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RESEARCH PAPER

Improvement of Detection Sensitivity of Triglyceride with Methylamine Formate as Ionization Enhancer in Reversed Phase Liquid Chromatography-Electrospray Ionization Mass Spectrometry

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Abstract: A novel ionization enhancer, methylamine formate, was proposed for improving the detection sensitivity of triglyceride in edible oil by reversed phase liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). The commonly used isopropanol-acetonitrile-methanol-water and isopropanol-acetonitrile were selected as the mobile phase. By using a reversed phase C18 column, and taking the corn oil in isopropyl alcohol as sample solution, we compared methylamine formate with ammonium formate, as ionization enhancers, for their effect on the detection sensitivity of triglyceride by LC-ESI-MS after screening other different ionization enhancers, such as formic acid, acetic acid, ammonium formate, ammonium acetate, butyl formate, dibutylamine formate, triethylamine formate, diethylamine formate, methylamine formate, and ethylamine formate. The result indicated that, using methylamine formate in mobile phase, the mass spectral peak response and the signal to noise ratio of trilinolein component were all about 5 times higher than that using ammonium formate. The effect of the concentration of methylamine formate ionization enhancer, the flow rate of the mobile phase and the flow rate of nebulizing gas on the detection of methylamine formate was investigated. The concentration of triglyceride components in corn oil starting to form aggregates was similar in different mobile phases in the electrospray process according to measurement of the relationship between corn oil concentrations and the total ion chromatogram peak area of triglyceride. In particular, the peak area of trilinolein was linear with its concentration in the range of 7×10^{-7}–2×10^{-4} M with the correlation coefficient $R^2 = 0.9997$, but increased slower in the higher concentration range. According to the experimental data, the mechanism for improvement of detection sensitivity of methylamine formate was suggested as that the addition of methylamine mono-charged ions with hydrophobic groups had lower solvation energy, which made the enriched addition ions easily evaporation from the droplet surface, thus improving the electrospray ionization efficiency. This method provided an effective way to improve the detection sensitivity of triglyceride in edible oil by LC-ESI-MS.

Key Words: Liquid chromatography mass spectrometry; Triglyceride; Sensitivity; Methylamine formate

1 Introduction

The edible oil is mainly composed of triglyceride compounds (TAGs) with similar physical and chemical properties. The analysis of the composition of TAGs is an important basis for identifying adulteration of edible oil. Separation and analysis of different TAGs are commonly used with liquid chromatography-mass spectrometry (LC-MS) methods, especially the electrospray ionization (ESI) method[1,2]. However, the method presents some problems like poor detection sensitivity at low concentration, and the lipid aggregating at high concentration[2], which results in a narrow

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linear range. Therefore, it is important to improve the sensitivity and extend the linear range of mass spectrometry for the analysis of the TAG in edible oil.

To improve the ESI response, it usually requires increasing the ionization efficiency of the sample. Ikeda *et al*[3] analyzed triglyceride by reverse phase LC-ESI-MS/MS using the gradient elution mobile phases of acetonitrile, methanol or water with isopropanol (all containing acetic acid-ammonia buffer), and obtained accurate identification using MS/MS. Niu *et al*[4] studied the effect of nebulizing conditions of electrospray ionization on the detection. By comparing the effect of formic acid with that of pH value on the component's MS response, it was found that formic acid had a significant improvement for the ionization, whereas the pH had not such effect. Segall *et al*[5] also used formic acid to improve ionization in the analysis of triglycerides in coffee. Zeb *et al*[6] added acetic acid, ammonium acetate and sodium acetate simultaneously in the mobile phase, to identify the triglyceride compounds using different addition ions ($[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$). Now, commonly used ionization enhancers are formic acid, ammonium formate, acetic acid and ammonium acetate, etc. Tarvainen *et al*[7] also used lithium ion as ionization enhancer to analyze component of epoxy, hydroperoxy, and hydroxy fatty acids, and acylglycerols in oxidized, hydrolyzed and simulated digested rapeseed oil samples by LC-ESI-MS. Mirabaud *et al*[8] used lithium ion as ionization enhancers to analyze triglycerides. Cech *et al*[9] suggested the components with easy charged and proper hydrophobicity to improve the ESI process, based on a systematic review of the ESI-MS research results. This provided a way to find different ionization enhancers to improve the efficiency of electrospray ionization mass spectrometry. Grossman *et al*[10] synthesized methylammonium formate (MAF), and used it replacing methanol (MeOH) effective in reversed-phase LC experiment according to its lower viscosity. The result illustrated that MAF effectively suppressed the peak broadening due to silanol interaction and enhanced the separation of nitrofuran drugs than MeOH. Compatibility of MAF with LC-MS was shown for both APCI and ESI modes, particularly at modest flow rates. It showed that methylamine or amines could improve the detection sensitivity in LC-MS systems.

In this research, with the isopropyl alcohol-acetonitrile-methanol-water and isopropanol-acetonitrile as the mobile phase for the separation of triglycerides, and corn oil in isopropanol solution as sample, a novel ionization enhancer, methylamine formate, was proposed to enhance the ionization efficiency of triglycerides and improve the sensitivity of mass spectrometry by reversed phase liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). And the minimum concentration of the triglyceride starting to aggregation in the process of ESI was also investigated[2]. The method provided a way for LC-MS to improve the detection sensitivity of triglycerides in edible oil.

2 Experimental

2.1 Instrumentations and reagents

The instruments used in this experiment were as follows: LCMS-2020 ultra performance liquid chromatography-mass spectrometer (Shimadzu Corporation, Japan); N341M nitrogen generator (Proton Energy Systems Inc, USA); SY7200-D ultrasonic cleaning machine (Shanghai sound source ultrasound equipment Co., Ltd.); XS105 dual range electronic balance (Mettler Toledo, Switzerland).

Acetonitrile, isopropanol and methanol (HPLC grade) were purchased from Shanghai Adamas, China. Butylamine, dibutylamine, triethylamine, diethylamine, methylamine (25%–30%) and ethylamine (65%–70%) were analytical reagent (AR) made in China. Formic acid, acetic acid, ammonium formate, ammonium acetate (HPLC grade) were purchased from Shanghai Anpel Experimental Technology Co., Ltd., China. Distilled water (H_2O) was product of Watson Group Ltd. (China). Corn oil was purchased in local supermarkets.

2.2 Experimental method

2.2.1 Sample preparation

The sample solutions (0.001–10 μL mL^{-1}) were prepared by respectively dissolving 1 μL, 5 μL and 10 μL of corn oil sample in 1000 μL of isopropano.

2.2.2 Chromatographic conditions

Ultimate UHPLC XB-C_{18} Chromatography column (150 mm × 2.1 mm, 1.8 μm, Welch Materials, Inc.) was used to separate the targets. The mobile phase (i) was isopropanol-acetonitrile-methanol-water (80:81.6:14:0.4, *V*/*V*) and mobile phase (ii) was isopropanol-acetonitrile (50:50, *V*/*V*), in which containing 5 mM ammonium formate (A) or 5 mM formic acid + 5 mM methylamine (B) as ionization enhancers. The separation and analysis were carried out by isocratic elution of four mobile phases of 1A, 1B, 2A, 2B which were constituted with them, respectively. The flow rate was 0.3 mL min^{-1} and the injection volume was 3 μL. Under the condition of ESI-MS direct injection mode, the columns in the above chromatographic conditions were replaced with two way connector.

2.2.3 Mass spectrometry conditions

The mass spectrometric analysis was performed under the conditions such as ESI ionization source, positive ionization mode, mass to charge ratio range of *m*/*z* 300–1200, nebulizing