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Detection of trans-fatty acids by high performance liquid chromatography coupled with in-tube solid-phase microextraction using hydrophobic polymeric monolith

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ABSTRACT

Based on in-tube solid-phase microextraction (in-tube SPME) using a hydrophobic poly (octadecyl methacrylate-co-ethylene dimethacrylate) [poly (OMA-co-EDMA)] monolith, a simple high performance liquid chromatography (HPLC) method has been developed for detection of trans-fatty acids (TFAs) as their fatty acid methyl esters (FAMEs). The poly (OMA-co-EDMA) monolithic column with high hydrophobicity was specially prepared for simultaneous microextraction, pre-separation and purification for the analytes. The pre-separation selectivity, the extraction efficiency, and the purification effect for FAMEs were investigated respectively. Furthermore, some operation parameters have been optimized in detail with respect to satisfactory extraction efficiency of the target compounds. Under the optimized conditions, the enrichment factors for model FAMEs were ranged from 58.3 to 70.9, wide linear range (0.01-1.00 mg/kg) and low detection limits (LODs) (3.0-7.1 μg/kg) were achieved, respectively. In addition, recoveries of the method were in the range from 83.0 to 106.4% with low relative standard deviations (RSDs) of 3.2-4.7% (n = 4) at spiking levels of 0.05, 0.25 and 0.5 mg/kg, respectively. Finally, the proposed method was successfully applied for detection of TFAs in practical samples, which possesses short time-consuming, low disturbance and high detection sensitivity.

1. Introduction

Trans-fatty acids (TFAs) had been widely applied in food processing to impart desirable physical characteristics for food. The health risks of trans-fatty acids have been discussed for decades. It has been pointed out that excess consumption of trans-fatty acids increases the risk of coronary heart disease, thrombosis and strokes [1–3]. World Health Organization (WHO) recommended the intake of TFAs should be limited to less than 1% of daily energy needs [4]. On June 16th, 2015, U. S. Food and Drug Administration announced that TFAs would be prohibited in the later 3 years. Thus, the development of a simple and sensitive method for the routine detection of TFAs was essential of substantial importance for food analysis.

Until now, a variety of analytical methods have been used to analyze TFAs, including infrared spectroscopy (IR) [5,6], gas chromatography (GC) [7–13], and HPLC [14–17]. Among those methods, IR is limited by its low limit of detection. GC is currently the most frequently used approach for the analysis of TFAs. However, as heat-labile compounds, long chain unsaturated fatty acids are unstable during the GC analysis [16]. In contrast to GC where the gas carrier is inert and serves to transport the analytes through the column to the detector, in HPLC the eluent can be modified by varying the proportion of water and nonaqueous components to effect changes in retention and resolution. Thus, the ability to modify retention by varying the mobile phase composition gives HPLC more flexibility than GC in this respect [18]. Due to the very similar chemical properties of trans- and cis- fatty acids, there were always overlapping of trans- and cis-isomers or formation of mixed peaks occurred when HPLC was applied as the separation method. Some reports have been proposed to improve the resolution of HPLC method for

Abbreviations: 9c-C18:1, methyl *cis*-9-octadecenoate; 9c,12c-C18:2, methyl linoleate; 9t-C18:1, methyl *trans*-9-octadecenoate; 9t,12t-C18:2, methyl linoelaidate; EDMA, ethylene dimethacrylate; FAMEs, fatty acid methyl esters; OMA, octadecyl methacrylate; SPME, solid-phase microextraction; TFAs, trans-fatty acids.

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TFAs detection by combining HPLC analysis with silver ion modified high performance liquid chromatography (Ag-HPLC) [19–21]. Unfortunately, these methods have some disadvantages including low reproducibility and short lifetime for the loss of silver ion during operation.

As we know, the detection of TFAs using HPLC-UV methodology is not sensitive for their weak UV response. Therefore, sample preparation is indispensable for the high-efficient extraction and enrichment of TFAs. In-tube solid-phase microextraction (in-tube SPME), which addresses the need to facilitate rapid and efficient sample preparation, was first developed by Pawliszyn et al. [22]. Since its invention, there have been major breakthroughs in the development of the method including the application in the separation processes and contribution to high throughput analyses [23–26]. The combination of in-tube SPME and HPLC can integrate sample extraction, pre-separation, purification, and detection into one step. Due to its simple, fast, and online features, this method has received great attention. As the core for SPME, the stationary phase plays an important role in the selective pre-separation, efficient extraction and purification. Considering that TFAs or their esters are a kind of highly hydrophobic compounds, a hydrophobic microextraction column should be suitable as the stationary phase of SPME. Various hydrophobic octadecyl (C18) adsorbents including the commercial C18 cartridges/adsorbents [27–31], hybrid absorbents of octadecylsilane (ODS), primary secondary amine (PSA) and graphitized carbon black (GCB) [32,33], mixed mode adsorbents of C18, strong cation (SCX) and anion exchange (SAX) sorbents [34] and magnetic core mesoporous shell microspheres with C18-functionalized interior pore-walls (C18-Fe_3O_4@mSiO_2) [35], have been utilized for solid phase extraction of target compounds from food, biologic and environmental samples. Compared with these particle-packed columns, monolithic columns have got increasing attention for their advantages of easy preparation, fast mass transfer and diverse surface functionalization [36,37]. So far, few works have been reported for in-tube SPME of TFAs based on hydrophobic monolithic column. In this study, octadecyl methacrylate (OMA) [38,39] as a frequently-used strong hydrophobic functional monomer, which can provide strong hydrophobic interaction with the target compounds, was selected to prepare the special monolithic column for in-tube SPME of TFAs.

In this work, based on in-tube SPME using hydrophobic poly (octadecyl methancrylate-co-ethylene dimethacrylate) [poly (OMA-co-EDMA)] monolith, a simple and sensitive HPLC method has been developed for detection of TFAs. TFAs were primarily transformed to fatty acid methyl esters (FAMEs) by methyl esterification [9,10,40,41]. Considering the fact that TFAs in food are mainly monoenoic TFAs and dienoic TFAs, 9t-C18:1 and 9t,12t-C18:2 were selected as model analytes for this study [42]. The morphology and permeability of the monolithic column have been investigated. The pre-separation selectivity, the extraction efficiency, and the purification effect for FAMEs were investigated respectively. The factors affecting the extraction and detection by the online in-tube SPME-HPLC, such as ACN content in the sampling solution, extraction flow rate, desorption flow rate and desorption time, have been optimized in detail. Finally, monoenoic TFAs (represented by 9t-C18:1) and dienoic TFAs (represented by 9t,12t-C18:2) in practical samples were successfully analyzed under the optimized condition by the proposed method.

2. Experimental

2.1. Reagents and materials

Methyl linoleate (9c,12c-C18:2), methyl linoelaidate (9t,12t-C18:2), methyl *cis*-9-octadecenoate (9c-C18:1), methyl *trans*-9-octadecenoate (9t-C18:1), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Octadecyl methacrylate (OMA), ethylene dimethacrylate (EDMA), 2,2-azobisisobutyronitrile (AIBN), 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS) were bought from Acros (New Jersey, USA). Cyclohexanol, 1,4-butanediol, HCl (AR, 37%), KOH, and petroleum ether were supplied by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Deionized water was obtained by Millipore Milli-Q purification system (Milford, MA, USA). Acetonitrile (ACN), methanol, ethanol, diethylether and *n*-hexane (Chemical Reagent Corporation, Shanghai, China) were of HPLC grade. The fused-silica capillary with dimension of 250 μm i.d. was obtained from the Refine Chromatography Ltd. (Yongnian, Hebei, China).

2.2. Preparation of poly (OMA-co-EDMA) monolithic column

In order to improve the stability of the monolithic columns, the inner wall of the capillary was treated with a bifunctional reagent, γ-MAPS, according to the procedure reported previously [43].

The pre-polymerization mixture was consisted of a monomer OMA (360 mg, 18%, w/w), a crosslinker EDMA (240 mg, 12%, w/w), porogenic solvents cyclohexane (1120 mg, 56%, w/w), 1,4-butanediol (280 mg, 14%, w/w), and initiator AIBN (1.8 mg, 0.3%, w/w with respect to monomer and cross linker) [39]. After purging with a N_2 stream for 30 min to remove the oxygen, the mixture was allowed to fill the capillary. The capillary was sealed at both ends with rubbers immediately and the reaction was initiated in a water bath at 60 °C for 24 h. The prepared monolithic capillary was washed with methanol for 30 min to remove the unreacted components and porogenic solvents.

2.3. Instrument and analytical conditions

As shown in Fig. S1 in Electronic Supplementary information (ESI), the in-tube SPME-HPLC system consisted of the pre-extraction segment, which included a Rheodyne 7725i six-port valve (valve 1), a LC-10AD pump (pump A) (Shimadzu, Kyoto, Japan) and a PEEK tube (0.03 in. i.d., 0.5 mL total volume), and the analytical segment, which included a LC-10AD pump (pump B) (Shimadzu, Kyoto, Japan), a VICI six-port valve (valve 2) with 15 cm poly (OMA-co-EDMA) monolithic column and a Shimadzu SPD-M20A photodiode array detector (Shimadzu, Kyoto, Japan). The online in-tube SPME-HPLC manipulation was referred to Fan et al. [44] with some modifications.

Before extraction, valves 1 & 2 were initially set at LOAD positions. The sampling solution (1% ACN solution) was driven by pump A to flow through the monolithic column for conditioning at 0.04 mL/min. The mobile phase was driven by pump B directly through the analytical column to obtain a stable baseline for chromatographic separation. Meanwhile, the PEEK loop was filled with the sample solution using a syringe.

When extraction began, valve 1 was directed towards INJECT position for a given time (13 min) and returned to LOAD position immediately to perform extraction. The sampling solution was kept to flow through the monolithic column for 90 s in order to eliminate the residual sample solution and reduce the interference.

Then, the extracted analytes were desorbed from the monolithic column by the mobile phase at a flow rate of 0.07 mL/min by simply switching the valve 2 to the INJECT position. When extraction had finished, valve 2 was switched to the LOAD position, and followed by adjusting the flow rate of the mobile phase to 1.0 mL/min for separation.

A Syncronis 5u C18 chromatographic column (250 × 4.6 mm) from Thermo (Boston, USA) was used for the separation. Experimental conditions for the online in-tube SPME-HPLC method were optimized as followed: the mobile phase for HPLC separation was

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