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Saturated fatty acid determination method using paired ion electrospray ionization mass spectrometry coupled with capillary electrophoresis

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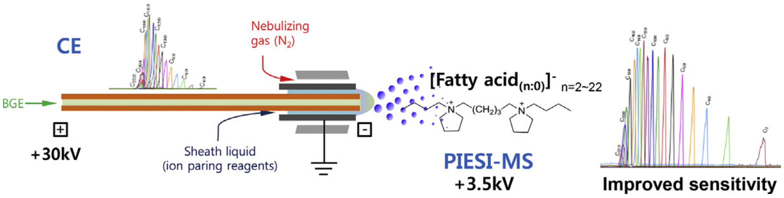
HIGHLIGHTS

- A new CE- PIESI MS method was developed to determine a wide range of fatty acids.
- It provides excellent sensitivity with good peak resolution for short-chain fatty acids.
- The method was validated and provided good linearity and repeatability.
- The amounts of fatty acids in cheese and coffee extracts were determined.

GRAPHICAL ABSTRACT



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ABSTRACT

A sensitive and selective capillary electrophoresis-mass spectrometry (CE-MS) method for determination of saturated fatty acids (FAs) was developed by using dicationic ion-pairing reagents forming singly charged complexes with anionic FAs. For negative ESI detection, 21 anionic FAs at pH 10 were separated using ammonium formate buffer containing 40% acetonitrile modifier in normal polarity mode in CE by optimizing various parameters. This method showed good separation efficiency, but the sensitivity of the method to short-chain fatty acids was quite low, causing acetic and propionic acids to be undetectable even at 100 mgL^{-1} in negative ESI-MS detection. Out of the four dicationic ion-pairing reagents tested, *N,N'*-dibutyl 1,1'-pentylenedipyrrolidium infused through a sheath-liquid ion source during CE separation was the best reagent regarding improved sensitivity and favorably complexed with anionic FAs for detection in positive ion ESI-MS. The monovalent complex showed improved ionization efficiency, providing the limits of detection (LODs) for 15 FAs ranging from 0.13 to 2.88 μg/mL and good linearity ($R^2 > 0.99$) up to 150 μg/mL. Compared to the negative detection results, the effect was remarkable for the detection of short- and medium-chain fatty acids. The optimized CE-paired ion electrospray (PIESI)-MS method was utilized for the determination of FAs in cheese and coffee with simple pretreatment. This method may be extended for sensitive analysis of unsaturated fatty acids.

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1. Introduction

Fatty acids (FAs) consisting of hydrocarbon chains and a carboxyl group perform important functions in the body, such as storing energy and serving as building blocks for lipids. Based on carbon chain length, FAs can be classified as short-chain FAs (SCFAs, 2–4 carbon atoms), medium-chain FAs (MCFAs, 6–10 carbon atoms), and long-chain FAs (LCFAs, 12–20 carbon atoms). Of these FAs, dietary saturated FAs come mainly from meat and dairy products as well as some plant-based oil, which are known to raise the level of cholesterol in blood [1]. Accordingly, their concentrations in food should be considered when assessing the edible quality of food related to health, which would contribute to increasing the commodity values of the food. Dietary saturated FAs have been used to assess acidity in coffee [2], the odor and taste of beer [3] and sake [4], and the authenticity in edible oil [5].

Typical analytical methods used for FA analysis employ derivatization steps to enhance the volatility and change the polarity to be amenable for separation in GC [6–8] or to improve the weak UV absorption of FAs in LC [9,10]. There is no doubt that these techniques provide competent sensitivity and selectivity for determining FAs. Nevertheless, these techniques include time-consuming processes used to achieve stable and reproducible performance, and sometimes, unexpected side reactions are generated during the process. In addition, FAs volatilities are considerably different depending on chain length of FAs so that pretreatment methods for extraction need to be differently considered to determine a wide range of FAs simultaneously because SCFAs are lost during derivatization for GC analysis [11]. An alternative CE method can provide a fast analysis time without the need for a derivatization step [12–16]. The most frequently used approaches for saturated FAs were indirect UV detection and micellar electrokinetic chromatography (MEKC), which are based on the use of chromophores and surfactants, respectively [13,17–19]. These separation techniques were adaptable for MCFAs and LCFAs, but their disadvantages are less sensitivity and selectivity as well as a limitation on the use of mass spectrometry due to contamination of the ion source and less ionization efficiency induced by non-volatile additives. Therefore, a limited number of investigations using volatile buffers have been reported for the application of FAs analysis by CE-MS [20,21].

For the determination of anionic compounds, such as FAs, by using MS detection, a negative ESI mode is the preferred method of detection. However, compared to positive ESI mode, this method leads to an unstable signal and higher background noise in addition to an increased possibility of electric discharge. Recently, paired ion electrospray ionization (PIESI) mass spectrometry was introduced for the sensitive detection of anionic compounds in positive ion mode [22–25]. This method allows anionic molecules to form anion/IPR associated complexes through the addition of multiple charged reagents by continuous infusion after separation. The positively charged complexes are detected using positive ESI mode with high sensitivity and stability. This result is especially true for low mass anionic analytes, such as small organic [26,27] and inorganic ions [28–30], because they yield higher m/z complexes. PIESI coupled with CE separation has the following advantages: high efficiencies and sensitivities for anionic compounds with less solvent consumption and requiring less sample volume than LC, and an easy infusion method using a sheath-liquid spray interface that utilizes three approaches for complex formation by either pre-, on-, or post-column introduction methods for ion-pairing reagents (IPR). A few papers based on CE-MS applications using IPR have been reported [31,32] in which di- and tri-cationic IPR were used as complexing agents to provide effective quantitative results for singly charged environmental contaminants in water and divalent anionic analytes for on-column complexation, respectively. These CE-PIESI-MS methods using IPR are applicable to determine charged anions in the fields of food, biological, and environmental studies.

In this study, we utilized the PIESI technique to develop an analytical method for the determination of a wide range of FAs by CE-MS with rapid and reproducible detection. With optimized CE separation of FAs in negative ESI detection, four types of dicationic IPRs were introduced into the sheath-liquid interface ESI source to form FAs/IPR complexes, which were detected in positive ESI mode to generate enhanced signal responses. Finally, the developed analytical method was validated and applied to cheese and coffee samples to quantify the concentrations of FAs.

2. Experimental

2.1. Reagents and solutions

The 21 linear FAs ($C_2 \sim C_{22:0}$) and four IPRs shown in Fig. 1 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium formate and ammonium hydroxide solutions (≥25% in H_2O) for preparing buffers were also obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN), 2-propanol (IPA), and water were obtained from Fisher Scientific (Pittsburg, PA, USA). A 1 M solution of sodium hydroxide and pure water were obtained from Agilent Technologies (Palo Alto, CA, USA) and used for conditioning fused-silica capillaries. Each of the 21 FA standard solutions of 10 mg/mL were prepared in IPA, and the stock mixture for method optimization was prepared at a concentration of 0.1 mg/mL. The pH buffer solution was adjusted by adding ammonium hydroxide into ammonium formate solution and was prepared at the appropriate concentrations according to the method studied.

2.2. Capillary electrophoresis/PIESI mass spectrometry

All CE separations were performed using an Agilent CE 7100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a bare fused-silica capillary (50 μm i.d. × 358 μm o.d., 85 cm in length) purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary was conditioned before its first use by rinsing with 1 M sodium hydroxide solution for 5 min and then rinsing with water for 5 min. Between each analysis, the capillary was flushed with buffer solution for 5 min. Samples were hydrodynamically injected at 50 mbar for 20 s. For the electrophoretic separation of FAs, 30 kV in normal polarity mode was applied across the capillary maintained at 30 °C. The optimized background electrolyte (BGE) was 30 mM ammonium formate in 40% ACN, which was adjusted to pH 10 with adding of 25% ammonium hydroxide. The CE system was coupled with an Agilent 6550 Q-TOF MS (Agilent Technologies, Palo Alto, CA, USA) with a coaxial sheath-liquid ESI source. IPR solutions were prepared at 250 μM in a methanol:water (1:1, v/v) solution, which was continually delivered to the ESI source as a sheath liquid at a flow rate of 10 μL/min by an Agilent 1260 Iso Pump(Agilent Technologies, Palo Alto, CA, USA) during the separation to maintain a stable CE current and improve the mass sensitivity. The source conditions were set to a Vcap of 3.5 kV in positive ESI mode, with a nozzle voltage of 2 kV, sheath gas temperature of 230 °C, and sheath gas flow of 11 mL/min. Mass spectra were recorded for the range of 100–700 m/z using a scan rate of 1 spectra/scan.

2.3. Calibration and method validation

The standard stock solution of FAs was diluted from 0.5 μg/mL to 150 μg/mL for calibration. Calibration curves were plotted with a minimum of 7 points using the peak area obtained from IPR/FAs