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Capillary electrophoresis with capacitively coupled contactless conductivity detection for the determination of cis/trans isomers of octadec-9-enoic acid and other long chain fatty acids

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ABSTRACT

A capillary electrophoresis (CE)–capacitively coupled contactless conductivity detection (C^4D) method for the simultaneous separation of eleven underivatized fatty acids (FAs), namely, lauric, myristic, tridecanoic (internal standard), pentadecanoic, palmitic, stearic, oleic, elaidic, linoleic, linolenic and arachidic acids is described. The separation was carried out in normal polarity mode at 20 °C, 30 kV and using hydrodynamic injection (50 mbar for 1 s). The separation was achieved in a bare fused-silica capillary (70 cm × 75 μm i.d.) using a background electrolyte of methyl-β-cyclodextrin (~6 mM) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (~8 mM) dissolved in a mixture of Na_2HPO_4/KH_2PO_4 (5 mM, pH 7.4):ACN:MeOH:*n*-octanol (3:4:2.5:0.5, v/v/v/v). C^4D parameters were set at fixed amplitude of 100 V and frequency of 1000 kHz. The developed method was validated. Calibration curves of the ten FAs were well correlated ($r^2 > 0.99$) within the range of 5–250 μg mL^{-1} for lauric acid, and 3–250 μg mL^{-1} for the other FAs. The method was simple and sensitive with detection limits (S/N = 3) of 0.9–1.9 μg mL^{-1} and good relative standard deviations of intra- and inter-day for migration times and peak areas (≤9.7%) were achieved. The method was applied to the determination of FAs in margarine samples. The proposed method offers distinct advantages over the GC and HPLC methods, especially in terms of simplicity (without derivatization) and sensitivity.

1. Introduction

Fatty acids (FAs) are carboxylic acids with long hydrocarbon chains. They are widely found in nature (e.g. food products, vegetable oils, and living organisms) and are the basic components of most naturally occurring lipids in both animals and plants. FAs can be categorized according to their degree of saturation, i.e., saturated fatty acids (SFAs) and those with different degrees of unsaturation, from monounsaturated fatty acids (MUFAs) to polyunsaturated fatty acids (PUFAs). The physical and chemical characteristics of FAs are highly dependent on the carbon chain length, number and position of double bonds, and cis–trans isomer conformations.

Consumption of fats and their effects on human health have long been an active area of research, thus the profiling of FAs is of great importance from commercial point of view and health impacts (e.g. toward the prevention of diseases) [1–5]. Lately, a considerable increase in the consumption of trans fats from industrial processes has been observed around the world. Trans fatty acid (TFA) is an important quality parameter in the oleofood industry and is routinely measured during the production and storage stages. Generally, TFAs are classified into two groups: ruminant and industrial TFAs. Ruminant TFAs (e.g. vaccenic and conjugated linolenic acids) are hypothesized to have beneficial physiological effects [6–8]. Some studies also revealed that some monounsaturated trans fatty acid, specifically vaccenic acid, could be involved in the production of conjugate linoleic acid (CLA), which are thought to have anti-carcinogenic properties (regulate tumor growth) [9,10]. Elaidic acid is the most common industrial TFA, being responsible for 80–100% of the total TFA found in processed foods [11,12]. The current upsurge in interest of TFA is fueled by the discovery that TFA is associated with increased risk of coronary heart diseases and type two diabetes [13,14]. Furthermore, numerous clinical studies have suggested that the consumption of TFA is associated with lower maternal and neonatal PUFA status, increased fetal loss and low birth weight infants [15–18]. Due to the adverse effects of TFA on humans, several countries (US, Canada, Denmark, etc.) have made it mandatory to include TFA in the labeling of food. The World Health Organization (WHO) has suggested that consumption of trans fat should not exceed 1% of the caloric value of the daily diet [19].

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Therefore, it is imperative to develop a suitable analytical method to determine TFA in view of their widespread presence in food. Conventionally, analysis of TFA has been performed using spectroscopic or chromatographic methods. Fourier transformed infrared (FTIR) spectrophotometric method based on the CH out-of-plane deformation band observed at 966 cm^{-1} has been reported [20]. However, this method is not specific as it does not provide details on the content of saturated and PUFAs in the sample. Moreover, the method is considered unreliable when quantifying trans fat concentrations of less than 5% (as % of total fat) [20,21]. The determination of TFAs has been dominated by chromatographic techniques, especially gas chromatography (GC). The analytically challenging task of separating the isomers has been capitalized by the distinct ability of transition metals such as silver to complex with unsaturated organic compounds [22–26]. Thus silver ion-impregnated stationary phases for thin layer chromatography (Ag^+-TLC) [22,23] and high performance liquid chromatography (Ag^+-HPLC) with ultraviolet (UV) detection [24–26] or mass spectrometer (MS) [27] were predominant. However, low reproducibility and contamination by trace silver salts into the fractions are some of the major difficulties of using the Ag^+-impregnated stationary phases [28]. Thus reversed-phased high performance liquid chromatography (RP-HPLC)–UV [23,27–30] or fluorescence [31] or MS [27] were also reported. Since FAs lack chromophores, derivatization is often needed to increase the sensitivity of HPLC analysis. Common derivatization agents such as 9-(2-hydroxyethyl)-carbazole [32], and 2-(2-naphthoxy)ethyl-2-(piperidino)ethane sulfonate [33] have been used. Additionally, LC methods also required long analysis time and consume large amounts of solvents.

For GC separations, the use of highly polar (e.g. based on ionic liquids [34,35]) and long columns (100 and 120 m) becomes mandatory, and these were used in conjunction with flame ionization (FID) [23,26–29,36] or MS [36,37] detectors. For a given double bond position on the carbon chain, the trans isomers were eluted before their corresponding cis homologues. However, overlaps and co-elution of the isomers still occur. To overcome this problem, fractionation techniques prior to GC and alternative chromatography techniques (e.g. Ag^+-TLC × GC [23,26], Ag^+-HPLC × GC [26], RP-HPLC × GC [23], dual column-LC [30] and GC × GC [25,36]) become necessary. Generally, GC methods involve the separation of either methyl or trimethylsilyl esters derivatives. The derivatization procedure (especially for the longer chain FAs) is necessary to increase the volatility and to overcome adsorption of the polar functional groups to the GC column [38]. However, derivatization reactions often produce incomplete conversion of the analyte and undesirable interfering side products. In addition, GC technique also increased the risk of isomerization of double bonds as higher temperature was required during the analysis.

CE with indirect UV detection has been developed for the analysis of TFAs [39–44]. No derivatization is needed, high separation efficiency, short analysis time, low sample consumption and simplicity in operation are some notable advantages of the method. However, the sensitivity of the method is low due to the high background noise and the short sample path length. In addition, the fluctuating baseline and incomplete separation of the major FAs also make the method unsuitable for routine analysis.

The popularity of capacitively coupled contactless conductivity (C^4D) detection has been growing steadily over the last few years. C^4D is a conductometric measuring approach that is based on the conductivity differences between the sample zones and the background electrolyte (BGE) [45]. C^4D overcomes the disadvantage of short path length that is encountered by on-column optical detections. Furthermore, it avoids the direct contact of working electrodes with BGE in the conventional conductivity detection [45] and eliminates the laborious work of fabricating detection cells in potential gradient detection [46]. Other main advantages include the capability to detect small organic molecules and non-chromophoric compounds, using simple electronic circuitry, required low maintenance and also relatively low cost [45]. C^4D has been applied in determining species including small inorganic and organic ions, biochemical species, etc. [45]. The use of C^4D for the determination of long chain FAs in oil samples in combination with CE [41,47], HPLC [48] or flow injection analysis (FIA) [49] has been previously reported. The reported CE–C^4D method enables good separation and determination of nine SFAs [47]. However, overlapping between palmitic and oleic acids (common fatty acids) when real samples were analyzed was observed. The addition of Brij 35 to the running buffer had overcome this problem [41]. Recently, HPLC coupled with C^4D had been also used to separate palmitic and oleic acids [48]. However, both of the methods can be applied for the analysis of a limited number of FAs and the separation of cis/trans forms of octadec-9-enoic acid was not targeted. Therefore, a simple method for the simultaneous determination of the most common SFAs, MUFAs, PUFAs and TFAs has yet to be done.

In this paper, we describe a novel CE–C^4D method for the rapid analytical separation and simultaneous determination of eleven FAs (SFAs (lauric, tridecanoic, myristic, pentadecanoic, palmitic and arachidic acids), MUFAs (oleic acid), PUFAs (linoleic and linoleic acids) and TFA (elaidic acid)). The use of different solvent polarities and dual cyclodextrins makes this method capable to differentiate the cis and trans isomers of octadec-9-enoic acid. The method was validated and the analytical practicality of the method was demonstrated in the determination of FAs in a few types of margarine samples. The chemical structures of the FAs studied are shown in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

Lauric ($C_{12:0}$; ≥99.5%), tridecanoic ($C_{13:0}$; ≥99%), myristic ($C_{14:0}$; 99%), pentadecanoic ($C_{15:0}$; ≥99.5%), palmitic ($C_{16:0}$; ≥99%), stearic ($C_{18:0}$; ≥99%), oleic ($C_{18:1c}$; 99%), elaidic ($C_{18:1t}$; ≥99%), linoleic ($C_{18:2}$; 99%), linolenic ($C_{18:3}$; ≥99%), and arachidic ($C_{20:0}$; ≥99%) acids, monobasic sodium phosphate (NaH_2PO_4), dibasic sodium phosphate (Na_2HPO_4), monobasic potassium phosphate (KH_2PO_4), methyl-β-cyclodextrin (MβCD), heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCD), 1-propanol (HPLC grade), 1-pentanol (HPLC grade) and *n*-octanol (HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide, boron trifluoride (20% solution in MeOH), HPLC grade hexane, methanol (MeOH), 1-hexanol, 1-heptanol and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Ethanol (HPLC grade) was purchased from Fisher Scientific (Hampton, New Hampshire, USA). HPLC grade 2-propanol and 1-butanol were purchased from Q-Rec (Auckland, New Zealand). Phosphoric acid (85%) was supplied by Univar (Ingleburn, Australia). Ultra pure water (18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, MA, USA).

2.2. Instrumentations

2.2.1. CE–C^4D

Separations were conducted on a $HP^{3D}CE$ capillary zone electrophoresis system, model 7100 (Agilent Technologies, Waldbronn, Germany) equipped with C^4D (eDAQ, Denistone East, Australia). The separations were performed using a bare fused-silica capillary 80.5 cm × 75 μm i.d. (detection length, 10.5 cm from the outlet end of the capillary) supplied by Agilent Technologies (Waldbronn,