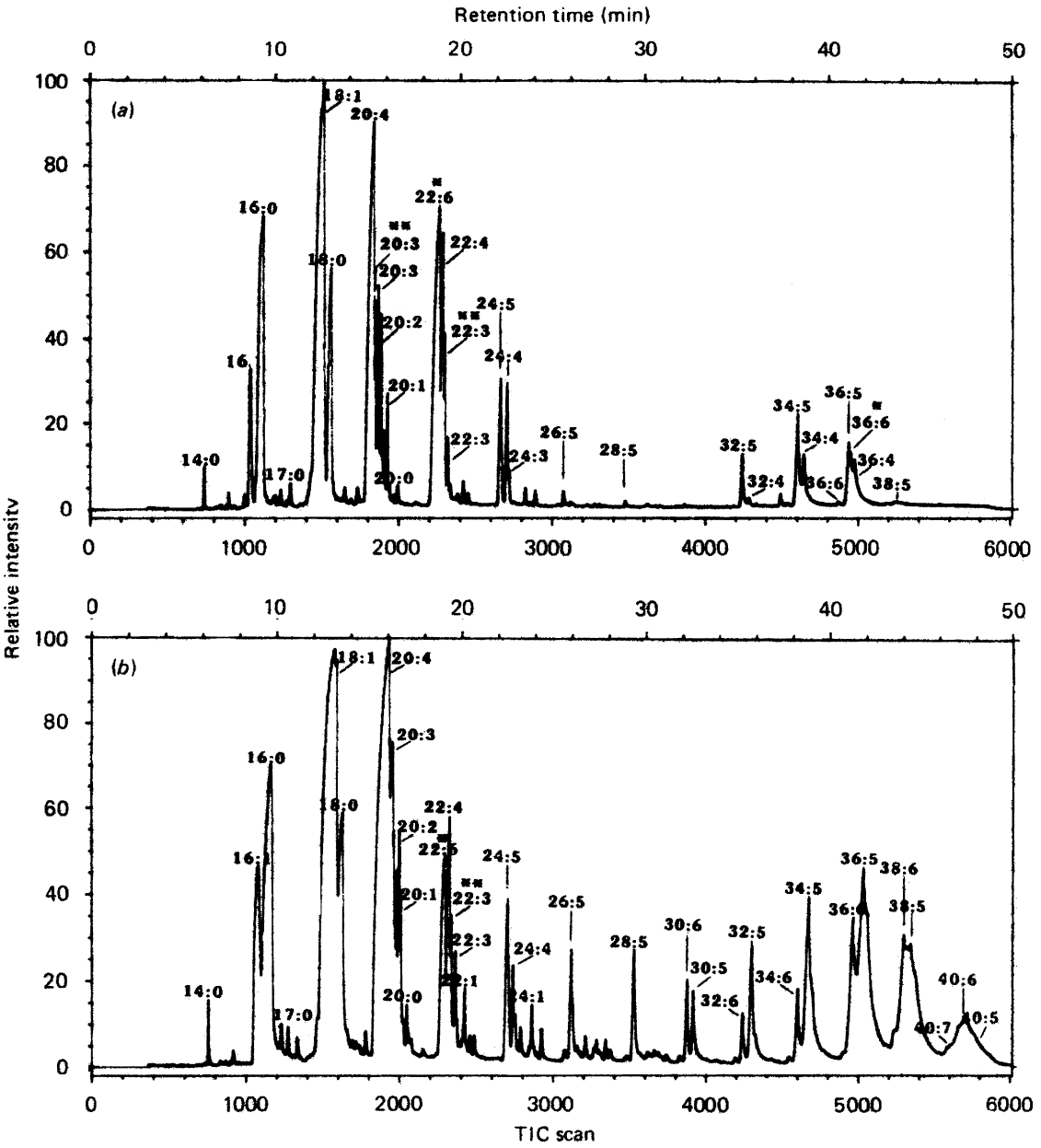

Fig. 1. GC–MS of normal and Zellweger brain phospholipid-bound FAs. FAME from normal (upper) and Zellweger (lower) brain [31]. Experimental conditions [106] are same as in Table 1.

Another technique used a partial degradation of unsaturated FA, and non-separation of homologues in the natural mixture that differ in the presence of one CH_2 group.

A paper describing the enrichment of the total FA fraction with VLCFA by HPLC (RP-1) technique on a semipreparative scale [38] was published. With the help of double chromatography, an amount of 32 mg

of VLCFA longer than 28:0 was prepared from 3.5 g of FAME. The same method was successfully applied in another study, when a C_{18} reverse-phase column was used [39].

Preparative HPLC of FAME from herring on cation/exchange column containing Ag^{+} ions was described [25]. The obtained PUFA fraction was subsequently separated on a polar capillary gas chromatography (GC). The structures of the individual FAME were identified by chemical ionization-mass spectrometry (CI-MS), comparing the *m*/*z* 109 and 151 ions characteristic of ω-3 and ω-6 double bonds. The even PUFA as large 32:6ω-3, 30:5ω-3 and 30:6ω-3, the odd acids (25:5 and 27:5) and a minor component (28:7ω-3) previously undetected in marine fish were identified.

The capillary GC, mass spectrometry (MS) (60 m polar capillary column with Supelcowax in the quadrupole mass spectrometer, i.e. QP-1000 from Shimadzu) were employed for the identification of long-chain monocarboxylic (fatty) and dicarboxylic acids (up to 36 and 33 carbon atoms) in peat, lignite, brown coal, bituminous coal, anthracite and graphite samples. As the FA enrichment technique was used solid-phase extraction in a cartridge system [40]. The other method for the enrichment of VLCFA fraction FA-containing samples was described in the paper [41]. The method is based on the use of RP-HPLC and/or solid-phase extraction cartridges with following identification of VLCFA by capillary gas GC–MS. By combining the two techniques, unusual VLCFA were identified in newly isolated oligotrophic bacteria

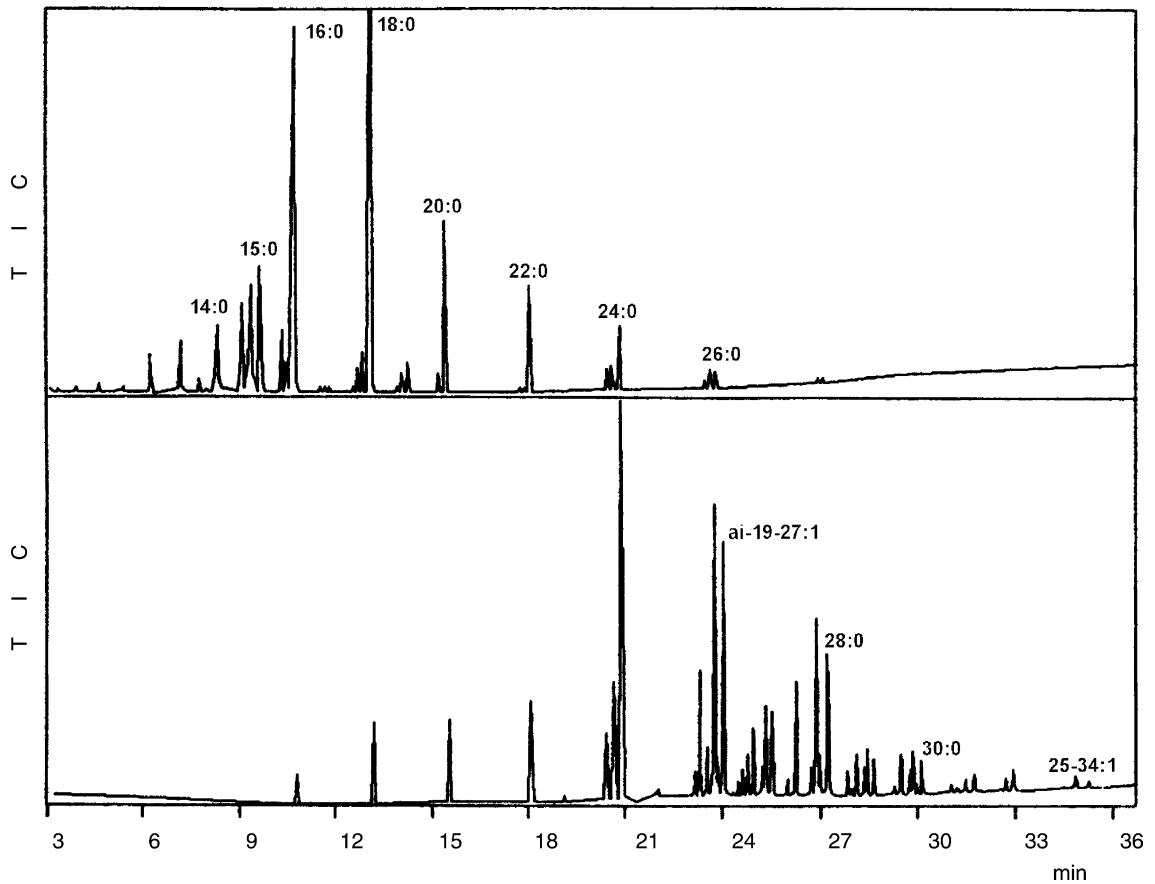

Fig. 2. FAME from soil oligotrophic bacteria *Renobacter vacuolatum*. Total FAME (upper); FAME after SPE (lower), [41]. For experimental conditions see Table 1.

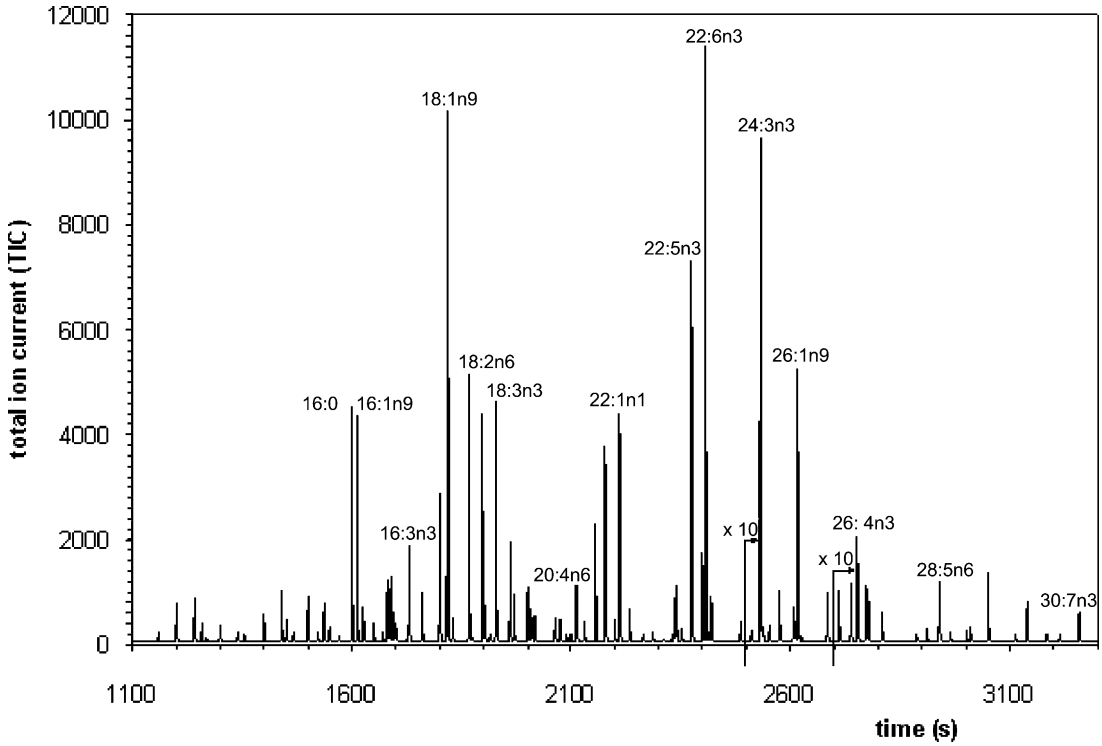

Fig. 3. GC–MS of FAME from *Bathynella natans*, [42]. For experimental conditions see Table 1.

(Fig. 2). In the past, VLCFA were identified and described in sulfate-reducing bacteria and in some wood-degradating fungi.

Total lipids of three crustacean species, *Bathynella natans*, *B. baicalensis* and *Baicalobathynella magna* from Lake Baikal and the caves of central Europe were analyzed for VLCPUFA using Ag^{+}-TLC and GC–MS [42] (Fig. 3). VLCPUFA in the total lipids were mainly ω-6 and ω-3 PUFA, i.e. 24:3ω-6, 24:3ω-3, 24:5ω-3, 26:3ω-6, 26:5ω-6 and 28:7ω-6. The major VLCPUFA components in all three samples were 24:3ω-6 (0.4–0.5% of total FAs) and 24:3ω-3 (similar to 1%), but 26:5ω-6 (0.08%) was also prominent in the species from Lake Baikal. The total lipids of the Lake Baikal species contained considerably more 26:5ω-6 and 28:7ω-6 than those from caves. The differences among the VLCPUFA in these species appear to be due mainly to different environmental conditions, dietary habits and geographical spreading.

In the further paper, the VLCFA from the same sources were determined by means of liquid chromatography (LC)–MS with atmospheric pressure chemical ionization (APCI) [43] (Fig. 4). This method enabled to identify more than fifty VLCPUFA. These acids were described in the crustaceans for the first time, predominantly 26:5ω-6, 28:7ω-6, 30:7ω-3 and 40:7ω-6.

A method for the enrichment of VLCFA from total FA of heterotrophically cultivated green freshwater algae *Chlorella kessleri* and their identification as picolinyl esters by means of LC–MS with APCI [44] was successfully verified. The method is based on the use of preparative RP-HPLC of 100 ml samples and their subsequent identification by microbore APCI LC–MS (Fig. 5). The combination of these two techniques was used to identify unusual VLCFA up to C_{47}, both saturated and monounsaturated, with two positional isomers (ω-9 and ω-26).

TLC analysis was used in study of FA radiolabelled by ^{14}C up to 36:4 that were isolated from rat brain. In the first development step the Ag-TLC method was applied. In the second one the RP-TLC was used

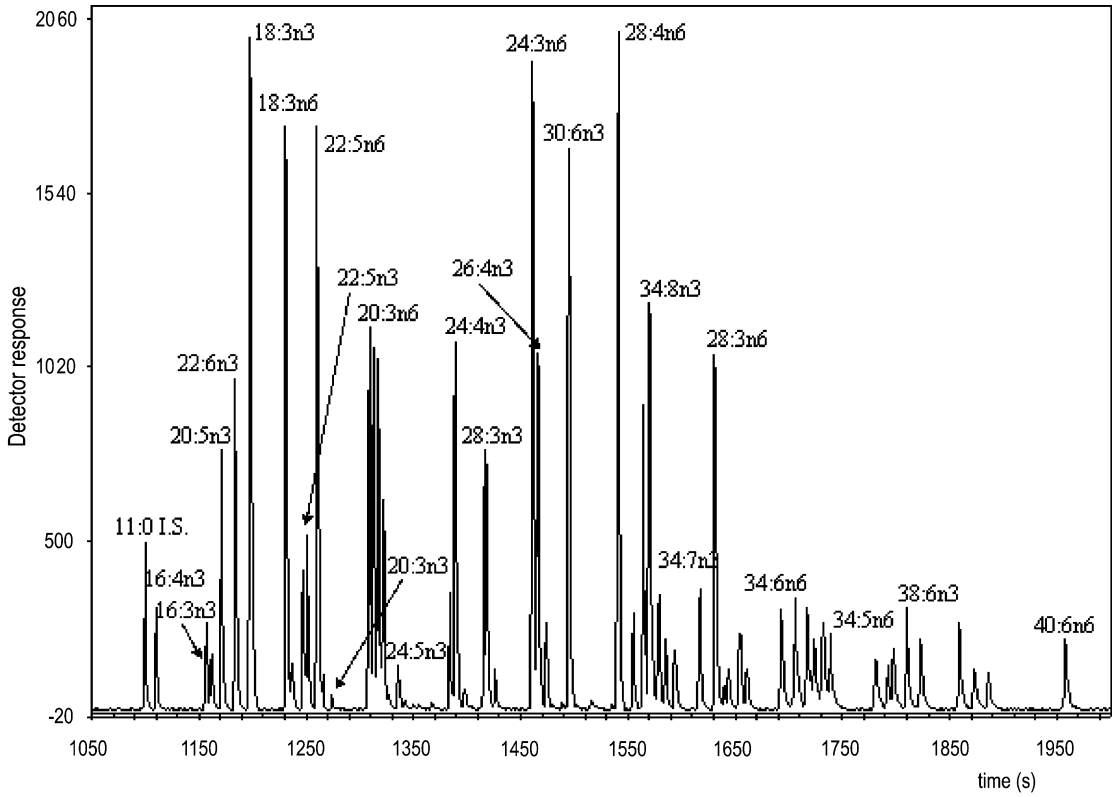

Fig. 4. Chromatogram (LC–MS with APCI) of VLCPUFA from *Bathynella baicalensis*, [43]. For experimental conditions see Table 2.

for separation of individual compounds [45]. The RP-TLC [46] technique was successfully applied for the separation of PUFA up to 28:4 from normal and Zellweger syndrome skin fibroblasts. Similarly, Aveldano and Sprecher [30] employed the method of TLC for the separation of VLCPUFA. Methyl esters of supraenoic species of PC were separated by using of silica-gel TLC (mobile phase = hexane:diethylether, 95:5). After separation, two bands were obtained where the upper one was enriched by VLCPUFA. For the further chromatographic separation of the upper band Ag^+-TLC technique with chloroform:methanol (95:5) was used. The procedure resulted in several fractions containing hexaenoic, pentaenoic, etc. The identification of FAME was performed on the basis of their retention characteristics that were obtained by analysis on a packed column in isothermal conditions. The pentaenoic and tetraenoic fractions were obtained nearly pure state; whereas the hexaenoic fraction was contaminated with FAME containing five unsaturated bonds.

Based on the analysis of VLCFA and phytanic acid in plasma or serum, most of peroxisomal disorders can be detected. Previous methods utilizing gas–liquid chromatography (GLC) are time consuming and are influenced by interference with cholesterol derivatives. Herein, we refer to GC–MS use for the simultaneous quantification of VLCFAs and phytanic acid. The method employs single ion monitoring with deuterated internal standards. Vallance and Applegarth [47] studied 38 normal controls and 12 patients with peroxisomal diseases. They found a complete discrimination between the two groups.

HPLC [48–50] technique allowed separating the esters of the acids from C_{22} to C_{32}. A fraction of FAME (as far as 30:5) was isolated from rat testicles. In latter separation step, the RP-18-phase RP-HPLC with an acetonitrile–methanol–water mixture as the

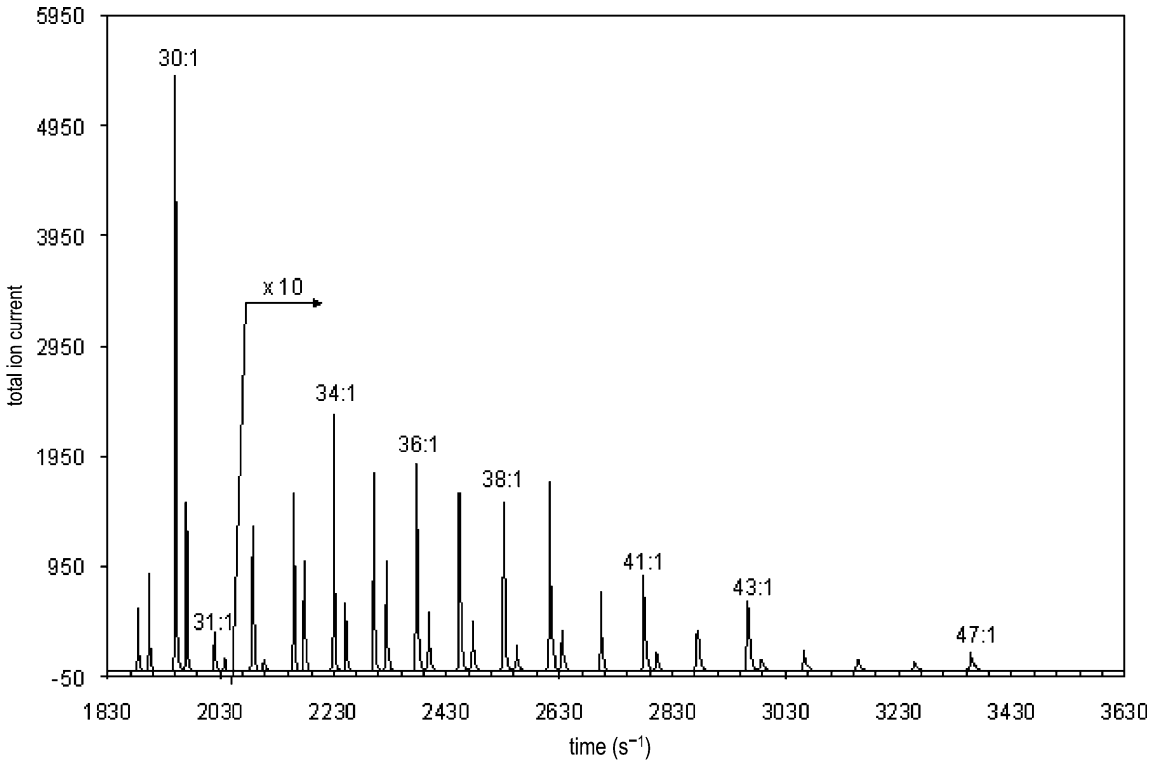

Fig. 5. Chromatogram (LC–MS with APCI) of VLCPUFA from *Chlorella kessleri* [44]. 1090 HPLC system with mass spectrometer system and two narrow-bore columns 250 mm × 2.1 mm, i.d. 5 μm phase particle. Conditions: vaporizer temperature 400 °C, capillary heater temperature 220 °C, corona current 5 μA, sheath gas (nitrogen) pressure 55 psi, and auxiliary gas flow rate 15 ml/min. Ions with *m/z* 200–900 were scanned with a scan time of 0.5 s. The flow (0.37 ml/min) was introduced into the APCI source without splitting. FA were separated using a gradient solvent program with acetonitrile, dichloromethane and propionitrile, linear gradient from (60:30:10, v/v/v) to (30:40:30, v/v/v) (3630 s); a peak threshold of 0.3% of intensity.

mobile phase [49] was applied. Other stationary phases have also been used (C_1 or C_8), namely for separation of VLCFA [48,50]. Minor FA in the range of C_{20}–C_{30} were isolated from patients suffering from adrenoleucodystrophy [51,52]. The fractions of higher FA having 30–56 carbon atoms in the chain were identified in *Mycobacterium tuberculosis*, by capillary GC and collision activation dissociation MS were also described in a paper [53]. These compounds were also separated as *p*-bromophenacyl esters by using a mixture of dioxane–acetonitrile as the mobile phase. Anthryl esters that exhibit a high fluorescence were separated by C_{18} RP-HPLC with acetonitrile as the mobile phase. The LC–MS method was employed for the analysis of anilides of even-number FA in the range of 16:0–30:0 [54]. For this purpose, a gradient separation from a mixture of methanol:isopropanol (95:5) into a mobile phase containing a greater amount of isopropanol was used. Pseudomolecular ions $(M + H)^+$ were scanned, all at once. These ions dominated in mass spectrum of all anilides during elution. A possibility of isolation of minor VLCFA from FA was described [55]. The procedure was linked with enrichment of VLCFA in an isolated fraction. The authors took advantage of well-known ability of the reverse phase to separate compounds according to the lengths of their chains. A mixture of FA can be selectively enriched with VLCFA by taking use of RP-TLC technique or better by RP-HPLC (Fig. 6).

In order to demonstrate the feasibility of the chromatographic procedures we outlined its ability for the separation of FA enantiomers. Usually, the chirality of

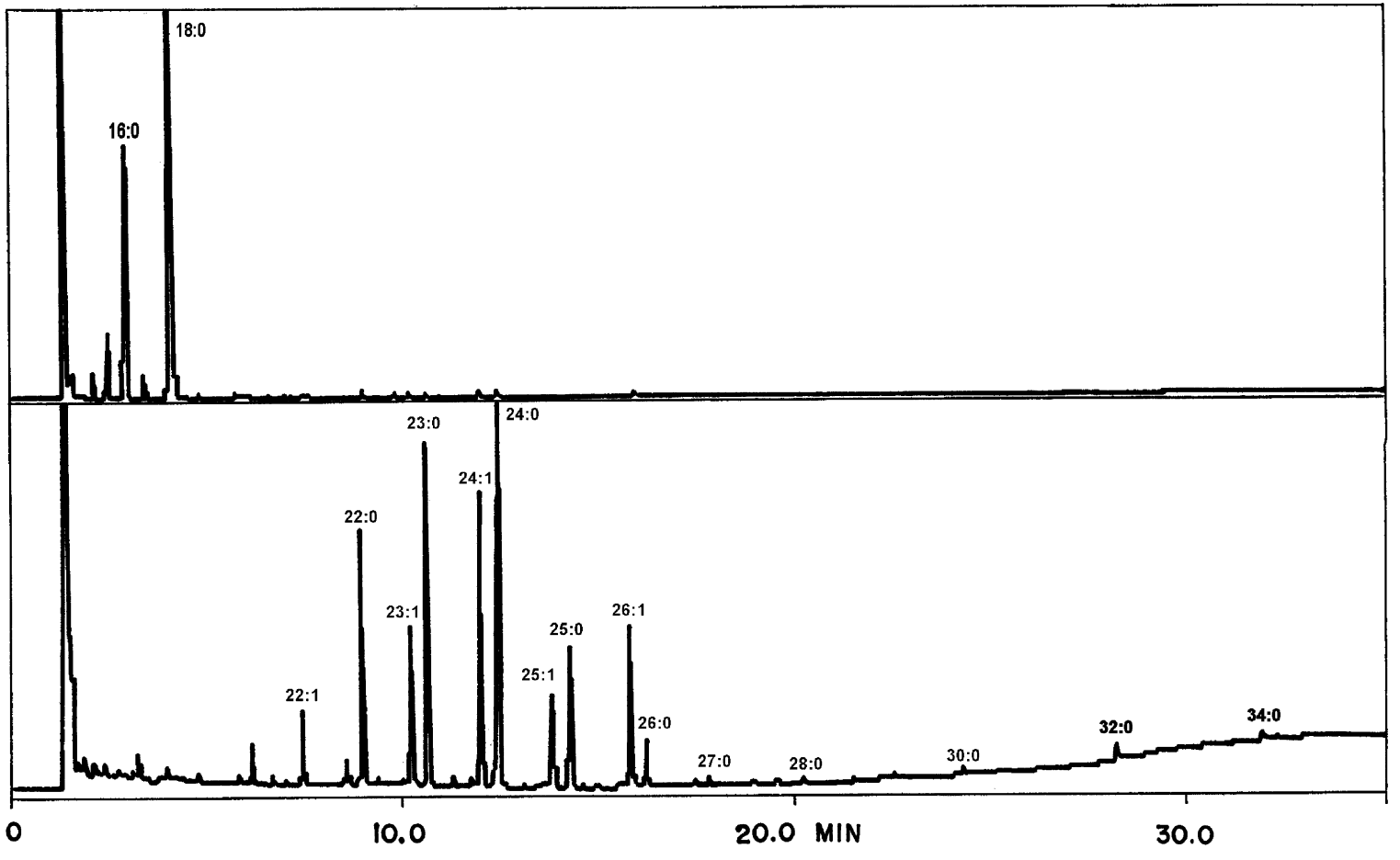

Fig. 6. GC–MS of FAME from fungus *Ganoderma applanatum*. Total FAME (upper trace); (lower trace) FAME after preparative RP-HPLC. Peaks: (1), 16:0; (2), 18:1, [55]. For experimental conditions see Table 1.

Table 1
GC of VLCFA

Column	Injection	Gas	Gradient	Detector	Compound	Source	Figure	Reference
20 × 0.3; OV-1		He 1.5 ml/min	80-4-260	FID	FAME (36:3 and 36:2)	Marine algae Emiliana	Yes	[91]
20 × 0.3; OV-1		He 2 ml/min		MS	FAME (36:3 and 36:2)	Marine algae Emiliana	No	[91]
25 × 0.25; OV-101	Split 1:8	N 0.5 ml/min	280 Isotherm	FID	FAME	Bovine brain cerebroside and sulphatide	Yes	[92]
28 × 0.32; SE-54	On-column		200-3-285	MS	Pyrrolidides up to 5,9-ai-27:2	Marine sponge Petrosia	No	[93]
30 × 0.32; SE-54	On-column		140-3-285	FID	FAME up to 5,9-ai-27:2	Marine sponge Petrosia	No	[93]
50 × 0.2; SP-2100		H 180 kPa	70 (0.5)-20-150-2-270 (40)	FID	FAME up to 32:0	Standards	No	[94]
28 × 0.32; SE-54				MS	2-MeO-FAME (PUFA) up to C_{30}	Marine sponge Higginsia	No	[95]
30 × 0.32; SE-54	On-column		70 (or 140)-3 (or 10)-290	FID	2-MeO-FAME (PUFA) up to C_{30}	Marine sponge Higginsia	No	[95]
25 × 0.25; Silar 10C	Split	H 30 cm/s	150-4-240	FID	FAME up to 34:0	Cigarette smoke	Yes	[96]
25 × 0.3; SE-54	Splitless	He 1.5 ml/min	70 (0.5)-30-130-8-300	MS	2-OH-FAME as TMS up to 26:0	Algae and blue-green algae	No	[97]
60 × 0.75 × 0.2; SP-2340	Splitless	Ar 20 cm/s	120 (10)-2-260	FID	FAME up to 30:5	Rat testis	Yes	[49]
15 × 0.32; SE-54	On-column	He	190-2-285	FID	Pyrrolidides up to 30:3	Marine sponge Aplysina	No	[98]
15 × 0.32; SE-54	On-column	He	70-3-280 or 130-2.5-280	FID	FAME up to 30:3	Marine sponge Aplysina	Yes	[98]
50 × 0.3 × 0.52; OV-1	On-column	He 2 ml/min	100-2-320	FID	FAME, pyrrol., picol. up to 29:0.	Meibomian gland (mouse)	Yes	[99]
50 × 0.3 × 0.52; OV-1	On-column	He 2 ml/min	100-2-320	MS	FAME, pyrrol., picol. up to 29:0	Meibomian gland (mouse)	No	[99]
30 × 0.32; SE-54			70-5-290	FID	FAME up to 30:0; n, br, monounst.	PL of marine sponge (Strongylophora)	No	[100]
30 × 0.31 × 0.25; SE-30		N 3 ml/min	75 (2)-8-300	FID	FAME up to 26:0; n, i, ai, monounst.	Meibomian gland (steer, human)	No	[101]
25; Sil 8 CB	Splitless	He	44 (1)-180-8-290	MS	FAME up to 26:0	Fibroblasts (Zellweger s.)	No	[102]
25; Sil 8 CB	Split 1:10	He 1 ml/min	160 (2)-5-230-10-285 (2)	FID	FAME up to 26:0	Blood (Zellweger s.)	Yes	[103]
30; SE-54			40-8-300	MS	FAME up to 28:0	Mycobacterium	Yes	[104]
50 × 0.2; OV-1	Split 170:1	1.5 ml/min	160-2-270	FID	FAME + OH-FAME up to 28:0 or OH-30:1	Grevillea (plant)	No	[105]
12 × 0.3 × 1.0; BP-1	On-column			MS	FAME up to 34:7	Zellweger brain	No	[106]
12 × 0.3 × 1.0; BP-1	Splitless	H 45 cm/s	165 (0.5)-4-320	FID	FAME up to 34:7	Zellweger brain	Yes	[106]
12 × 0.3 × 1.0; BP-1				MS	FAME up to 34:7	Zellweger brain	No	[106]
25 × 0.25; RSL-300			100-4-300	MS	FAME up to C_{30}	Green algae	Yes	[107]
30 × 0.32; SE-54	On-column		130-5-290	FID	FAME up to 26:3	Marine sponges	No	[27]
30 × 0.32; SE-54	On-column		130-5-290	FID, MS	FAME up to 2-AcO-30:0	Marine sponges	No	[108]
30 × 0.32; SE-54	On-column		190-10-290	FID, MS	FAME up to 2-AcO-30:0	Marine sponges	No	[108]
30 × 0.32 × 0.25; DB-1	Splitless	He 30 cm/s	210 (1)-6-330	FID	FAME up to 26:0	Zellweger syndrome	Yes	[109]
60 × 0.25 × 0.25; DB-1	On-column	He 30 cm/s	110 (0.5)-16-240-3-310 (10)	MS	FAME up to 26:0	Zellweger syndrome	No	[109]
12 × 0.22 × 0.25; BP-1	Splitless			MS	FAME up to 34:6	Mammalian spermatozoa	Yes	[110]
15 × 0.25; DB-1	Splitless	He	70 (1)-30-290	MS	FAME up to 34:6	Bovine retina	No	[30]
30 × 0.32; SE-54			190-10-290	FID	FAME up to 26:3	Marine sponges	No	[111]
30 × 0.33 × 0.25; SE-30			150-4-290	FID	FAME up to C_{32}, OH-FAME as TMS ethers	Finnish peat	Yes	[112]
30 × 0.32 × 0.25; SE-30	Split	H 2 ml/min	150-4-285	FID	FAME (and other acids) up to C_{32}	Finnish peat	No	[113]
30 × 0.3; SE-30			150-4-290	FID	FAME up to C_{28}, OH-FAME as TMS ethers	Peat (Sphagnum)	Yes	[114]
50 × 0.20; OV-1 HP	Splitless	H 1 ml/min	50-10-160-4-300	FID	FAME up to 26:1	Bacteria Nitzschia	No	[75]
50 × 0.20; OV-1 HP	Splitless	He	100 (0.5)-3 or 4-300	MS	FAME up to 26:1	Bacteria Nitzschia	No	[75]
50 × 0.20; OV-1 HP		H 40 cm/s	70-20-150-2-270	FID	FAME up to 32:0	Mangrove sediments	No	[115]
12 × 0.22 × 0.25; BP-1		He 1 ml/min	160-4-320	MS	FAME up to 36:5	Zellweger syndrome	Yes	[116]
12 × 0.22 × 0.25; BP-1	Splitless		160-4-320	MS-s	FAME up to 34:6	Zellweger syndrome	Yes	[82]
20 × 0.32; SE-52	On-column	He	125-4-310	FID, MS	FAME up to 26:0, i, ai, 3-OH-FAME	Marine sediments	Yes	[117]
30 × 0.32 × 0.3; OV-1				CADMIKE	Perfluorobenzyl esters of FA up to 27:1	Mycobacterium	Yes	[79]
25 × 0.32; SE-54	On-column		130-5-290	FID	FAME up to 30:3	Marine sponges	No	[118]
25 × 0.32; SE-54			170-5-320	FID	FAME up to 30:3	Marine sponges	No	[118]
30 × 0.25; DB-5	Split 10:1	He 1.3 atm	250-4-320	MS-s	Pyrrolidides up to 34:X, n, i, ai, monounst	Skin of monkey(Macaca)	No	[119]

30 × 0.25; DB-5	Split 50:1	He 1.3 atm	200-4-320	FID	FAME up to 34:X, n, i, ai, monounst	Skin of monkey(Macaca)	No	[119]
25 × 0.2; OV-1			100-6-280	MS	FAME up to C_{24}	Earthworm	No	[120]
28 × 0.28; SE-54			170-3-240	MS	DMOX up to 24:1	Standards	Yes	[121]
25 × 0.3; SE-54			170-5-320	FID	FAME up to 30:3	Marine sponge	No	[122]
25 × 0.3; SE-54			170-5-320	FID	FAME up to 30:3	Marine sponge (Pseudaxinyssa)	No	[122]
25 × 0.32 × 0.25; OV-1701	Splitless	He 3 ml/min	50 (2)-16-200 (0.5)-4-240	FID	FAME up to 26:0	Refsum's disease	Yes	[123]
12.5 × 0.2; Me silic. HP	Splitless	He	150 or 200-3 or 4-320	MS-q	TMS alcohols up to 46:0	Cabbage looper (Trichoplusia)	Yes	[124]
25 × 0.53 × 0.15; OV-1		H 2.5 ml/min	70-4-290	FID	FAME up to 35:0, c, i, ai, monounst.	Marine sediments	Yes	[125]
30 × 0.32 × 0.25; DB-1		He	80-32-210-6-300-25-350 (3)	FID	FAME up to C_{26}	Human blood Zellweger syndrom	No	[126]
26 × 0.32 × 0.13; CPSil-5		He	130-4-330	MS	FAME up to 30:0	Freshwater sediment	Yes	[127]
26 × 0.32 × 0.13;CPSil-5	On-column	He	100-130-4-330	FID, MS	FAME up to 30:0, n, i, ai, monounst., B, ω-OTMS	Lake sediments	Yes	[127]
25 × 0.2 × 0.33; OV-1	Split 1:10	He 1 ml/min	180-2-350	MS	picolinylesters up to C_{26}	Ear wax	Yes	[128]
25 × 0.2 × 0.33; OV-1	Split 10:1	He 1 ml/min	180-2-350	MS	TMS-alcohols and FA up to C_{26}	Human cerumen(ear wax)	No	[128]
50 × 0.3 × 0.52; OV-1	Split 15:1	He 2 ml/min	130-2-380	FID	TMS-alcohols and FA up to C_{26}	Human cerumen(ear wax)	Yes	[128]
25 × 0.2 × 0.33; OV-1	Splitless	He 1 ml/min	150-2-350	MS	TMS-alcohols and FA up to C_{33}	Gerbil meibomian gland	No	[129]
60 × 0.25; DB-5		He	60 (2)-20-160-2-290 (20)	MS	FAME up to C_{28}	Marine sediments	No	[130]
30 × 0.32; DB-17	Splitless		100 (1)-24-210-2-225	FID	FAME up to 30:1	Human brain-peroxisomal diseases	No	[131]
25 × 0.25 × 0.25;CPSil8CB	Solid inject	N 1.2 ml/min	230 (6)-3-285-(1)-10-300 (5)	MS	FAME up to 28:0	Firoblasts from peroxisomal disorders	Yes	[132]
30 × 0.32 × 0.3; OV-1	Solid inject	He 2 ml/min	230 (6)-3-285(1)-10-300 (5)	MS	FAME up to 28:0	Firoblasts from peroxisomal disorders	No	[132]
50 × 0.25 × 012; SIL 8	Solid inject	He 2 ml/min	285 (1)-5-300	MS	FAME up to C_{26}	Human plasma-peroxisomal diseases	Yes	[132]
25 × 0.25; OV 101	Split 1:60		240	MS	FAME up to 24:6	Marine coelenterates	No	[133]
25 × 0.32 × 1; MPS-50		He 20 ml/min	100-8-290	MS	TMS-2-OH-dioic up to C_{24}	Human uline	Yes	[134]
25 × 0.20; superox	Splitless	H	150-2-240	FID	FAME up to C_{26}	Serum human	Yes	[135]
15 × 0.32 × 0.1; OV-1	On-column	H	90-35-180-5-280 (10)	FID, MS	FAME up to C_{35}	Leaves of sorghum	No	[136]
12 × 0.22 × 0.25; BP-1		He 1 ml/min	160-4-320 (10)	MS	FAME up to $C_{38:6}$	Rat brain	Yes	[137]
12 × 0.33 × 1; BP-1		H 45 cm/s	165 (0.5)-4-325 (7)	FID	FAME up to $C_{38:6}$	Rat brain	Yes	[137]
23 × 0.32 × 0.2; CW 20M	On-column	H 90 kPa	80-15-145-3-195-5-230 (20)	MS	FAME up to 24:2	Human plasma	Yes	[138]
25 × 0.2; SE-30		He 1.2 ml/min	120-8-280	MS	FAME up to 26:1	Mycobacteria	Yes	[139]
25 × 0.2 × 0.33; OV-1	Split 10:1	He 1 ml/min	130-2-380	MS	picolinyl-TMS up to 32:1	Hamster meibomian glands	Yes	[140]
30 × 0.25; DB-1				MS	pyrroline 22-2	Marine sponge	No	[141]
50 × 0.22; CPWAX 52			195-2-205-15-215 (40)	FID	I-PR FA up to 24:4	Erythrocyte	Yes	[142]
25 × 0.2 × 0.11; HP-5		He	60-4-300	MS	FAME up to C_{32}	Sediments	Yes	[143]
25 × 0.3; SE-54			170-5-320	FID	FAME up to 30:5	Marine sponge	No	[144]
28 × 0.32; SE-54			200-3-290	MS	FAME up to 28:3	Marine sponge	No	[145]
30 × 0.25; DB-23	PTV	He	160-2-240	MS	FAME up to 26:1	Plant seeds	Yes	[146]
12 × 0.22 × 0.25; BP-1		He 1 ml/min	160-4-320	MS	FAME up to 34:1	Synthetic standards	Yes	[147]
25 × 0.31; SE-54	Splitless	He	160-1-200	MS	FAME 24:5 w-6	Marine coelenterates	No	[148]
25 × 0.2; HP-1	Splitless		180 (2)-10-290 (15)	MS	FAME up to 26:1	Human blood Zellweger syndrom	Yes	[149]
25 × 0.32; MPS-50	Splitless	He 0.8 ml/min	100-8-290	MS	FAME up to C_{24}	Urine acids	Yes	[150]
25 × 0.2; OV-1	Split 10:1	He 1 ml/min	150-2-350	MS	FAME up to C_{26}	Quinea pig Harderian glands	Yes	[151]
50 × 0.2; HP-1	Splitless	He	40 (1)-30-100-4-300	MS	FAME up to 26:1	Oceanic particulate matter	Yes	[152]
30 × 0.25; DB-1				MS	FAME up to 30:3	Marine sponge	No	[153]
30 × 0.25; DB-5		He 2 ml/min	100-2-300	MS	FAME up to C_{34}	Marine plancton	No	[154]
12 × 0.32; BP-1		H 2 m/s	60-30-165-4.5-320 (1.5)	FID	FAME up to 38:5	Human brain-peroxisomal diseases	Yes	[155]
12 × 0.33; HT-5		He	80-2-250	MS	PFB up to C_{24}	Corynebacteria	Yes	[156]
25 × 0.25; SPB-1		He 26 cm/s	150 (4)-3-270	FID	FAME up to 27:1	Yeast	No	[157]
25 × 0.2; OV-1	Split 10:1	He 1 ml/min	150-2-350	MS	FAME up to C_{24}	Rabbit Harderian glands	Yes	[158]
50 × 0.25; SE-52			40 (1)-10-80 (1)-5-280 (30)	MS	FAME up to C_{32}	Rains	Yes	[159]

Table 1 (*Continued*)

Column	Injection	Gas	Gradient	Detector	Compound	Source	Figure	Reference
50 × 0.32; CP-SIL-5CB		He 1 kPa	50-10-150-4-310	MS	FAME up to C_{30}	Sediment and plant leave	No	[160]
25 × 0.2; HP-5	Splitless	He	60-4-300	MS	FAME up to C_{32}	Sediments	Yes	[161]
25 × 0.32; OV-351	Split		70 (2)-3-230 (20)	MS	FAME up to C_{28}	Baltic herring	Yes	[162]
50 × 0.32; Silar 10C		He 0.8 bar	120-4-220 (20)	FID	FAME up to 26:1	Ocean sediment	No	[163]
30 × 0.25; DB-23	Splitless		130-1.5-170-2-245	MS	FAME and oxazoline up to C_{25}	Fruits	No	[164]
15 × 0.25; DB-5			40-10-325	MS	OH-FAME up to C_{28}	Earthworm	No	[165]
35 × 0.32; Carbowax 20M		H	150-3-250	MS	FAME up to C_{30}	Sediments	No	[166]
12 × 0.22; BP-1			160-4-320 (10)	MS	monoenoic FAME up to C_{28}	Human brain	Yes	[167]
50 × 0.32; HP-1		H	150-30-250-2-300 (5)	MS	TMS-OH-FAME up to C_{30}	Bacteria	Yes	[168]
25 × 0.32; SE-52	Splitless	He 69 kPa	220 (3)-5-320 (12)	CI-MS	picolynyl esters up to 26:1	Standards	Yes	[169]
30 × 0.2; Silar 10C		N 14 ml/min	165 (5)-2-220 (20)	FID	FAME up to C_{27}	Freshwater molluscs	No	[170]
25 × 0.32; OV-1		He	200-3-310	MS	pyrrolidides up to 30:3	Marine sponge	No	[171]
30 × 0.32; DB-1		He	180-3-310	MS	FAME up to 30:3	Marine sponge	No	[172]
30 × 0.32; DB-5		He	60-10-200-4-280 (10)	MS	FAME up to 24:4	Mussels	Yes	[173]
50 × 0.32; CP SIL 5	On-column	H	50-260	FID	FAME up to 30:1	Adrenoleukodystrophy patients	No	[174]
25 × 0.2; OV-101			250 Isothermal	FID	FAME up to 26:3	Freshwater sponge	No	[175]
25 × 0.31; OV-101		He	230-4-290	MS	pyrrolidides up to 26:2	Marine sponge	No	[176]
25 × 0.32; CP-Sil-5	Split	He 0.8 bar	40 (2)-30-100-2-280	MS	FAME up to C_{34}	Sea grass	No	[177]
30 × 0.32; SPD-5	On-column	He 35 cm/s	240-40-320-2-360	MS	FAME up to C_{32}	Wax of canola oil	Yes	[178]
60 × 0.32; DB-1		He 140 kPa	70-15-1305-4-190 (40)	FT-IR	FAME up to 28:3	Marine sponges	Yes	[179]
50 × 0.2; ULTRA-3		He 0.5 ml/min	70 (2)-40-160-3-280	MS	FAME up to C_{28}	Soils	No	[180]
60 × 0.31; DB-1		He 2 ml/min	70 (2)-15-290 (40)	FT-IR	pyrrolidides up to 30:3	Marine sponge	Yes	[181]
30 × 0.25; PS-2330	Split	He 15 psi	140-3-200	FID	FAME up to C_{26}	Peroxisomal disorders	No	[182]
30 × 0.25; DB-23	Split	He 2.7 ml/min	140 (1)-1.5-200	MS	FAME up to C_{25}	Yeasts	Yes	[183]
30 × 0.25; HP-5		He 10 psi	130 (2)-3-270 (40)	MS	FAME up to 30:3	Marine sponges	No	[184]
25 × 0.25; Carborax 20M		H	170 (3)-4-225 (25)	MS	FAME up to 28:3	Marine sponge	No	[185]
30 × 0.2; DB-5		He	50-10-120-4-300	MS	FAME up to C_{28}	Marine sediments	No	[186]
30 × 0.32; SPB-1			50-20-240-2-260	MS	FAME up to 30:1	Invertebrate	No	[187]
60 × 0.32; DB-1		He	70-15-130-4-290	MS	pyrrolidides up to 24:5	Marine Gorgonian	Yes	[188]
50 × 0.32; CP Sil-5-CB		He	50-10-150-4-310	CI-MS	FAME up to 37:2	Marine alga	Yes	[189]
25 × 0.2; HP-1		He	130-2-300 (20)	MS	furan FA	Human blood	Yes	[190]
12 × 0.2	Splitless		70-20-150-8-300 (5)	MS	FAME up to C_{26}	Wool wax	No	[191]
30 × 0.32; OV-1		He	140-2-290	MS-MS	pentafluorobenzyl esters up to 36:5	Mycobacteria	Yes	[192]
30 × 0.32; Omegawax 320		He	210 or 245 Isothermal	FID	FAME up to 28:7	Mammal—seals	Yes	[193]
25 × 0.32; CP-SIL-5		He	70-4-320 (5)	MS	FAME up to C_{28}	Eelgrass	Yes	[194]
30 × 0.25; HP-5		He	130 (2)-3-270 (40)	MS	pyrrolidides up to 24:6	Marine Gorgonian	No	[195]
60 × 0.32; DB-1		He 140 kPa	70-15-130-4-290 (40)	MS	FAME up to AI 30:3	Marine sponges	No	[196]
25 × 0.22; BP-70	Split	He 2.2 ml/min	110-3-320 (5)	MS	FAME up to 36:6	Rat retinas	Yes	[197]
25; DB-1			150-3-200-4-300 (30)	MS	FAME up to 36:2, dioic methyl esters	Bacteria	Yes	[198]
30 × 0.53; DB-1		He 20 ml/min	140 (2)-4-310 (10)	MS	FAME up to C_{26}	Peroxisomal diseases	Yes	[199]
30 × 0.25; HP-5			130-3-260	MS	dioic acids methyl esters up to C_{32}	Bacteria	No	[200]
60 × 0.25; DB-Wax		H 1.15 ml/min	200	FID	standards of FAME up to C_{25}	Standards	Yes	[201]
30 × 0.3; DB-5	On-column	He	80 (5)-3-300 (20)	MS	FAME up to C_{32}	Cyanobacterial Mat	Yes	[202]
10 × 0.2; SOP-50	On-column	H 0.5 m/s	70 (1)-12-250-4-380	MS	FAME up to 30:0	Plant oil	Yes	[203]
30 × 0.25; DB-5	On-column		50-16-150-4-200-4-300 (6)	MS	FAME up to 26:0	Sediments	Yes	[204]
25 × 0.32; CP-SIL-5	On-column	He	70-20-130-4-320 (10)	MS	TMS OH-FAME from C_{28} to C_{34}	Algae	No	[205]
30 × 0.25; DB-225		He	100-12-300	FID	FAME up to 26:0	Mycobacteria	Yes	[206]
30 × 0.25; SPB-5	Split	He	230	MS	FAME up to 24:6	Marine invertebrate	No	[207]
30 × 0.32; DB-1		He	50-40-200-3-300 (30)-3-320	MS	TMS of FA up to C_{28}	Plant wax	Yes	[208]
25 × 0.25; HP-Ultra-1		HE 1.5 ml/min	100-3-230 (20)	MS	FAME up to C_{26}	Seed oils	No	[209]
30 × 0.25; DB-5		He	100-3-320	MS	FAME up to C_{30}	Sediments	No	[210]
25 × 0.25; CP-SIL-5CB		He 50 kPa	60 (1)-30-160-5-230-20-320	MS	TMS ester up to C_{26}	Peroxisomal diseases	No	[211]
30 × 0.32; DB-5		He	40 (1)-6-320 (20)	MS	TMS ester up to C_{30}	Sediments	No	[212]

25 × 0.32; CP-SIL5		He	70-20-130-4-320 (15)	MS	OH-FAME up to 34:1	Green algae	No	[213]
25 × 0.25; CP-SIL-5CB			60 (10)-3-300 (30)	MS	FAME up to C_{32}	Peat	No	[214]
30 × 0.25; HP5-MS	Splitless	He	60 (4)-4-310 (15)	MS	FAME up to C_{32}	Sediments	Yes	[215]
25 × 0.25; CP-SIL-5CB			60 (10)-3-300 (30)	MS	FAME up to C_{32}	Peat	No	[216]
30 × 0.25; OV-1			140 (1)-8-280 (10)	MS	FAME up to C_{26}	Peroxisomal diseases	No	[217]
50 × 0.3; DB-5	On-column	He	80 (5)-4-300 (20)	MS	FAME up to 26:2	Marine sponge	Yes	[218]
50 × 0.32; HP-5		He	45 (1)-30-140-3-310 (12)	MS	FAME up to 28:8	Marine dinoflagellates	Yes	[219]
30 × 0.25; PH-5		He	150 (5)-5-310 (20)	MS	TMS-OH FAME up to 30:0	Bacterium	Yes	[220]
60 × 0.25; DB-5	Splitless	He 1.3 ml/min	150 (4)-7-280 (40)	MS	TMS of FA up to C_{28}	Gray mold	No	[221]
30 × 0.25; DB-5	On-column	He 1.4 ml/min	50-30-170-5-325 (30)-2-350	MS	TMS of FA up to C_{32}	Insect	No	[222]
60 × 0.32; BPX-70			60-50-170-2.5-200-15-255	CI-MS	FAME up to 28:8	Marine dinoflagellates	Yes	[223]
50 × 0.2; CBP-1		He 0.38 ml/min	50 (1)-10-100-5-320	MS	FAME + TMS-OH-FAME up to C_{32}	Vernix caseosa	Yes	[224]
25 × 0.2; PH-Ultra-2	Split	He 132 kPa	230 (3)-15-290 (5)-5-320	MS	pentafluorophenyldimethylsilyl FA up to C_{26}	Human plasma	Yes	[225]
30 × 0.25; HP-5		He	150 (5)-5-310 (20)	MS	TMS-OH FAME up to 30:0	Bacterium	Yes	[226]
50 × 0.32; OV-1701	Split	He 4 ml/min	100-3-280	MS	FAME up to 30:1	wax of Vanilla	No	[227]
30 × 0.25; HP-5MS			130 (2)-3-270 (40)	MS	FAME up to C_{26}	FA from Bacillus	No	[228]
30 × 0.25; SPB-1			100 (1)-4-280	MS	dioic FA up to C_{35}	Horsetails	No	[229]
60 × 0.25; Supelcowax-10		He 70 cm/min	100 (1)-7-230-2-280 (10)	MS	FAME up to 30:7	Freshwater invertebrate	Yes	[42]
25 × 0.25; OV-101			200-5-280	MS	2-OH-FA up to 26:0	Yeast	No	[230]
77 × 0.5; SE-30		He 1.5 ml/min	160-4-280 (30)	MS	FAME up to 30:1	Freshwater algae	Yes	[231]
60 × 0.25; SPB-1	Split		160-6-250	MS	FAME up to 24:1	Mouse	Yes	[232]
60 × 0.32; SPB-1	Splitless	He	100-4-300	MS	dioic up to C_{34}	Horsetails	Yes	[233]
60 × 0.25; SPB-1	Split		160-6-330	MS	FAME up to C_{34}	Fungi	Yes	[55]
60 × 0.25; SPB-1	Splitless	He	100-4-320	MS	FAME up to C_{34}	Bacteria	Yes	[234]
15 × 0.25; SPB-1		He 70 cm/s	100 (1)-20-230-3-320	MS	FAME up to C_{40}	Bacteria	Yes	[235]
15 × 0.25; Supelcowax-10		He 70 cm/s	100 (1)-6.5-230-2-280 (10)	CI-MS	FAME up to 32:6	Herring	Yes	[25]
25 × 0.2; HP-1		H 75 cm/s	100 (1)-20-230-4-339	MS	picolinyl esters up to 32:6	Herring	Yes	[236]
60 × 0.32; SPB-1	Splitless	He	100-4-320	MS	FAME up to C_{26}	Soil bacteria	No	[237]
60 × 0.32; SPB-1	Split	He	100-4-320	MS	FAME up to C_{36}	Coals	Yes	[40]
60 × 0.32; SPB-1	Split	He	100-4-320	MS	FAME up to 34:1	Soil bacteria	Yes	[41]
60 × 0.25; Supelcowax-10	Splitless	H 60 cm/min	100 (1)-20-180-2-280 (1)	MS	isoprenoic-FA up to C_{26}	Freshwater sponge	No	[238]
60 × 0.25; Supelcowax-10	Splitless	H 60 cm/min	100 (1)-20-180-2-280 (1)	MS	FAME up to 30:3	Freshwater sponge	No	[239]
60 × 0.25; Supelcowax-10	Splitless	H 60 cm/min	100 (1)-20-180-2-280 (1)	MS	FAME up to 30:5	Freshwater sponge	No	[240]
77 × 0.5; SE-30		He 1.5 ml/min	100 (2)-240-2-290 (50)	MS	FAME up to 36:1	Freshwater alga	No	[241]
30 × 0.25; HP-5			130 (1)-3-270 (30)	MS	FAME up to 27:2	Marine sponge	No	[242]
25 × 0.32; OV-1		He	210	FID	FAME up to 26:3	Freshwater sponge	No	[272]

separated compounds is caused by hydroxy or methyl substituents. As the both groups of compounds can be found regularly outdoors, the attempt at separation of enantiomers was limited to lower FA up to C_{24}. Hydroxy FAs from C_{10} to C_{25} with different position of hydroxy group (from ω-12 to ω-2, mainly hexa and octadecanoic acids were separated by GC–MS of their *t*-BDMS ether-methyl esters [56].

It was reported the application of supercritical fluid chromatography (SFC) for the separation of free FAs in the range of 24:0–30:0 [57]. Liquid CO_2 was used as the mobile phase in a column having the following characteristics: 20 mm × 0.1 mm i.d.; film thickness 0.4 μm; DB-1 methylsilicone phase. The elution time of the last component was about 20 min. A flame ionization detector was used for detection of the eluted components. A wide range of methyl esters of saturated and unsaturated FAs from C_{16} to C_{24} was separated by SFC on a packed silica column from 25 to 175 cm. The retention times were primarily dependent on the number of double bonds and the chain length of the parent acid had a negligible influence [58].

On the present, the great attention is paid to the separation of FA by means of capillary electrophoresis [59]. A number of examples of successful separation of VLCFA up to C_{26} is showed in the Table 1 of that paper. It recapitulates operating conditions, capillary types, mobile phase and methods of detection, too.

Novel esters, i.e. cyanomethyl esters [60] were prepared and identified by nitrogen–phosphorus detector. The method was applied in medical research of the role of VLCFA by patient that suffering with X-linked adrenoleukodystrophy.

Pentafluorophenylsilylesters of FAs up to C_{26} were analyzed by GC–MS [61]. The ions $(M - 15)^+$ are the most intense in mass spectra and detection limits are about 1 pg.

The abnormal phospholipids, phosphatidylcholine and phosphatidylethanolamine, both of which contain a very long-chain fatty acyl residue (1-melissoyl-2-oleolyl-sn-glycero-3-phosphocholine and 1-melissoyl-2 - oleolyl - sn - glycero - 3-phosphoethanolamine), accumulated in fas2 strains of the fission yeast *Schizosaccharomyces pombe* were determined [62].

Pattern recognition analysis on the levels of the VLCFA in plasma is described for the visual discrimination of X-linked adrenoleukodystrophy patients from normal healthy group. Plasma VLCFA compositions of 58 normal subjects and 16 X-ALD patients were examined by GC as their methyl esters to determine the area percentages of 22:0, 24:0 and 26:0 in the total FAs [63].

Extracts of the silk from the golden orb weaver, *Nephila clavipes*, were studied by GC–MS and chemical derivatizations. The major group of the lipids consisted of methyl-branched 1-methoxyalkanes (methyl ethers) with up to four methyl groups in the chain (chain length between C_{28} and C_{34}), which are unique to spiders. The position of the methyl branches was determined by conversion into VLCFA, which allowed easy location of methyl branches [64].

The gammarid amphipods (*Eusirus perdentatus* and *Orshomene rossi*) were collected near the south Shetland Islands. VLCPUFA (C_{24} and C_{26}) comprised 0.6–2.8% of total FAs in almost all 1998 amphipods [65].

A capillary GC–MS with electron-capture negative-ion method was developed for the quantitative determination of C_8–C_{26} total FAs in plasma. FA were analyzed as pentafluorobenzyl esters. Fourteen saturated and twenty-five unsaturated FAs are quantified by selected ion monitoring in ratio to thirteen stable-isotope-labeled internal standards [66].

The extracts of the roots of *Cymbopogon martinii* var. *motia* have been investigated to afford mainly FAs. A new hydroxy unsaturated FA, namely, 16-hydroxypentacos-14Z-enoic acid, has also been isolated [67].

4. Unsaturated bond identification

The location of the unsaturated bonds position in VLCFA is rather difficult. We can find a lot of papers that refer to usual FA up to C_{22} [68–70]. Generally, they can be applied in either destructive or non-destructive techniques. The destructive methods cleave the chain of the molecule at an unsaturated-bond site by OsO_4, $KMnO_4$ or KJO_4 oxidation and oxidative or reductive ozone splitting. The non-destructive methods preserve the length of the chain and the agent reacts with the carbon atoms participating in the double bond, e.g. reduction by deuterium and its compounds is involved or an oxidation resulting in the formation of vicinal diols. Most frequently, these methods use the chromatography combined with GC–MS.

The position of unsaturation, chain branching and other structural features of FAs are not often apparent from the mass spectra of common derivatives such as methyl esters. Usually, it can be attributed to a charge location at the carboxy terminus and to the migration of double bonds. On the other hand, the spectra of nitrogen derivatives such as picolinyl esters contain fragment ions that provide this information. The esters are synthesized by reaction of the acids with thionyl chloride to form the acid chloride that is reacted with 3-pyridylcarbinol to give the ester. Under electron impact conditions in the mass spectrometer, an electron is removed from the nitrogen of the pyridine ring and a hydrogen atom is abstracted from the alkyl chain to this electron-deficient site. This process produces a radical site in the chain that initiates chain cleavage. Hydrogen atoms can be removed from any position of the chain with varying probability, depending on the chain structure. Thus, diagnostic ions are produced from each type of FA whose masses and relative abundances reflect the structure of the alkyl chain and any substituents. Patterns of fragmentation for straight-chain, branched-chain, unsaturated and cyclic FAs are described together with those containing hydroxy-, epoxy-, keto-, and ether groups [71].

In current literature, we can find many review papers that are dedicated to the MS of FA derivatives [68,72,73].

GC–MS technique of the picolinyl esters [74] or 4,4-dimethyloxazoline derivatives of FAs is a powerful technique for elucidation of their chemical structure. A comparison of the mass spectral properties of derivatives, unsaturated FAs and FAs with other functional groups was presented. The paper describes the treatment of complex natural samples.

Nichols et al. [75] described the application of the adduct of dimethyl disulfide with monoenoic acids for the identification of the position and geometry of the double bonds. Dimethyl disulfide was applied after the separation of compounds on GC–MS. A sample of FAME isolated from the marine diatom *Nitzschia cylindrus* which contained 13-*cis*, 15-*cis*-24:1, 15-*cis* and 17-*cis*-26:1 was used as a model mixture.

Scribe et al. [76] reported that the earlier mentioned method is limited, since longer homologues are not sufficiently volatile or they degrade at higher temperatures.

For determination of the double-bond position in polyunsaturated C_{24}–C_{30} FAs from marine organisms, methoxy derivatives were prepared. Diagnostic mass spectral fragment as well as molecular ion intensities was obtained by adjusting the ion source optics in the presence of ammonia at a lower source pressure than used conventionally. A lower detection limit was observed compared to conventional methane chemical ionization, which is a more favorable condition for capillary GC. Analysis of FAs from the sponge *Calyx niceaensis* showed the double-bond position of eighht unsaturated FAs, including a new cyclopropane-containing FA, i.e. 19,20-methylene-hexacosanoic acid [77].

For the identification double bonds, the FAB method combined with MS/MS was applied [78]. The combined method MIKES/CID offers a possibility for location of the position of double bonds. It was used for the identification of monoenoic acids from *Mycobacterium phlei* [79,80]. As an excellent example, the identification of position of double bonds of monoenoic acids in *M. phlei* can serve. Monoenoic fraction was prepared by argentation chromatography and the resulting acids were detected on a capillary column as perfluorbenzyl esters. When CAD MIKE technique was used both the position isomers 24:1 and 26:1 and branched chain isomers such as br_{15}-4-24:1 and br_{16}-5-25:1 were identified.

The method of FAB of phosphatidylethanolamin, phosphatidylinositol, cardiolipin and phosphatidic acid is suitable for both the detection of the molecular ion and determination of characteristics of acyl carbanions. The modification FAB-MS/MS was convenient. Having applied the above soft-ionization methods, both the length of the acyl chain bound to glycerol and the position of double bonds and of the chain branching (by use of daughter ions) could be determined [81].

The further method [82] for the location of positions of $n-3$, $n-6$ and $n-9$ double bonds on the basis of the splitting of PUFA (VLCPUFA) methyl esters during GC–MS (various relative proportions of m/z 108, 150 and 192 ions) was described. In order to identify the individual isomers, the structure of the methylene-interrupted double bonds of PUFA must be verified on the basis of the molecular ions and diagnostic fission ions having m/z of 79 and 91. It is necessary to separate all position isomers of the

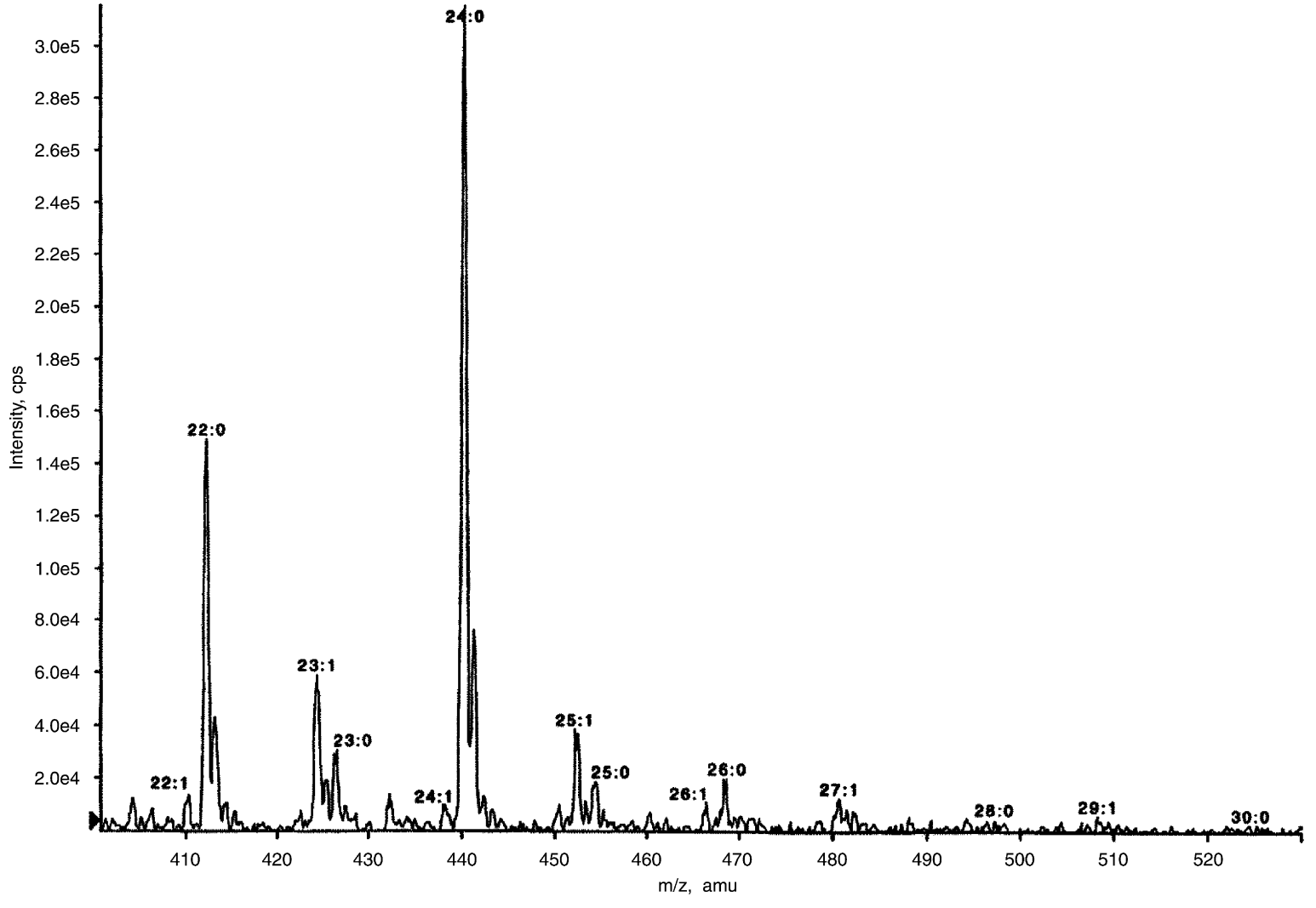

Fig. 7. Neutral loss of *m/z* 45 scan of the DMAE esters of FAs from 10 μg of beewax [88]. Neutral loss of 45 Da scan, on a PE SCIEX API 365 tandem mass spectrometer.

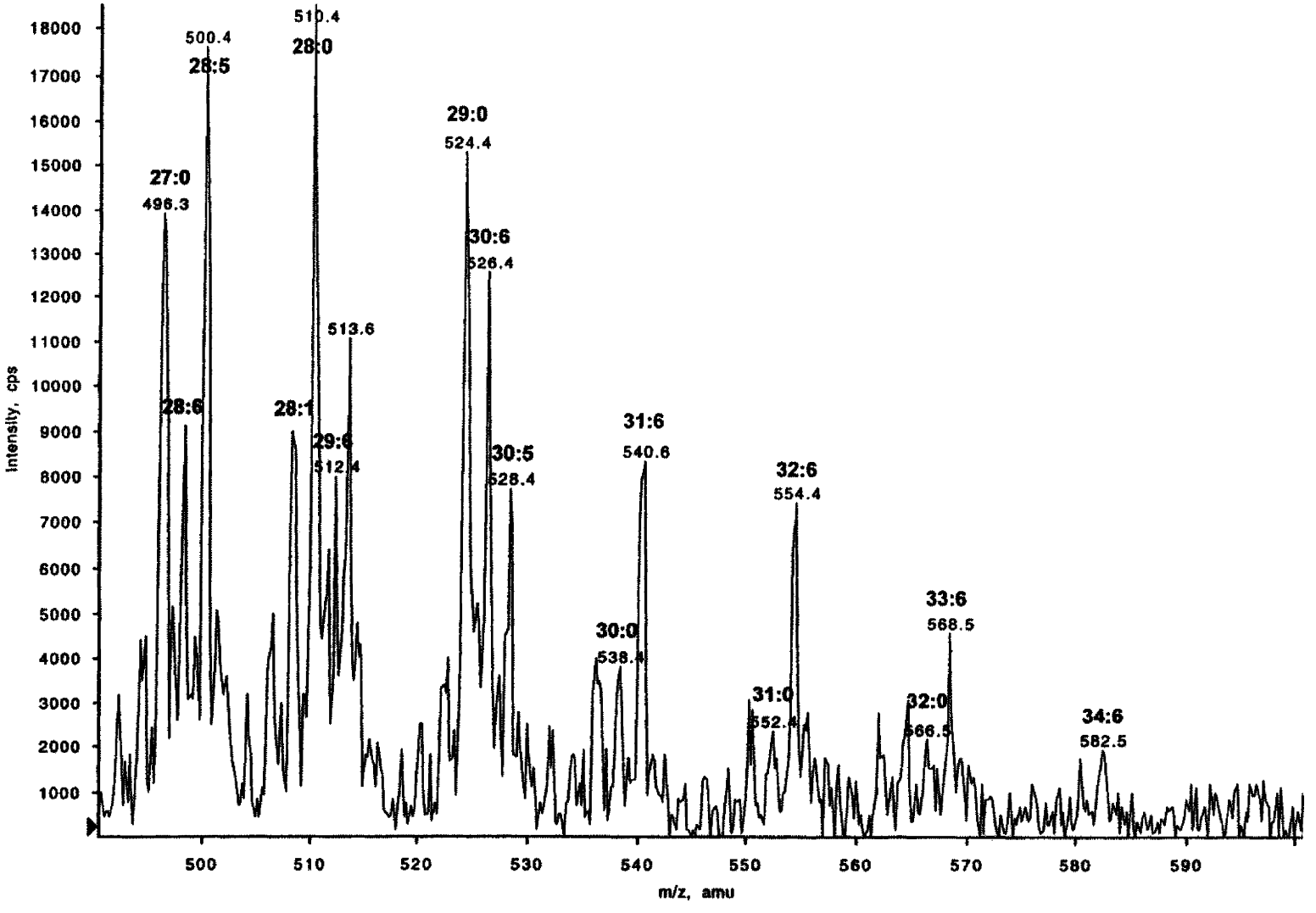

Fig. 8. VLCFA as TMAE from plasma sample (1 μl) of a patient with a peroxisomal biogenesis defect [89]. Neutral loss of 59 Da scan, on a PE SCIEX API 365 tandem mass spectrometer.

VLCPUFA on a capillary column. On the understanding of elution order of the splitting in MS (i.e. presence of the three above-mentioned diagnostic ions), one can precisely determine the position of double bonds.

The combination of RP-HPLC and GC was applied for the separation and identification of minor picolinyl esters from fish oil [73,83]. In this study, the combinations of Ag^+-ionex-HPLC and RP-HPLC for the preparation of milligram amounts of 28:7ω-6 and 28:7ω-3 acids were applied. The total of picolinyl esters sample was separated into six fractions on by ionex chromatography. However, only the last fraction contained a mixture of VLCFA having six and seven double bonds. Except for the 28:7 fraction, the application of RP-HPLC allowed to separate all esters. The 28:7 fraction was identified by use of GC–MS, separately. The elution temperature for 28:7ω-6 was about 314 °C. It was by 56 °C higher than that one of methylesters of the same acid [84].

In the last decade, a new class of methods for double bonds identification was developed. Most of them take advantage of the technique of soft ionization of appropriate derivatives of FA.

The mass spectral behavior of cationized saturated FA derivatives has been studied in order to gain insight into the loss of molecular hydrogen during cesium ion bombardment. It is shown that molecular hydrogen and hydrogen radical loss occurs for protonated and alkali (Li^+/Na^+)-cationized FA methyl esters and that molecular hydrogen loss is dependent upon the acyl chain length. Investigation of ion structures by collision-induced dissociation tandem MS indicates that in the case of alkali-cationization a hydrogen radical is lost from all positions of the saturated acyl chain, whereas in the case of protonated molecules a hydrogen radical is predominantly eliminated from the protonated ester group [85].

Acetonitrile was used as chemical ionization reagent to locate double bonds in unsaturated FAs [86,87]. Adducts of acetonitrile and FAME give characteristic splitting in chemical ionization mass spectra (CI-MS/MS). Unfortunately, the method was used only to FAME up to C_{26}.

Further derivatives e.g. dimethylaminoethyl esters or dimethylaminoethyl esters iodies were used for electrospray ionization tandem mass spectrometry (ESI-MS/MS) [88,89] (Figs. 7 and 8). ESI-MS/MS gives the best results for biological samples, e.g. plasma, blood and urine. Very high sensitivity makes it possible to analyze VLCFA from 3.6 μl of blood [90].

5. Conclusion

Tables 1 and 2 characterize two basic chromatographic techniques that can be used for VLCFA identification. The examples and overview of VLCFA separation on capillary column is summarized in Table l. In this connection, it is necessary to emphasize that the number of papers that were ascribed to the application of packed bad columns is negligible. The table gives a reader an idea about the basic parameters of separation, including a category of analyzed compound, its origin etc. Unfortunately, in the most of papers, the significant data for chromatographic analysis were not given. Nevertheless, in the next, we summarize a few general rules that can help the reader to select a suitable method. At first, the most important is the selection of an elution temperature. Secondly, the choice of a technique for preparation of derivative of VLCFA is substantial. Quite unrivalled derivatives are methyl esters of VLCFA. At third, the selection of stationary phase of a column is vital. A polar stationary phase was used less than in the third of cited papers. Fourthly, it is necessary to take into account the long elution time of VLCFA. For example, when we analyze a C_{22} FAME fraction and we finish the analysis soon then we can pass up the detection of VLCFA. At fifth, as a detector MS is recommended. In most cases, the application of MS is a unique technique for chain length and double bond detection.

Table 2 summarizes the methods that use a liquid mobile phase. The RP-HPLC technique is prevailing. The performance of its use differs in a selection of mobile phase, gradient elution type and choice of suitable derivative of VLCFA. Based on their citation frequency, the more obsolete SCF method is out of use, now. As emphasizes above, for success in VLCFA analysis, it is crucial to specify the separation conditions, to estimate the VLCFA concentration and to identify a suitable biological resource of these compounds.

Among the other papers concerning of FA, this paper is a first review that summarizes the current knowledge on the chromatography of very long chain FAs. Based on authors own experiences in the field,

Table 2
LC of VLCFA

Stationary phase	Detector	Compound	Source	Figure	Reference
MeOH; 1.5 ml	RI	FA; preparation of 5,9-i(ai)-27:2	Marine sponge Petrosia	No	[243]
AcN; 0.5 ml/min	UV 205 (sat) 192 monounst, FFA	FFA up to 28:0 or 32:1	Bovine brain SPH	Yes	[48]
MeOH; 1.5 ml/min	RI	br_{20}-5,9-26:2, br_{22}-5,9-28:2	Marine sponge Aplysina	No	[244]
AcN; 2 ml/min	FL exc. 365 nm; emis. 412 nm	9-Anthrylmethyl esters up to 26:0	Adrenoleukodystrophy	Yes	[52]
AcN:15% MeOH in W 60:40–100:0 (1 h); log gr; 2 ml/min	UV 215 nm	FAME up to 30:5	Rat testis	Yes	[49]
MeOH:W (98:2)	RI	2-MeO sat. or unst. FAME up to 28	Marine sponge Higginsia	No	[245]
AcN; 1 ml/min	UV 254 nm	*p*-Br-phenacyl unsat. FA C_{22}–C_{30}	Mycobacterium	Yes	[246]
Diox:AcN (2:3); 1 ml/min	UV 254 nm	*p*-Br-phenacyl unsat. FA C_{32}–C_{47}	Mycobacterium	Yes	[246]
Diox:AcN (3:7); 1 ml/min	UV 254 nm	*p*-Br-phenacyl dicyclopropyl FA C_{30}–C_{40}	Mycobacterium	Yes	[33]
Diox:AcN (1:1); 1 ml/min	UV 254 nm	*p*-Br-phenacyl FA C_{40}–C_{50}	Mycobacterium	Yes	[33]
Diox:AcN (9:11); 1 ml/min	UV 254 nm	*p*-Br-phenacyl methoxy FA C_{41}–C_{56}	Mycobacterium	Yes	[33]
MeOH	RI	FA; 5-monounst., br, sat.	Marine sponge Strongylophora	No	[247]
AcN:W (75:25–100:0); gr (95 min); 1.6 ml/min	FL exc. 365 nm; emis. 415 nm	9-Anthrylmethyl FA up to 26:0	Adrenoleukodystrophy, PC and PS	Yes	[248]
AcN:W (90:10–100:0); gr (95 min); 1.6 ml/min	FL exc. 365 nm; emis. 415 nm	9-Anthrylmethyl FA up to 26:0	Adrenoleukodystrophy	Yes	[249]
AcN:15% MeOH in 3 mM acid 60:40–100:0; log gr (1 h); 2 ml/min	UV 215 nm	FAME of PUFA	Human cells	Yes	[250]
AcN:15% MeOH in 3 mM acid 55:45–100:0; log gr (1 h); 2 ml/min	UV 215 nm	FAME of PUFA	Human cells	Yes	[250]
Diox:AcN (3:17); 3 ml/min	UV 254 nm	*p*-Br-phenacyl FA C_{24}–C_{35}	Mycobacterium	Yes	[251]
Diox:AcN (3:2); 3 ml/min	UV 254 nm	*p*-Br-phenacyl FA C_{35}–C_{55}	Mycobacterium	Yes	[251]
W:MeOH (12:85–0:100); gr (20 min); 1.5 ml/min	UV 254 nm	*p*-Br-phenacyl FA up to 26:0	Peroxisomal disordes	Yes	[252]
AcN:W (75:25–100:0); gr (90 min);	FL	9-Anthrylmethyl FA up to 26:0	Adrenoleukodystrophy	Yes	[51]
MeOH-*i*-PrOH (95:5); gr 1 ml/min	API-MS	Even anilide of FA up to 30:0	Standards	Yes	[54]
Acet:AcN (20:80); 0.3 ml/min	FL exc. 365 nm; emis. 415 nm	9-Anthrylmethyl FA up to 24:0	Standards	Yes	[253]
Acet:AcN (20:80); 0.3 ml/min	EI-MS (20 eV); SIM *m/z* 191	9-Anthrylmethyl FA up to 24:0	Standards	Yes	[253]
Liquid CO_2; non-linear gr; 2800–3800 psi; 110 °C	FID	Even FA up to 30:0	Standards	Yes	[57]
AcN–W	Radio	FAME	Cockroachs	Yes	[254]
AcN; 1 ml/min	UV 254 nm	*p*-Nitrobenzoyl up to C_{24}	Human brain and blood glycolipids	Yes	[255]
CO_2	FID + UV 190 nm	FFA	Standards up to C_{26}	Yes	[256]
CO_2	FID	FA up to 30	Standards	Yes	[57]
AcN:W (9:1)	FID	FAME up to 28:1	Peroxisomal disordes	No	[257]
MeOH; 1.5 ml/min	FL exc. 365 nm; emis. 412 nm	9-Antrhyl derivatives up tp C_{27}	Adrenoleukodystrophy	Yes	[258]
MeOH; 1.5 ml/min	UV 254 nm	Phenacyl bromide up to 28:0	Standards	Yes	[259]
MeOH phosphate buffer gradient	UV 213 nm	Hydroxamic FA	Standards	Yes	[260]
40 mM Tris, NMF:dioxan:water (5:4:1)	IPD 259 nm	FFA up to C_{31}	Standards	Yes	[261]
MeOH:water (95:5); 1.2 ml/min	FL exc. 235 nm; emis. 366 nm	Naphtoxyethyl FA up to C_{26}	Standards	Yes	[262]
MeOH:ACN:Hex (300:200:22.5); 0.6 ml/min	FL exc. 298 nm; emis. 462 nm	br-FA 12-methylpentadecanoic	Marine dinoflagellates	Yes	[263]
NMF:dioxan (3:1); pH 10.4	UV 264 nm	Anthraquinone-2-carboxylic acids up to C_{26}	Standards	Yes	[264]
Tris, water:ACN (1:1)	UV 214 nm	FA up to C_{24}	Standards	Yes	[264]
ACN:water (80:20); gr; 2 ml/min	UV 254 nm	FA up to C_{24}	Standards	Yes	[265]

Table 2 (*Continued*)

Stationary phase	Detector	Compound	Source	Figure	Reference
ACN:water (91:9); 1.3 ml/min	FL exc. 321 nm; emis. 390 nm	Brommethylmethoxycoumarines up to C_{26}	Peroxisomal diseases	Yes	[266]
Liquid CO_2; 52 °C	FID	FFA standards up to C_{26}	Standards	Yes	[267]
ACN–water; gr; 2 ml/min	UV 242 nm	Phenacyl esters up to 24:5	Retinal FA	Yes	[268]
ACN:MeOH:AcOEt (11:4:4); 2 ml/min	RI	br-FA up to C_{26}	Marine sponge	No	[269]
MeOH:water (85:15); gr; 1.1 ml/min	FL exc. 365 nm, emis. 412 nm	Standards ADAM up to 24:0	Standards	Yes	[270]
MeOH:water (50:50); gr; 2 ml/min	UV 210 nm	FAME up to C_{30}	Green alga	Yes	[271]
ACN:PrCN:DCM (6:2:2); gr	APCI-MS	FAME up to 40:7	Freshwater invertebrate	Yes	[43]
AcN:W (40:60–100:0); (3 h); log gr	UV 254 nm	*m*-MeO-phenacyl FA up to 32:0	Bacteria on the skin	Yes	[273]

it summarizes the information on pre-separation techniques that are used for enrichment of samples for further chromatography, it discusses the experience with separation and isolation of VLCFA and finally it makes a survey of unsaturated bond and chemical structure identification.

The review pointed out the limits of particular techniques of chromatography including GC, TLC GC–MS, RP-HPLC, LC–MS, etc.

Many practical examples of VLCFA applications are described, in order the help the reader to orient himself in this, experimentally difficult field.

It is emphasizes that the lack of pure standards requires the use of expensive and sophisticated analytical devices such as GC–MS or LC–MS.

Finally, as a method of a first choice GC–MS can be recommended. It suited well for the analysis of a sample that contains both as saturated or monoenic VLCFA. On contrary, the proper even more expensive LC–MS technique is more convenient when it is necessary to analyze VLCPUFA. Right in this method, we foresee the further development of VCLFA analysis and its remarkable role in the study of metabolism of recently examined organisms.

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