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Rapid and sensitive determination of fatty acids in edible oil by liquid chromatography-electrospray ionization tandem mass spectrometry

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A sensitive and robust on-line LC/MS method was developed for quantitative determination of linoleic acid, docosahexaenoic acid and docosanoic acid from edible oil samples. The oil samples were dissolved in chloroform-isopropyl alcohol (20:80, *v*:*v*) solution and the three fatty acids were separated by HPLC with a C4 column using 10 mmol/L ammonium acetate-isopropyl alcohol-acetonitrile (20:40:40, *v*:*v*:*v*) mobile phase in isocratic elution. Electrospray ionization mass spectrometry with the selected ion recording monitoring was used to detect and quantify the fatty acid. The calibration curves were linear in the range of 10.00–5000 pg/mL for linoleic acid and docosanoic acid, and 1.000–500.0 pg/mL for docosahexaenoic acid. The limit of detection was 2.0 pg/mL for linoleic acid, 3.0 pg/mL for docosanoic acid, and 0.20 pg/mL for docosahexaenoic acid. The results showed that the method described in this paper could be utilized for rapid determination of three fatty acids at picogram levels in edible oils.

liquid chromatography-mass spectrometry, linoleic acid, docosahexaenoic acid, docosanoic acid, edible oil

1 Introduction

Fatty acids (FAs) play important roles in a number of diseases, including atherosclerosis [1], cystic fibrosis [2], type II diabetes [3], depression and epilepsy [4–6], inflammatory bowel diseases [7], and breast and ovarian cancers [8–10]. FAs are mainly acquired from food and oil. Therefore, information of FAs content in the food and edible oils is important. In this report, a rapid and accurate simultaneous determination of linoleic acid (LA, $C_{18}H_{32}O_2$, M_w 280), docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$, M_w 328) and docosanoic acid (BA, $C_{22}H_{44}O_2$, M_w 340) was described. These three FAs (Figure 1) were examined not only because of their wide distribution in various foods such as dairy products, meat, and edible oils, but also because of their important physiological functions. For example, LA was shown to have anticarcinogenic and antiatherogenic effects [11], and DHA could affect membrane receptor activities and cellular signaling [12]. Since processing, storage and preparation of food and oil might affect their FAs content, it is important to develop a simple, rapid and accurate method for determining their levels in food and oil.

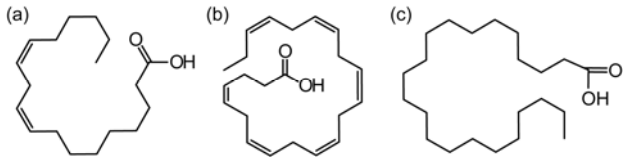

Figure 1 Chemical structures of LA (a), DHA (b) and BA (c).

To date, several methods have been employed to analyze

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FAs, such as thin-layer chromatography (TLC) [13–15], gas chromatography (GC) [13, 16, 17], high performance liquid chromatography (HPLC) [18–20], enzyme-linked immunosorbent assay (ELISA) [21], gas chromatography-mass spectrometry (GC-MS) [22, 23], and liquid chromatography-mass spectrometry (LC-MS) [10, 24]. Among these methods, high selectivity and sensitivity can be achieved by GC-MS [25]. However, the required derivitization steps are time-consuming. ELISA method is simple but does not provide good selectivity, limit of detection (LOD) and limit of quantification (LOQ). In contrast, LC-MS with multi-reaction monitoring (MRM) and selective ion monitoring (SIM) provides high selectivity and sensitivity without the need of derivitization steps. Here, we report a simple and effective method of preparing samples of edible oils, and a rapid and accurate LC-MS method for simultaneous determination of LA, DHA and BA contents in these oils.

2 Experimental

2.1 Chemicals and standards

Unless specified otherwise, all chemicals including solid and powder were analytical reagent grade and obtained from the Beijing Chemical Factory Co. Ltd. (Beijing, China). Acetonitrile (ACN) and isopropyl alcohol (IPA)) were of HPLC grade and purchased from Fisher Scientific Co. Ltd. (Hampton, NH, USA). Trichloromethane (TCM) was provided by Fuyu Chemical Factory Co. Ltd. (Tianjin, China). Linoleic acid (LA, purity > 99%), docosahexaenoic acid (DHA, purity > 98%) and docosanoic acid (BA, purity > 99%) were obtained from Sigma-Aldrich Co. Ltd. (St Louis, MO, USA). Eight different oils (corn oil, walnut oil, sunflower seed oil, peanut oil, grape seed oil, olive oil, quality cooking oil and sesame oil) were obtained from local supermarket. An ultra-water system from SG Water Purification System (Barsbuttel, Germany) was used to obtain ultrapure water.

2.2 Preparation of standard solution

Individual standard stock solutions (1000 ng/mL) of LA, BA and DHA were prepared in IPA-ACN (1:1, *v*:*v*) solution. Mixed standard stock solution of the three FAs was prepared in IPA-ACN (1:1, *v*:*v*) with the concentration of 100.0 ng/mL for LA, 10.00 ng/mL for DHA, and 100.0 ng/mL for BA. The standard solutions for calibration curves were 10.00–5000 pg/mL (10.00, 50.00, 100.0, 1000, and 5000 pg/mL) for LA and BA, 1.000–500.0 pg/mL (1.000, 5.000, 10.00, 100.0, and 500.0 pg/mL) for DHA. Each assay was performed in triplicate.

2.3 Sample preparation

1.0 mL oil sample and 5.0 mL TCM-IPA (2:8, *v*:*v*) were added and vigorously mixed with the HS3120 BENCHTOP CLEANERS supersonic wave mill (Beijing, China) for 5 min. Then, a 10 μL mixed sample solution was added to 1.0 mL IPA-ACN (1:1, *v*:*v*). The diluted oil sample was filtered and then transferred to vials for HPLC/MS analysis.

2.4 Instrumentation

Chromatographic separation was performed in the Waters Alliance 2695 HPLC system, equipped with a column oven. HPLC columns used include Waters XBridge C_{18} (100 mm × 2.1 mm i.d., 3.5 μm), Symmetry C_4 (100 mm × 2.1 mm i.d. 3.5 μm) and Atlantis HILIC Silica (100 mm × 2.1 mm i.d., 3 μm) columns. The mobile phases composed of A (10 mmol/L ammonium acetate), B (isopropyl alcohol) and C (acetonitrile). The samples were eluted with four different mobile phases (Table S1, in the Supporting Information online) and eluted at a flow rate of 0.3 mL/min.

Quattro Premier XE Mass Spectrometer (Waters, UK) was operated in the negative ESI ion mode for the quantification of fatty acids. The source temperature was 120 °C, desolvation temperature was 350 °C, and the gas desolvation for BA, and the cone gas was set at 30 L/h. Selected ion recording (SIR) was used to detect molecular ions mass. In all cases the product ions were the *m*/*z* 279 for LA, *m*/*z* 327 for DHA and *m*/*z* 339 for BA. The dwell time was 0.2 s. All mass spectral data were acquired in the centroid mode. Data acquisition and processing were performed using Masslynx 4.1 Analyst Software Quanlynx.

3 Results and discussion

3.1 Sample preparation

Three different solvent systems including isopropyl alcohol, chloroform-isopropyl alcohol and chloroform-acetonitrile were used to dissolve the standard FAs and different oil samples. It was found that all samples dissolved better in the chloroform-isopropyl alcohol mixed solvent (data not shown). Thus, sample preparation was optimized by dissolving 1.0 mL edible oil in 1.0 mL chloroform and then diluted with 4.0 mL isopropyl alcohol. Then 10 μL of the mixed sample solution was further diluted by adding to 1.0 mL isopropyl alcohol-acetonitrile (1:1, *v*:*v*) solution. The final sample solution was homogeneous and compatible with the mobile phase and MS system.

3.2 Choice of ion source for fatty acids

The quadrupole mass spectrometer system includes the atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) ion sources. Generally, APCI is used for the analysis of samples with less polarity and ESI is for polar compounds. Since the polarity for the three FAs under investigation is medium polarity, it is necessary to optimize the ion source for their analysis. The optimum

condition for APCI was determined as follows: mass scanning range, *m/z* 250–350; scan time, 0.5 s; vaporizer temperature, 400 °C; capillary heater temperature, 150 °C; corona current, 5.0 μA; gas desolvation nitrogen, 500 L/h; and cone gas (also nitrogen), 30 L/h. The MS spectra for three FAs were obtained and shown in Figure 2. The optimum conditions for ESI were shown in the section 2.4, and the MS spectra were shown in Figure 2(a).

Under the optimized MS conditions, the signals at *m/z* 279, 327, and 339 for LA, DHA, and BA were shown clearly under both full-scan-ESI/MS and APCI/MS ionization modes, respectively (Figure 2). However, the signal intensity for three FAs by ESI (intensity scale of 1.33×10^8, 4.11×10^8, and 8.22×10^7, Figure 2(a)) under each of their optimized condition was greater than that by APCI (intensity scale of 6.36×10^6, 6.76×10^6, and 1.63×10^7, Figure 2(b)), respectively. Thus, ESI ion source was employed in the following experiments.

Using the optimized ESI ionization mode, the negative molecular ion $[M–H]^-$ of each FAs was selected as the precursor ion, and their MS/MS spectra were obtained. As shown in Figure 3, it could be seen that the signal intensity of product ions were much weaker than the parent ions. This strongly suggested that all parent ions with $[M–H]^-$ at *m/z* 279, 327 and 339 were stable and suitable for sensitive detection of three FAs by SIR method.

3.3 Optimization of HPLC program and column

We have also evaluated four different elution programs (Table S1) and three different columns for HPLC separation of FAs. The elution programs were first tested in Symmetry C4 column, and the results are shown in Figure S1. It could be seen that elution program 1 and 3 provided longer retention time than program 2 and 4. However, among these programs, only program 2 resulted in symmetrical peaks,

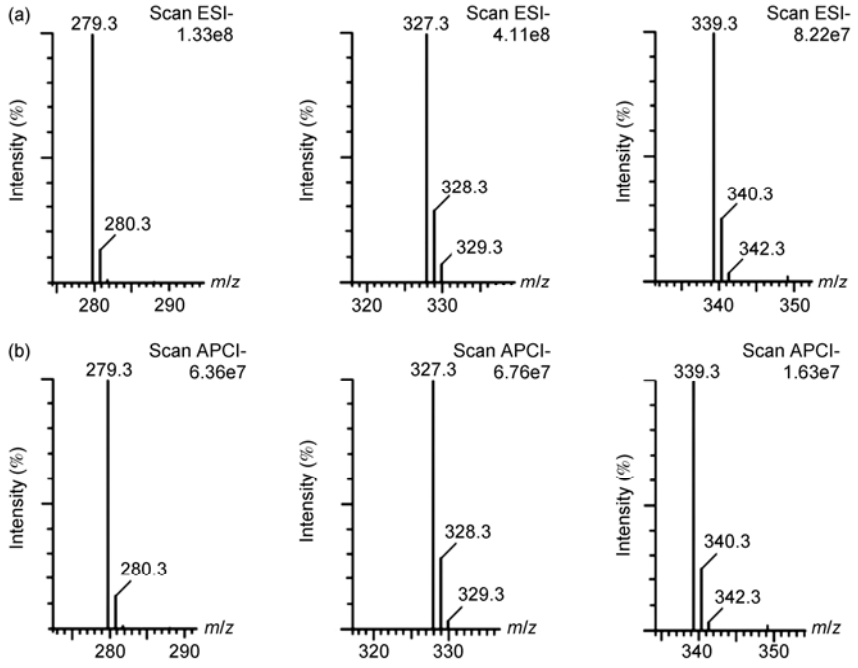

Figure 2 MS spectra with ESI source (a) and APCI source (b) for LA (*m/z* = 279), DHA (*m/z* = 327) and BA (*m/z* = 339).

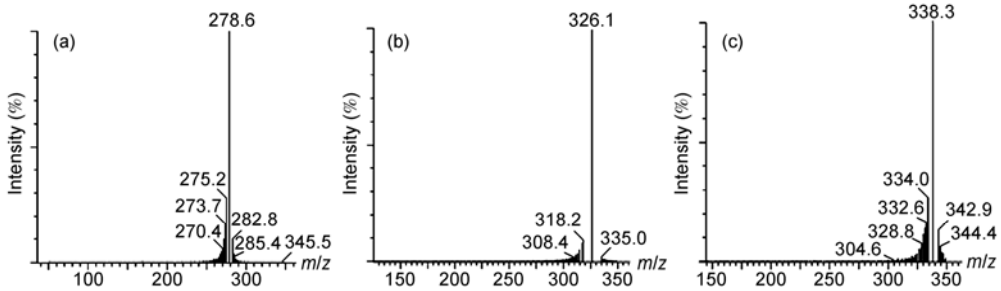

Figure 3 MS/MS fragmentation of LA (a), DHA (b) and BA (c) with MRM mode.

providing better sensitivity and accuracy for the assay. Thus, elution program 2 was adopted.

Firstly, the void volumes of the columns were tested at 0.7, 0.6 and 0.5 min, respectively. Using the optimized elution program 2, separation of three FAs in the three HPLC columns (XBridge C18, Symmetry C4, and Atlantis HILIC Silica) were compared.

As shown in Figure 4, three FAs are not retained in the silica column, but are well retained in the C18 and C4 columns. Their retention times are longer in C18 resulting in the tailing peaks. In C4 column, the peaks were symmetrical. Therefore, C4 column and elution program 2 were adopted. Under this condition, three FAs were separated within 3 min (Figure 5(a)).

3.4 Analytical parameters

Using HPLC/MS method with ESI ion source and SIR mode, the linearity between the peak area and the concentration (pg/mL) for three FAs were determined. Their linear regression equations were given in Table S2. Their LOD (signal-to-noise ratio, S/N = 3) and LOQ (S/N = 10) were also calculated and shown in Table S2. These data demonstrated that the developed HPLC/ESI/SIR/MS method was sensitive. The three FAs can be detected at pg/mL level.

The precision of the method was assessed by the coefficients of variation (CV) of the quality control (QC) samples at lower (LQC), middle (MQC) and higher (HQC) concentration levels. The QC samples of three FAs were determined in triplicate on three separate days, and the CV results are shown in Table S3.

The accuracy of the method was evaluated by assessing the recovery of three FAs standards. Three QC samples at three levels (LQC, MQC and HQC) were determined and the results are shown in Table S4. Nearly full recovery of the three FAs demonstrates that the developed method is reproducible and accurate.

3.5 Application

We developed a simple HPLC/ESI-MS method to quantify FAs in oil. A typical SIR chromatogram of an oil sample is

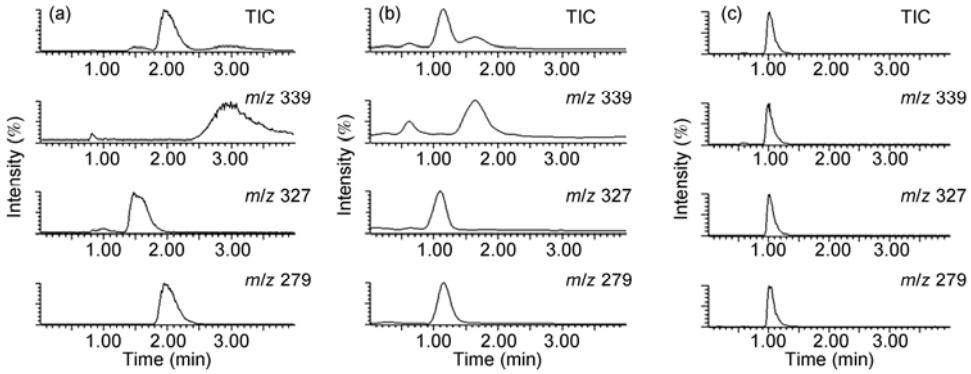

Figure 4 HPLC/ESI/SIR/MS spectra of three standard fatty acids. (a) The XBridge C18 column (2.1 mm×100 mm); (b) the Symmetry C4 300 column (2.1 mm×100 mm); (c) the Atlantis HILIC Silica column (2.1 mm×100 mm). Program 2 was used for all experiments in this figure.

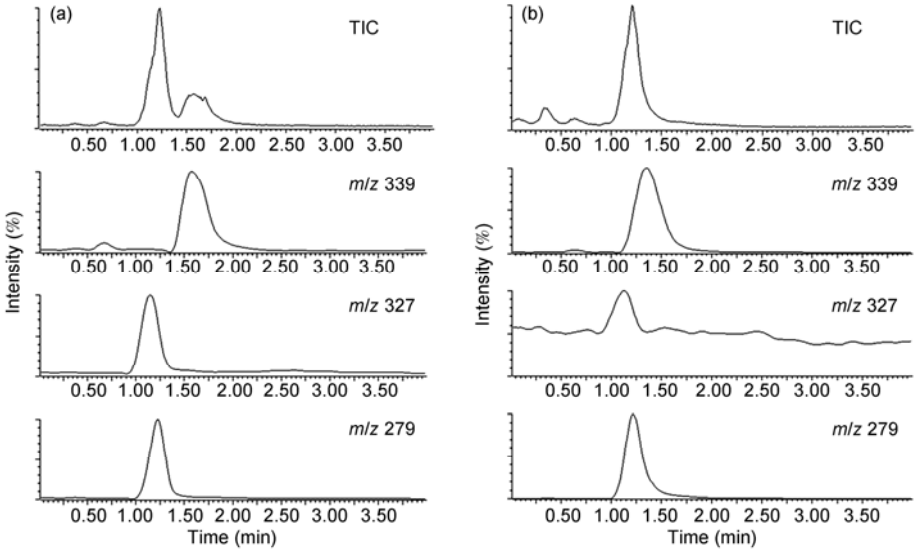

Figure 5 HPLC/ESI/SIR/MS spectra. (a) Three standard fatty acids; (b) three fatty acids from oil sample. All the experiments were performed under the optimized conditions.

Table 1 Determination of three fatty acids in eight oil samples (ng/mL)

Sample	LA	DHA	BA
Corn oil	71.49 ± 2.941	1.242 ± 0.1054	420.0 ± 58.86
Walnut oil	477.6 ± 27.64	1.013 ± 0.0985	609.6 ± 84.4
Sunflower seed oil	247.3 ± 37.62	1.657 ± 0.1270	580.7 ± 33.18
Peanut oil	890 ± 48.23	1.879 ± 0.04060	636.7 ± 36.24
Grape seed oil	217.9 ± 2.674	1.896 ± 0.2760	438.6 ± 5.533
Olive oil	63.47 ± 1.505	1.369 ± 0.0813	887 ± 10.83
Quality cooking oil	137.8 ± 0.4793	2.085 ± 0.07844	616.2 ± 3.870
Sesame oil	1.914×10^4 ± 721.5	339.3 ± 11.89	2997 ± 731.1

shown in Figure 5(b). It clearly shows that LA, DHA and BA can be separated in a single run and can be completed within 3 min. The result of this simple and rapid analysis of FAs is comparable to that of the complicated method of Pichini's [3]. Quantitative data of three FAs in 8 oil samples are shown in Table 1. These results demonstrate that different oil samples contain different amounts of LA, DHA and BA. This information will be useful for consumers to select suitable oil for their dietary needs.

4 Conclusions

A simple, sensitive and rapid HPLC/MS method for the simultaneous quantification of linoleic acid, docosahexaenoic acid and docosanoic acid in eight different oil samples has been developed in this paper. The FAs were obtained by dissolving with 20% trichloromethane in isopropyl alcohol as dissolve solvent. The improved performance for FAs in oil samples was demonstrated. The preparation was easily applied to the real samples and also brought satisfactory accuracy results. The HPLC/ESI-MS method described in this paper is highly applicable to analysis of FAs samples because it does not need derivatization which can affect the accuracy results. What's more, the HPLC method with C4 column under 10 mmol/L ammonium acetate-isopropyl alcohol-acetonitrile (20:40:40, *v*:*v*:*v*) can analyze in 5 min. The selective SIR scanning mode allows the detection of FAs at pg/mL (or femtomole) level.

In sum, this method is suitable for the determination of linoleic acid, docosahexaenoic acid and docosanoic acid in oil samples. Especially, the preparation process of samples described in this paper is eligible for the quatification analysis of the oil with high throughput workload. Furthermore, this method can be readily modified to quantitate FAs content in other types of food.

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