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Establishment of reliable mass spectra and retention indices library: Identification of fatty acids in human plasma without authentic standards

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ABSTRACT

Gas chromatography mass spectrometry (GC–MS) is routinely employed to analyze small molecules in various samples. The more challenge of GC–MS data processing is to identify the unknown compounds in samples. Mass spectra and retention indices library searching are commonly used method. However, the current libraries are often built through collecting data from different groups. To unknown compounds with similar mass spectra and retention indices (e.g. geometric (cis/trans) isomers), the inaccurate results sometime are supplied. In this case, the costly standard compounds have to be used in every analysis. In this report, taking identification of fatty acids as an example, we proposed a strategy of establishment of special database constructed by equivalent chain length (ECL) values in **uniform conditions** and mass spectra of fatty acid methyl esters (FAMEs). The mass spectral characteristics were firstly used to identify all expected straight saturated fatty acids, and subsequently calculate the ECL for fatty acids in the sample. Finally, the ECL values of fatty acids in the sample were compared with those of fatty acids in the customized database to identify their structures. The results showed that the method developed in this report could effectively identify similar unknown compounds (FAMEs in the human plasma) after validated by the authentic standards.

1. Introduction

Gas chromatography mass spectrometry (GC–MS) is one of the most versatile and widely applied technology platforms in various fields [1]. The major challenge is the identification of the components in the complex samples. The widely available identification method is mass spectral and retention indices library searching [2] or comparison of mass spectra and retention indices with the results of authentic standards. In practice, many mass spectra and retention indices libraries are available to the analysts [3–5]. Among them, the library established by National Institute of Standards and Technology (NIST) [5] is the commonly used one. However, some compounds have many isomers in one sample, both whose mass spectra and retention indices are similar or even identical with each other. In this case, mass spectra and retention indices often provide little evidence of these positional and geometrical isomers. Therefore, it is necessary to establish more reliable mass spectra and retention indices library to obtain accurate identification results without authentic standards. In this study, we use fatty acid methyl esters to illustrate our strategy to build and use a more reliable library.

Fatty acids are one of the most fundamental categories of biological lipids [6,7]. They play active roles in biological tissues and influence their properties such as fluidity, integrity and the activities of membrane-bound enzymes as constituents of lipids in biological membranes [8]. Fatty acid analysis of biological samples shows great importance for the understanding the relationships between dietary lipids and development of diseases such as diabetes and cardiovascular diseases. Recently, researches showed fatty acids were significant signal materials to the development of obesity and associated metabolic diseases such as type 2 diabetes, hypertension, and cardiovascular disease [9–12]. So, the analysis of the fatty acid composition of human blood and plasma samples was applied to diagnosis of disease and pharmacological analysis [13–15].

To obtain a complete knowledge of the involvement of lipids in physiological processes and to develop compounds of therapeutic interest, it is important to quantify changes in their metabolites and reveal modification of biochemical pathways and interaction network maps of lipids. To meet these requirements, the positive identification and exact quantification of fatty acids are crucial. For

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the analysis of unknown fatty acids, the equivalent chain length (ECL) [16,17] and fraction chain length (FCL) [18,19] are preferred systems [20,21]. However, the authentic standards are required to calculate the ECL and FCL of FAMEs by the external calibration algorithm. Moreover, since the influence from conditional differences, traditional method of comparison with the ECL values and mass spectra in reference library could sometimes inaccurately identify the isomers. So, without further derivatization and reference standards, the unambiguous identification of FAMEs is still the main problem for the analysis of fatty acids.

To address this question, the combination of retention index and the structural information obtained from mass spectra might be the most promising method to identify FAMEs in complex samples. Herein, we proposed a strategy of establishment of special database constructed by ECL values and mass spectra in **uniform conditions** to reduce the conditional influence as more as possible. In this method, the mass spectral characteristics of FAMEs were employed to automatically recognize saturated FAMEs, which were subsequently used to calculate the ECL values for the unsaturated FAMEs in sample. Finally, the ECL values and mass spectra were applied to the identification of FAMEs.

2. Experimental

2.1. Material and reagents

The plasma sample was from a type 2 diabetes mellitus (T2DM) patient in the Xiang'ya Hospital of Hunan of China. The present study was approved by the ethics committee of the University and adhered to the tenets of the Declaration of Helsinki. Additionally, informed consent to the study protocol was obtained from patient.

Supelco 37 component FAME mixture (No. 47885-U) was purchased from Sigma (St. Louis, MO, USA). 11-Octadecenoic acid (C18:1n-7, >97.0 purity) and 7-hexadecenoic acid methyl ester were purchased from Sigma (St. Louis, MO, USA). The solution 5% H_2SO_4/CH_3OH was freshly prepared by diluting H_2SO_4 (>98.0 purity) by chromatographic grade methanol, and NaOH–CH_3OH (0.4 M) was freshly prepared in our laboratory by dissolving a reagent grade NaOH in methanol.

2.2. Procedure of extraction and derivatization

The method proposed in our pervious studies [13–15] was used as a standard method. The blood sample was immediately centrifuged at $3000 \times g$ for 10 min and plasma was transferred into a clean Eppendorf tube. The anticoagulant was EDTA–Na_2. The plasma sample was stored at −80 °C until analysis. Aliquots (200 μL) of plasma were spiked with internal standard (I.S.) working solution (25 μL C17:0 and 25 μL C17:0 methyl ester) and 2 mL 0.4 M NaOH–CH_3OH was added, vortex-mixed for 30 s and placed at room temperature for 30 min. Then, these were extracted with 2 mL of hexane twice using a vortex mixer for 30 s. The hexane phase was the esterified fatty acid methyl esters, removed this phase and some anhydrous sodium sulfate were added to remove traces of water and 2 mL 5% H_2SO_4–CH_3OH was added to the residuary phase of plasma, reacted at 70 °C water bath for 30 min. Then, extracted with 2 mL of hexane twice using a vortex mixer for 30 s and the non-esterified fatty acid methyl esters were obtained. Samples were evaporated to dryness under N_2 gas. Hexane (100 μL) was added to each tube before analysis.

2.3. GC–MS analysis

The analyses were performed on a Shimadzu (Kyoto, Japan) GC-2010 gas chromatograph interfaced to a Shimadzu (Kyoto, Japan) QP2010 mass spectrometer.

In the gas chromatography system, a fused silica capillary column DB-23 (30 m × 0.25 mm × 0.25 μm) was used. Helium (99.999% purity) was used as carrier gas at a flow-rate of 1 mL min^{-1}. The column was first set at 70 °C, temperature was subsequently increased to 150 °C at the rate of 20 °C/min, then to 180 °C at the rate of 6 °C/min, finally to 220 °C at the rate of 20 °C/min, which was held for an additional 9 min (total program time, 20 min). Injector, interface and ion-source were kept at 250, 250 and 200 °C, respectively. Splitting ratio was 1:10. Electron impact mass spectra were taken at 70 eV. Scans were acquired from 50 to 500 amu with 0.2 s/scan.

2.4. Identification of FAMEs

Since many isomers have very close retention indices as well as similar mass spectra, the severe overlapping of their peaks are present in the chromatograms. In this case, the chemometric methods are essential to obtain the high-quality mass spectra and retention times. In this method, the overlapping peaks were revolved by the Heuristic Evolving Latent Projections (HELP) [22,23] which was proven to be an effective method to revolve the overlapping peaks [24,25].

Retention times and mass spectral characteristics were combined to identify the FAMEs in the biological sample. A novel algorithm was proposed to identify all expected straight saturated fatty acids from extracted ion chromatograms, and subsequently calculate the ECL for fatty acids in the sample. Finally, the ECL of fatty acid in the sample was compared with those of fatty acids in the customized database. The detailed procedure is described as follows:

(1) After background subtraction and denoising, extracted ion chromatograms of m/z 74, 87, 55, 67 and 79 were employed to identify all expected saturated FAMEs in the GC–MS data. Threshold signal to noise ratio of peak was set to 3 in our method. The retention times of the expected and verified saturated FAMEs were transferred into ECL (the ECL value is by definition set to equal the number of carbon atoms in the fatty acid carbon chain.).
(2) The background subtracted GC–MS data was resolved using Heuristic evolving latent project (HELP) to obtain the pure chromatographic profile and pure mass spectrum for each component.
(3) The retention times of the straight saturated FAMEs were employed as calibration series, while ECL value of each fatty acid was calculated using the straight saturated FAMEs eluting immediately before and after the compound of interest by Eq. (1). If retention time of interest is out of straight saturated FAME, extrapolation will be employed by using retention times of two nearest straight saturated FAMEs.

$$\mathrm{ECL}(x) = n + \frac{RT(x) - RT(n)}{RT(n+1) - RT(n)} \quad (1)$$

where n and $n+1$ represents the number of carbons of the straight saturated FAME eluting immediately before and after the compound of the interest, respectively.

Determine the type of FAME using the characteristic ions (e.g. m/z 87), base peak (e.g. m/z 74, 55, 67 or 79), the ECL of FAMEs in the sample were compared with those in the custom database to identify structure of the fatty acid.

Table 1
Database of commonly used FAMEs.

Serial no.	Compounds	ECL	FCL	Characteristics of mass spectra
1	10:0[a]	10	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
2	11:0	11	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
3	12:0	12	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
4	13:0	13	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
5	14:0	14	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
6	14:1n-5c[b]	14.4079	0.4079	*m/z* 55; M-32, M-74, M-116
7	15:0	15	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
8	15:1n-5c	15.4200	0.4200	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
9	16:0	16	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
10	16:1n-7c	16.3305	0.3305	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
11	17:0	17	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
12	17:1n-7c	17.3414	0.3414	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
13	18:0	18	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
14	18:1n-9t	18.1501	0.1501	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
15	18:1n-9c	18.2647	0.2647	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
16	18:2n-6t	18.4938	0.4938	*m/z* 67,81; M-31, M-32, $M^{+\bullet}$
17	18:2n-6c	18.7643	0.7643	*m/z* 67,81; M-31, M-32, $M^{+\bullet}$
18	18:3n-6c	19.0925	1.0925	*m/z* 79,67,150,194; $M^{+\bullet}$
19	18:3n-3c	19.4362	1.4362	*m/z* 79,67,108,236; $M^{+\bullet}$
20	20:0	20	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
21	20:1n-9c	20.2780	0.2780	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
22	20:2n-6c	20.8427	0.8427	*m/z* 67,81; M-31, M-32, $M^{+\bullet}$
23	21:0	21	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
24	20:3n-6c	21.1778	1.1778	*m/z* 79,67,150,222; $M^{+\bullet}$
25	20:3n-3c	21.3806	1.3806	*m/z* 79,91; M-31
26	20:4n-6c	21.5140	1.5140	*m/z* 79,67,108,264; $M^{+\bullet}$
27	22:0	22	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
28	20:5n-3c	22.0722	2.0722	*m/z* 79,91; M-31
29	22:1n-9c	22.3024	0.3024	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
30	22:2n-5c	22.8996	0.8996	*m/z* 67,81; M-31, M-32, $M^{+\bullet}$
31	23:0	23	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
32	24:0	24	0	*m/z* 74,87; M-31, M-43, $M^{+\bullet}$
33	24:1n-9c	24.3531	0.3531	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
34	22:6n-3c	24.4217	2.4217	*m/z* 79, 91; M-31
35[c]	16:1n-9c	16.2203	0.2203	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$
36[c]	18:1n-7c	18.3587	0.3587	*m/z* 55; M-32, M-74, M-116, $M^{+\bullet}$

[a] Numbers before and after a ":" mean number of carbon atoms and number of multiple double bonds present, respectively.
[b] The number after "-" represents the position of double bond; c refers to cis-, while t to trans-.
[c] Mass spectral characteristics, ECL and FCL obtained by single reference standard at the same conditions.

2.5. Nomenclature and software

The most common name and abbreviated formula of the target compounds using the shorthand annotation according to A:Bn-*i z* are shown in Table 1, where A is the number of carbon atoms in the fatty acid chain, B is the number of double bonds, n-*i* is the location of the double bond on the *i*th carbon–carbon bond, counting from the terminal methyl carbon towards the carbonyl carbon and *z* is the geometrical configuration expressed as c for *cis* and t for trans.

Data handling was performed on a Pentium 4 personal computer. All programs, including HELP, automatic search tool for straight saturated FAMEs, automatic calculation of theoretical and experimental ECL, were coded in Matlab 6.5 for windows (The Mathworks, Natick, MA).

3. Results and discussion

In order to accurately identify fatty acids without standards, the combination of mass spectra and retention times is necessary. For identification of constitutional isomers and homolog, our previous studies [24,25] proposed methods to combine mass spectral characteristics and rules of retention times/indices. Additionally, for geometric isomers, we developed a strategy for establishment of special library with mass spectra and retention indices [8,15]. However, in identification of geometrics isomers, the retention indices and mass spectra between isomers are so slight that chromatographic shift could influence results of identifications. In this study, to obtain the best repeatability of retention indices, we established a special library of mass spectral characteristics and retention indices (ECL for fatty acid methyl esters) at **uniform conditions**. All unknown samples were subsequently analyzed at the same conditions.

Straight saturated fatty acids make up 10–40% of the total fatty acids in most natural lipids [4]. Additionally, mass spectra of straight saturated FAMEs are relatively simple and can be therefore applied to unambiguously identify the fatty acids alone. In this study, mass spectral characteristics of saturated FAMEs were used to identify all expected saturated FAMEs whose retention times were subsequently used to calculate the ECL of other FAMEs in the analyzed sample according to Eq. (1) in Section 2.4. This method involves three steps as follows: (1) an automatic mass spectral search is conducted to identify all expected straight saturated FAMEs; (2) The ECL for unsaturated FAMEs can be calculated by employing the retention times of straight saturated FAMEs; and (3) characteristic ions and ECL of FAMEs are employed to identify the structure of compounds by comparing with those in the library.

3.1. Construction of database of commonly used FAMEs

In the analysis of fatty acids, analytical conditions have a significant effect on the ECL values especially when the polar columns were employed in the GC–MS system. So, analytical conditions might be important for construction of a database involving mass spectra and ECL values of commonly used FAMEs. In this report, our database was built on analytical conditions proposed in our previous studies [13–15] including a fused silica capillary column

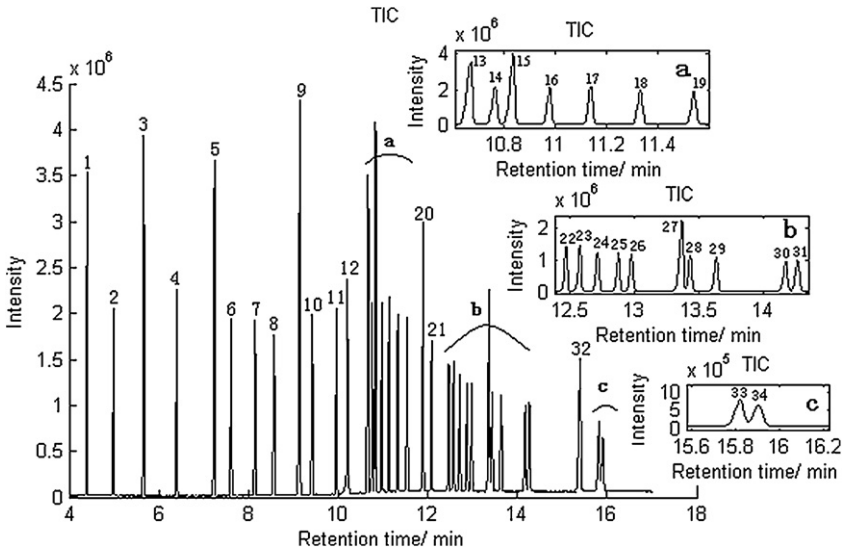

Fig. 1. Total ion current of 34 reference FAMEs. 1, 10:0; 2, 11:0; 3, 12:0; 4,13:0; 5, 14:0; 6, 14:1n-5c; 7, 15:0; 8, 15:1n-5c; 9, 16:0; 10, 16:1n-7c; 11, 17:0; 12, 17:1n-7c; 13, 18:0; 14, 18:1n-9t; 15, 18:1n-9c; 16, 18:2n-6t; 17, 18:2n-6c; 18, 18:3n-6c; 19, 18:3n-3c; 20, 20:0; 21, 20:1n-9c; 22, 20:2n-6c; 23, 21:0; 24, 20:3n-6c; 25, 20:3n-3c; 26, 20:4-6c; 27, 22:0; 28, 20:5n-3c; 29, 22:1n-9c; 30, 22:2n-5c; 31, 23:0; 32, 24:0; 33, 24:1n-9c; 34, 22:6n-3c.

DB-23 (30 m × 0.25 mm × 0.25 μm) and fixed temperature program described in Section 2.

Through tested by more than 30 reference FAMEs, these analytical conditions are suitable to the analysis of fatty acids whose carbon atoms are from 10 to 24. As shown in Fig. 1, the complete baseline separation could be achieved for geometric isomers, for example 18:1n-9t (at 10.76 min) and 18:1n-9c (at 10.83 min), 18:2n-6t (at 10.98 min) and 18:2n-6c (at 11.14 min). It is well-known that it could not obtain the best separation of all peaks for the various samples at uniform analytical conditions. In this case, multivariate curve resolution could compensate for this disadvantage. Recently, there are many chemometric methods proposed for the resolution of two-way data. Among them, heuristic evolving latent project (HELP), which was proven to be an effective method to revolve the overlapping peaks [24,25], was employed to deconvolve GC–MS data of sample.

As illustrated in Table 1, more than 30 commonly used FAMEs were collected in the database. Surely, according to analytical requirement and potential composition of practical samples, more FAMEs could be added into this database in future. It is worth noting that ECL values are calculated using the Eq. (1) for programming temperature ECL.

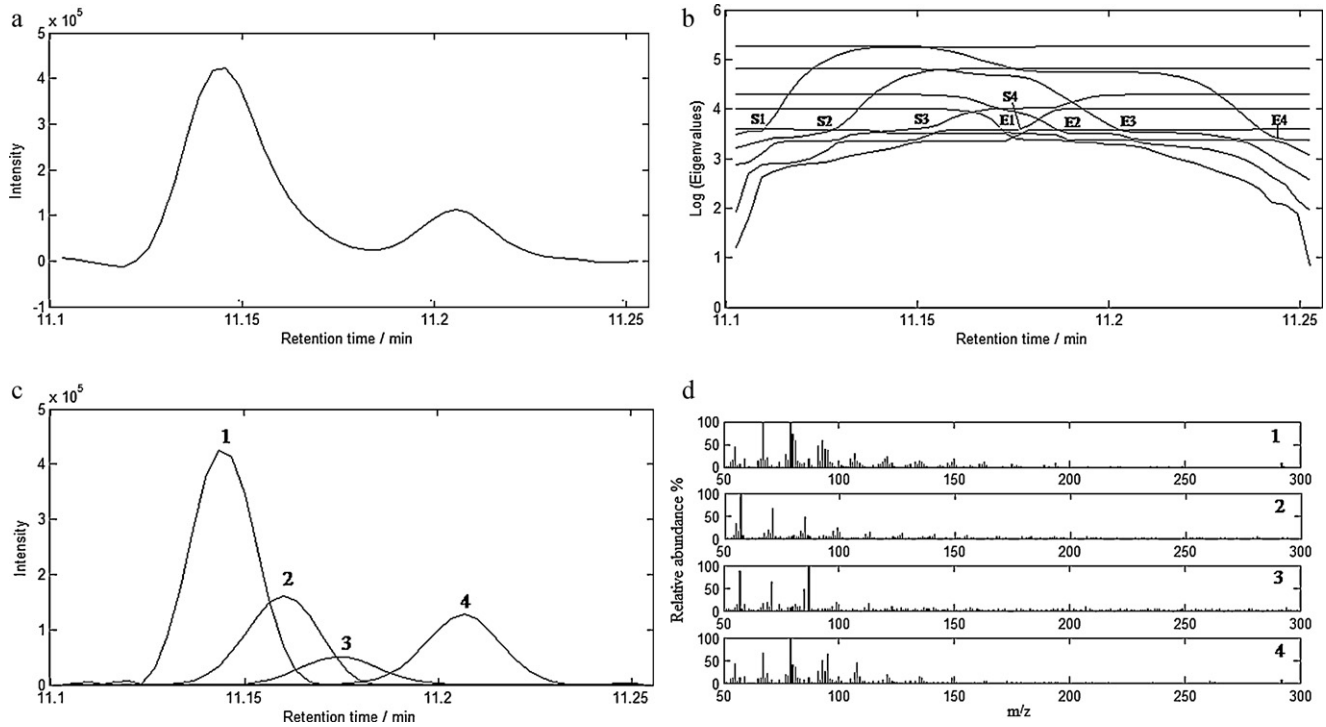

Fig. 2. (a) Total ion current chromatogram of the overlapping peaks in the range of 11.1–11.3 min; (b) evolving factor analysis (EFA) plot of the data shown in (a); (c) the pure chromatographic profiles for three components in the overlapping peaks; and (d) pure mass spectra of three components.

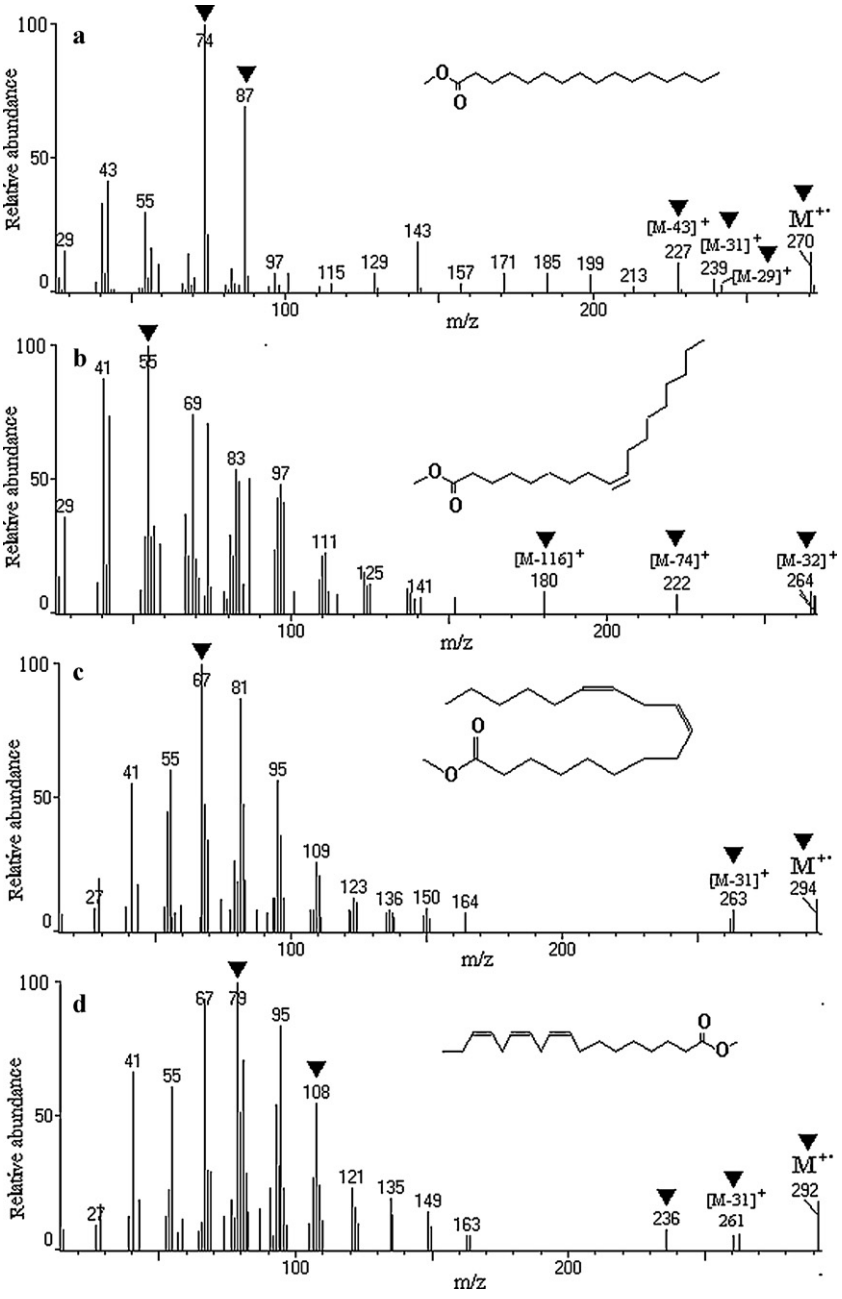

Fig. 3. Mass spectrum and structure of palmitic acid methyl ester (a), Oleic acid methyl ester (b), Linoleic acid methyl ester (c) and Linolenic acid methyl ester (d).

3.2. Resolution of overlapping peaks by HELP

To obtain stable ECL of fatty acids, the uniform conditions are employed for all samples. So, the overlapping peaks inevitably appear in the two-way data. In this case, chemometric methods (such as HELP used in this study) might be useful to obtain the pure mass spectra and chromatographic profiles. For the sake of brevity, only the simple description of HELP is given for the detailed theories and procedures could be found elsewhere [22,23]. The chromatographic peaks in the range of 11.10–11.25 min were taken as example to simply describe how HELP works. Fig. 2a plots the total ion chromatogram (TIC) of this data, while evolving eigenvalues obtained through evolving factor analysis (EFA) are shown in Fig. 2b. From Fig. 2b, four components can be determined in the overlapping peaks and their elution regions are marked. Using the information of elution regions, the two-way data matrix of the overlapping peaks can be uniquely resolved into pure chromatographic profiles and mass spectra for all components. The resolution results are shown in Fig. 2c and d. With the help of this chemometrics method, the accurate chromatographic profile and high-quality mass spectrum of this fatty acid could be obtained for the subsequent identification.

3.3. Mass spectral fragmentation of FAMEs

The classic researches of Stenhagen and his coworkers in 1950s on the mass spectra of FAME was probably the first clear demonstration of the value of mass spectrometry for deducing the structure of complex natural product molecules [26]. The primary fragmentations of some types of FAMEs are briefly summarized.

Mass spectra characteristics of straight saturated FAMEs are relatively simple. As shown in Fig. 3a, the molecular ion, which usually

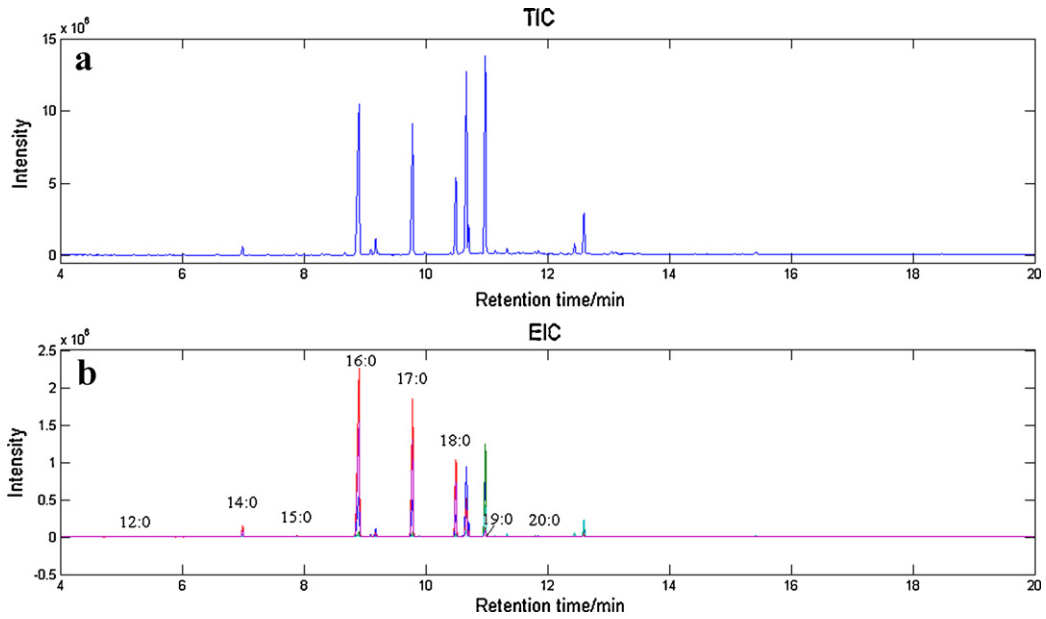

Fig. 4. Identification results of straight saturated FAMEs (a) total ion current chromatogram of one sample of human plasma, and (b) extracted ion chromatograms of one sample of human plasma.

appears in mass spectrum, could be therefore used to conclude the molecular weight. Additionally, the base peak of all mass spectra of straight saturated FAME appears at *m*/*z* 74 [26,27]. Neutral losses 29 D (loss of ethyl), 31 D (loss of methoxy group) and 43 D (loss of propyl) are other important mass spectral characteristics of this kind of FAMEs. More detailed differences between mass spectra of straight saturated FAME and those of branched saturated FAME are described in the Lipid Library [4]. Although mass spectra of branched FAMEs are similar with straight saturated FAMEs, the content of this kind of fatty acids are relative low and their ECLs are obviously different from those of straight saturated FAMEs. So, we can eliminate the influence from branched saturated FAMEs.

For fatty acids in biological samples, the *m*/*z* of molecular ion could be used to determine the molecular weight and the type of a methyl ester. The molecular ion of monoenoic FAME might be sometimes absent. However, the characteristic neutral losses including 32 D (loss of methanol), 74 D (loss of methanol and propylene), 116 D (Fig. 3b) supply the clue to infer the molecular weight. The base peak of mass spectra of monoenoic FAME often presents at *m*/*z* 55, while that of the dienoic FAME usually appears at *m*/*z* 67 or 55. As shown in Fig. 3c, the present of molecular ion and neutral loss 31 D (loss of methoxy group) are characteristics of their mass spectra. Contrast to monoenoic and dienoic FAMEs, mass spectra of trienoic FAMEs provide more information for identification of the structure of molecules. The odd ions, *m*/*z* 108 and 236 for linolenic acid methyl ester (Fig. 3d), suggest that the octadecatrienoic acid methyl ester is an n-3 polyunsaturated fatty acid, while those at *m*/*z* 150 and 194 mean that this FAME is an n-6 polyunsaturated fatty acid [28–30]. After summarizing of the above characteristic fragmentation and data mining of the standard mass spectra, the detailed mass spectral characteristics were achieved and listed in Table 1.

3.4. Identification of the FAMEs in human plasma

To test this method and demonstrate the protocol of identification of FAMEs, a GC–MS dataset of human plasma was taken as an example (Fig. 4a).

From above analysis of mass spectral characteristics of FAMEs, it is obvious to find that the major differences among types of FAMEs are the mass-to-charge ratio of base peak. At the first step, these mass-to-charge ratios (including 74, 55, 67 and 79) are therefore used to automatically identify the type of FAMEs from their mass spectra. As shown in Fig. 4, the extracted ion chromatograms of *m*/*z* 74, 87, 55, 67 and 79 were used to identify all expected straight saturated FAMEs from the background subtracted GC–MS data of human plasma. All of peaks whose signal-to-noise ratios (S/Ns) were more than 3 were analyzed. After automatic implementing this detection, nine straight saturated FAMEs, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0 and 20:0 included, were singled out (Fig. 4b).

At the second step, the background-subtracted GC–MS data were resolved by HELP. The pure chromatograms and pure mass spectra of 37 compounds, most of which are fatty acid methyl esters, were obtained after deconvolution. Among them, there were 15 components whose S/Ns were more than 5 (Table 2). Meanwhile, the peak areas of 15 components were obtained using the chemometric method. Their mass spectra were used to infer the type of FAME using the mass spectral characteristics of each type above mentioned.

At the third step, the 9 straight saturated FAMEs were subsequently used to calculate the ECL of peaks using the Eq. (1). The retention time of apex of each peak, whose S/Ns were more than 5, was taken as the retention time of the corresponding compounds.

Finally, ECL of the interest was compared with those in the library and the FAME with most similar ECL was determined to be the candidate. The threshold of difference between the experimental ECL and corresponding ECL in the database is set to 0.1. Taking the peaks at 9.110 and 9.194 min as examples, first the both of components were identified as the mono-FAME (16:1) through the base peak and molecular ion in their mass spectra; then the theoretical ECLs of mono-FAMEs were calculated and compared with that of the interest; Finally, the components at 9.110 and 9.194 min were identified as 16:1n-9c and 16:1n-7c, respectively. Using the same method, as shown in Table 2, 15 components were identified. The results were validated by the authentic standards.

Table 2
Results of identification of the FAMEs in human plasma.

Retention times	Type	ECL	Theoretical ECL	Relative area	Area (%)
5.453	12:0	12.000	12.000	0.005095	<0.05
6.993	14:0	14.000	14.000	0.05615	0.9433
7.890	15:0	15.000	15.000	0.01076	<0.05
8.923	16:0	16.000	16.000	1.5859	26.64
9.110	16:1	16.215	0.2203 (16:1n-9c)	0.04536	0.7621
9.190	16:1	16.307	0.3305 (16:1n-7c)	0.1180	1.983
9.794	17:0	17.000	17.000	1.0000	IS
10.497	18:0	18.000	18.000	0.5276	8.864
10.673	18:1	18.303	18.2647 (18:1n-9c)	1.3423	22.55
10.706	18:1	18.360	18.3587 (18:1n-7)	0.1530	2.570
10.984	18:2	18.840	18.7643 (18:2n-6c)	1.3922	23.39
11.077	19:0	19.000	19.000	0.004811	<0.05
11.673	20:0	20.000	20.000	0.006184	<0.05
12.442	20:3	21.230	21.1778 (20:3n-6c)	0.07170	1.205
12.600	20:3	21.473	21.3806 (20:3n-3c)	0.2885	4.847

4. Conclusion

The identification of unknown compounds in GC–MS data is a major challenge. To accurately identify geometrics isomers, we established a special library of mass spectral characteristics and retention indices (ECL for fatty acid methyl esters) at **uniform conditions** and analyzed the unknown samples at the same conditions. Furthermore, the straight saturated FAMEs were picked out with the help of mass spectral characteristics and employed to calculate the ECL for other FAMEs. This method could be used to accurately identify the FAMEs without authentic standards. Additionally, it eliminates most of the error from run-to-run drift in retention times. After tested by the sample of human plasma and validated by the authentic standards, our method was proven to be used to identify fatty acids in the complex GC–MS data of biological sample. Through this study, it indicates that the strategy proposed in this study for establishment of special mass spectral and retention indices library should be also extend to identify the isomers with very similar mass spectra and similar retention indices (e.g. ECL for FAME).

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