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Quantitation of triacylglycerols in edible oils by off-line comprehensive two-dimensional liquid chromatography–atmospheric pressure chemical ionization mass spectrometry using a single column

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ABSTRACT

In this investigation, off-line comprehensive two-dimensional liquid chromatography–atmospheric pressure chemical ionization mass spectrometry using a single column has been applied for the identification and quantification of triacylglycerols in edible oils. A novel mixed-mode phenyl-hexyl chromatographic column was employed in this off-line two-dimensional separation system. The phenyl-hexyl column combined the features of traditional C18 and silver-ion columns, which could provide hydrophobic interactions with triacylglycerols under acetonitrile conditions and can offer π–π interactions with triacylglycerols under methanol conditions. When compared with traditional off-line comprehensive two-dimensional liquid chromatography employing two different chromatographic columns (C18 and silver-ion column) and using elution solvents comprised of two phases (reversed-phase/normal-phase) for triacylglycerols separation, the novel off-line comprehensive two-dimensional liquid chromatography using a single column can be achieved by simply altering the mobile phase between acetonitrile and methanol, which exhibited a much higher selectivity for the separation of triacylglycerols with great efficiency and rapid speed. In addition, an approach based on the use of response factor with atmospheric pressure chemical ionization mass spectrometry has been developed for triacylglycerols quantification. Due to the differences between saturated and unsaturated acyl chains, the use of response factors significantly improves the quantitation of triacylglycerols. This two-dimensional liquid chromatography–mass spectrometry system was successfully applied for the profiling of triacylglycerols in soybean oils, peanut oils and lord oils. A total of 68 triacylglycerols including 40 triacylglycerols in soybean oils, 50 triacylglycerols in peanut oils and 44 triacylglycerols in lord oils have been identified and quantified. The liquid chromatography–mass spectrometry data were analyzed using principal component analysis. The results of the principal component analysis enabled a clear identification of different plant oils. By using this two-dimensional liquid chromatography–mass spectrometry system coupled with principal component analysis, adulterated soybean oils with 5% added lord oil and peanut oils with 5% added soybean oil can be clearly identified.

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1. Introduction

As a primary constituent of the human diet, edible oils serve as an important source of energy, essential fatty acids and fat-soluble vitamins, etc. The disproportion of oils may lead to several human diseases such as obesity, dyslipidemia and coronary heart disease [1]. The price of plant oil depends on the production cost and availability of plant sources; higher prices for high-quality plant oils can lead to efforts of falsification using cheaper oils with a

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lower quality and less beneficial nutritional properties. This type of adulteration of the product includes the replacement of high-cost constituents with lower-grade substitutes [2]. Triacylglycerols (TAGs) are the main components of plant oils and animal fats (95–98%). The physicochemical and nutritional properties of oils and fats are determined by the TAG molecules present. Therefore, TAGs in edible oils are considered as good fingerprints for authenticity control [3,4]. However, the profiling of TAGs in edible oils is a difficult and challenging task because oil consists of a complex mixture of TAGs with similar physicochemical properties. Therefore, the development and improvement of analytical methods that enable the thorough identification of TAGs in edible oils is very important for authenticity control [5].

The variety of TAG species is enormous due to a high number of fatty acids (FAs) present in natural samples. They differ in fatty acyl chain lengths, number, positions and *cis*-/*trans*-configuration of double bonds (DBs) and their position on the glycerol skeleton (regioisomers, enantiomers), all having great differences in their biological and nutritional properties [6]. TAGs are usually analyzed by gas chromatography (GC) and high-performance liquid chromatography (HPLC). GC is a routine method for fatty acid profiling after the transesterification of TAGs to fatty acid methyl esters (FAMEs). However, GC is strictly limited for TAG analysis because of the complexity of derivatization required before separation. High temperature (HT) GC is employed to directly analyze TAGs, however, some unsaturated TAGs are easily degraded at high temperature. In contrast to GC, HPLC is the most widely used separation technique for TAGs analysis with good reproducibility and high resolution. Non-aqueous reverse-phase HPLC (NARP-HPLC) and silver-ion HPLC (Ag-HPLC) are the two main methods used for the separation of complex TAG mixtures in natural samples. In the NARP-HPLC mode, the retention of TAGs depends on equivalent carbon numbers (ECNs), defined as the total carbon number in the acyl chains minus two times the number of DBs [7–9]. However, the NARP system has a low selectivity for the separation of TAGs with the same ECN. The silver-ion HPLC system is based on the formation of weak silver ion complexes with the π-electrons of the DBs present in the TAGs [10–13]. TAGs are separated according to the number and position of the DBs. However, Ag-HPLC suffers from a lower selectivity of TAGs differing only in the length of alkyl chains. When compared with the use of a single column, multiple chromatographic columns connected in series can enhance the peak capacity, but unfortunately suffers from very long retention times (up to 200 min) causing peak broadening, which is not practical for routine use [14–17].

When compared with one-dimensional (1D) liquid chromatography, the resolution and peak capacity of two-dimensional (2D) liquid chromatography have been improved tremendously. The off-line or on-line 2D system coupling NARP and Ag-HPLC is an effective way of realizing efficient TAG analysis [18,19]. Online and automated approaches are faster but require specific interfaces and are more difficult to operate. Furthermore, for two separation modes with different retention mechanisms, the incompatibility of the 2D solvents must be considered. Off-line approaches are easy to carry out and optimize [20]. However, off-line approaches are generally time-consuming and laborious. Firstly, it needs the replacement of two columns; secondly, between the two-dimensional separation, it needs to rinse the pipe of the separation system with isopropanol to replace the reverse-phase solvent used in the first dimension because of the immiscibility of the solvents in the first reverse-phase dimension and the second normal-phase dimension; after that it needs to use the normal-phase mobile phase to balance the Ag column in the second dimension. The entire procedure needs to switch columns repeatedly, which is not only tedious and time-consuming, but also requires a large amount of organic solvent, which may subsequently lead to additional environmental pollution in the analysis [21]. Therefore, a relatively convenient, rapid and stable off-line 2D separation system is urgently required.

In recent years, mixed-mode chromatography has received a lot of attention due to its great advantages over the single chromatographic mode, such as a great flexibility for retention and selectivity. In regard to TAG analysis, the combination of two or more different separation modes (hydrophobic and π-complexation interactions) may significantly increase the number of resolved TAGs found in natural oil samples [22,23]. A phenyl-hexyl column is comprised of a phenyl-hexyl-bonded silica stationary phase and is commercially available [24]. The stationary phase in the phenyl-hexyl column can be considered as a mixed-mode stationary phase, in which the phenyl moieties can provide π–π interactions with the π electrons of DBs and the hexyl ligands can provide hydrophobic interactions. The results of our previous study have shown that a phenyl-hexyl column eluted with acetonitrile brings selectivity with respect to the ECN value, while hindering the selectivity with respect to the number of double bonds. The use of methanol as the eluent conserves the selectivity of the π–π interactions, but exhibits a lower selectivity toward the ECN elution order [23]. Therefore, a phenyl-hexyl column combines the features of traditional C18 and silver-ion columns, which could provide hydrophobic interactions with TAGs under acetonitrile conditions and offer π–π interactions with TAGs under methanol conditions and would be suitable for the LC × LC separation of TAGs.

The coupling of HPLC and mass spectrometry (HPLC/MS) is a powerful tool in lipid analysis because it provides both structural information and usually the highest sensitivity among all the available chromatographic detectors [25]. Atmospheric pressure chemical ionization (APCI) is the most frequently used ionization technique for TAG analysis because it is easily coupled to non-aqueous mobile phase systems used in NARP-HPLC and shows a high ionization efficiency for non-polar TAG molecules. Furthermore, it can provide valuable information concerning fatty acid structure and position [26,27]. Due to the enormous number of TAGs occurring in natural edible oil samples, commercial standards are only available for a limited range of TAGs. Moreover, the cost of collecting large numbers of TAG standards is too high, hence, quantification based on calibration curves for "all" TAGs is extremely difficult, even if only the major TAGs are taken into account. For this reason, quantitation of TAGs is usually based on the relative areas of the chromatographic peaks neglecting the potential differences in the relative responses of TAGs differing in the number of double bonds and acyl chain lengths, but it may lead to significant systematic errors in the determination of TAG concentration [20]. Several authors have attempted to quantify complex natural TAG mixtures using a more sophisticated approach than normalized chromatographic peak areas or the limited number of TAG standards. The first approach is based on the measurement of calibration sets of six single-acid TAGs with APCI-MS and the use of response factors (RFs) calculated in different ways and related to a deuterated d_{12}-PPP standard [28]. In another approach, the quantitation of TAGs is based on the use of RFs determined with authentic single-acid TAG standards and related to triolein. The RFs of mixed-acid TAGs are calculated as the arithmetic mean of the RFs of individual FAs present in each TAG [29,30].

The main goal of our work is to develop a method suitable for reliable characterization and quantitation of TAGs in complex natural samples based on HPLC–MS. In this investigation, off-line comprehensive 2D liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS) using a single mixed-mode phenyl-hexyl column has been applied for the identification and quantification of triacylglycerols (TAGs) in edible oils. When compared with traditional off-line comprehensive 2D liquid chromatography, novel off-line comprehensive 2D liquid chromatography using a single column can be achieved by simply

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