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An alternative method for rapid quantitative analysis of majority cis–trans fatty acids by CZE

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ABSTRACT

An alternative methodology for simultaneous analysis of majority cis–trans fatty acids such as stearic (C18:0), elaidic (C18:1t), oleic (C18:1c), palmitic (C16:0), linoleic (C18:2cc) and linolenic (C18:3ccc) by capillary zone electrophoresis (CZE) under indirect detection was proposed in this work. The CZE methodology was optimized through the 2^3 central composite design (2^3 CCD) with three replicates in central point, having as factors Brij 35, acetonitrile and 1-octanol. The background electrolyte (BGE) for the optimum separation condition consisted of: 15.0 mmol L^{-1} of NaH_2PO_4/Na_2HPO_4 buffer at pH ≈ 6.86, 4.0 mmol L^{-1} of SDBS, 8.3 mmol L^{-1} of Brij 35 and 45% v/v of ACN, and 2.1% of 1-octanol was achieved by analyzing of the 2^3 CCD together with the principal component analysis (PCA). The FA quantification was performed through response factor (R_f) approach, which provided high analytical throughput for the real samples analysis. The CZE method optimized was successfully applied to the analysis of FA in samples of olive oil, soy oil, hydrogenated vegetable fat, butter, margarine and filled cookie. The results obtained were compared with AOCS GC official method (Ce 1j-07) through paired sample t test and no significant difference was found within 95% confidence interval.

1. Introduction

Fatty acids (FA) are aliphatic monocarboxylic acids which act as the building lipids blocks. They can be classified as either saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) or polyunsaturated fatty acid (PUFA) depending on the presence or absence of double bonds. On the other hand, the physical and chemical characteristics of FA such as freezing point, solubility and nutritional properties depend on the numbers of carbon atom, position and conformation (*cis–trans* isomers) of the double bond. Most FA which are usually present in the nature have even numbers of carbons atoms and long chain FA containing 12 to 22 carbons atoms without ramifications (Gurr, Harwood, & Frayn, 2002).

The determination of FA is an important quality parameter for several samples present in everyday life such as edible oils and dairy products. Nowadays determining the level of cis–trans FA in food is a requirement of the governmental health agencies, since it is necessary to give people information about lipid composition in the food they are eating, especially for those who have health problems like heart disease, obesity and hypertension. Thus, the development of new or alternative, rapid, efficient, precise and reliable methods for FA analysis is very important (Ascherio et al., 1994).

The classical separation technique for FA analysis is the gas chromatography with flame ionization detector (GC-FID). This FA methodology analysis involves firstly lipid fraction extraction, saponification reaction and then derivatization of the total FA content into fatty acid methyl esters (FAME) before injection in the GC-FID equipment (Bailey-Hall, Nelson, & Ryan, 2008; Ichihara et al., 2002; Lepage & Roy, 1984). However, others FA analysis methods can be applied such as high performance liquid chromatography under UV detection (HPLC-UV) which uses derivatization reactions with phenacyl and naphthacyl esters (Jordi, 1978), thin layer chromatography impregnated with silver (HPLC-Ag^+), fourier-transform infrared (FTIR) (Al-Alawi & Voort, 2004; Voort & Ismail, 1995) and infrared total attenuated reflectance (ATR-IR) have been reported in the literature (Mossoba, Yurawecz, & McDonald, 1996).

Since the 1990s, long chain FA analysis by capillary electrophoresis (CE) has aroused interest in the scientific community due to its versatility, short analysis time and absence of derivatization reaction in sample preparation procedure. Several different matrixes such as oils, fats, foods and biological samples can be analyzed by this method. Among the CE methodologies used to FA analysis different modes and detection possibilities can be highlighted such as non-aqueous capillary electrophoresis (NACE) with near-infrared fluorophore detection (Jr & Johnson, 2000), non-aqueous capillary zone electrophoresis (CZE) combined with indirect fluoresce detection (Wang, Wei, & Liz, 1998), hydroorganic CZE-UV under direct or indirect detection (Barra et al., 2012; Castro et al., 2010; Drange &

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Table 1
2^3 CCD matrix containing levels, factors and responses for resolution between critical pairs C18:1t/C18:1c and C16:0/C18:2cc.

Trial	Brij 35	1-octanol	ACN	$R_{C18:1t/C18:1c}$	$R_{C16:0/C18:2cc}$
1	−1	−1	−1	1.26	1.05
2	1	−1	−1	0.95	0.97
3	−1	1	−1	1.10	1.05
4	1	1	−1	1.01	1.06
5	−1	−1	1	1.18	1.00
6	1	−1	1	0.96	0.94
7	−1	1	1	1.25	1.00
8	1	1	1	1.05	1.07
9	−1.68	0	0	1.44	1.16
10	1.68	0	0	1.02	1.06
11	0	−1.68	0	1.00	0.99
12	0	1.68	0	0.97	0.97
13	0	0	−1.68	1.01	1.17
14	0	0	1.68	1.09	0.92
15	0	0	0	1.10	1.10
16	0	0	0	0.94	1.04
17	0	0	0	1.20	1.05

Brij 35 (mmol L^{-1}): (−1) 9.0, (0) 10.0, (1) 11.0, (−1.68) 8.3, (1.68) 11.7.
1-octanol (% v/v): (−1) 1.8, (0) 2.0, (1) 2.2, (−1.68) 1.7, (1.68) 2.3.
ACN (% v/v): (−1) 44.0, (0) 45.0, (1) 46.0, (−1.68) 43.3, (1.68) 46.7.

Lundanes, 1997; Oliveira, Micke, Bruns, & Tavares, 2001; Oliveira, Solis, Gioelli, Polakiewicz, & Tavares, 2003; Porto, Souza, & Oliveira, 2011), micellar electrokinetic chromatography under UV detection (MECK-UV) (Collet & Gareil, 1997; Gareil & Collet, 1996) and capillary electrophoresis with contactless conductivity detection (CE-C^4D) (Oliveira, Lago, & Tavares, 2003; Surowiec, Kamla, & Kenndler, 2004).

Due to the chemical FA features, which are of low molar absorptivity, low solubility in aqueous medium and presence of FA homologues and isomers, the most common background electrolyte (BGE) systems used for FA analysis by CE take into account the optimization of variables, such as: buffers like phosphate or Tris/HCl; buffers chromophore like Tris/p-hydroxybenzoate or p-anisato (Gareil & Collet, 1996); chromophore agents like sodium dodecylbenzenesulfonate (SDBS) (Oliveira, Solis, et al., 2003); organic solvents like methanol (Liu, Cao, & Chen, 2005), acetonitrile (ACN) (Surowiec et al., 2004) and/or 1-octanol (Oliveira, Solis, et al., 2003); surfactant agents like sodium dodecyl sulfate (SDS) (Bohlin, Ohman, Hamberg, & Blomberg, 2003), polyoxyethylene 23 lauryl ether (Brij 35) (Oliveira, Solis, et al., 2003), and chiral selectors like cyclodextrins (Gareil & Collet, 1996; Liu et al., 2005). Nevertheless, works in the literature report univariate methods for optimization step, where each variable of the system is investigated separately while others are held constant. However, this procedure does not allow the study of interaction effects among factors considering different levels. Then, the multivariate approach, in contrast to the univariate one, presents more comprehensive understanding of the investigated system through simultaneous evaluation of variables combined with a reduced number of trials, which results in less spending of reagents, solvents and laboratory time (Neto, Scarminio, & Bruns, 2007).

In spite of the excellent methodologies developed for FA analysis by CE reported in the literature of the last twenty years, according to our knowledge, none addressed more effort for quantitative considerations in real sample analysis, or performed comparison with the official methodology. Within this context, a rapid and efficient methodology for quantitative analysis of majority cis–trans long chain FA under indirect UV detection by CZE has been optimized, and no derivatization and extraction procedures for all sample preparation were highlighted.

2. Materials and methods

2.1. Chemicals and materials

All reagents were of analytical grade and the water was purified by deionization (Milli-Q system; Millipore, Bedford, MA, USA). The solvents

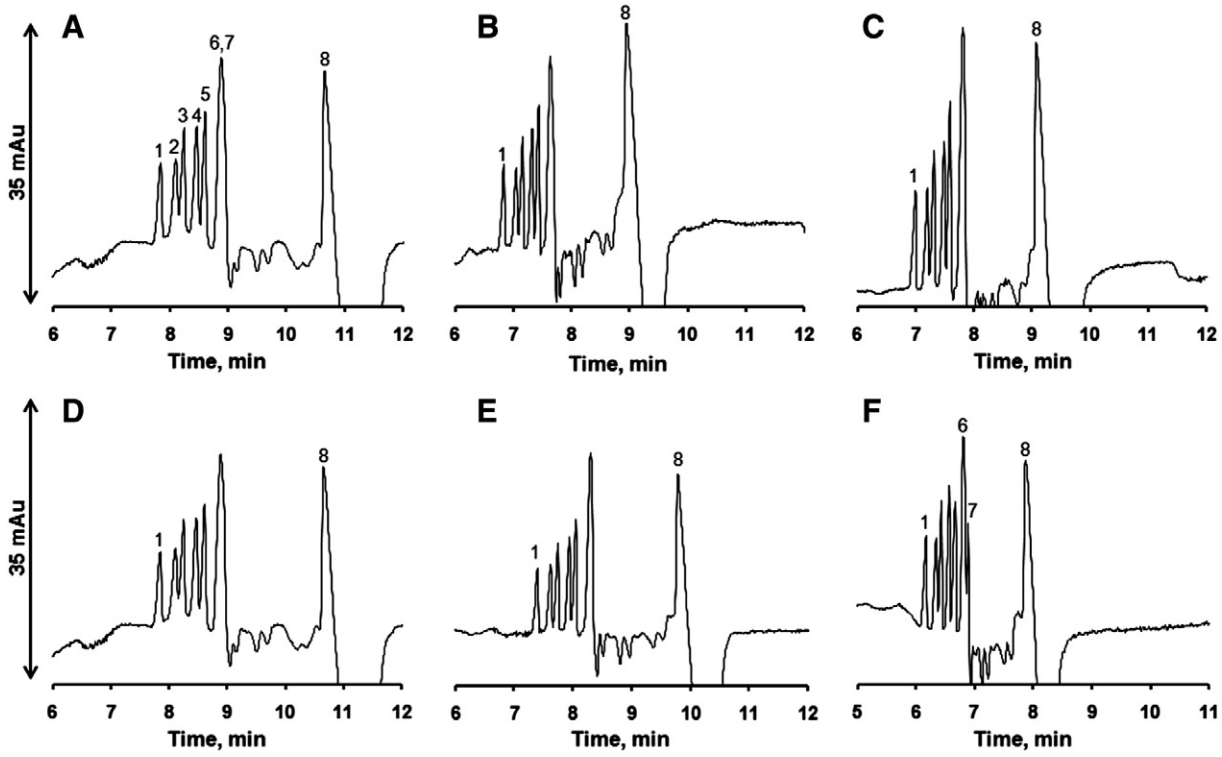

Fig. 1. Standard FA electropherograms obtained from 2^3 CCD (1) C18:0, (2) C18:1 9t, (3) C18:1 9c, (4) C16:0, (5) C18:2cc, (6) C18:3ccc, (7) C15:0 and (8) C13:0, all with a concentration of 0.50 mmol L^{-1} using conditions describe by 2^3 CCD for ACN, Brij 35 and 1-octanol. A — trials 12, 2, 6, 11, 16, and 5 with similar electrophoretic profiles, B — trials 4, 10, and 8 with electrophoretic similar profiles, C — trials 17, 1, and 7 with similar electrophoretic profiles, D — trials 3, 15, and 14 with similar electrophoretic profiles, E — trial 9, F — trial 13. All electrolytes were added with 4.0 mmol L^{-1} of SDBS and 15.0 mmol L^{-1} of buffer NaH_2PO_4/Na_2HPO_4. Operational conditions: injection 5 s × 12.5 mbar, voltage +19 kV, indirect detection at 224 nm and 25 °C temperature inside the cartridge, TSH capillary with 48.5 cm long (40 cm effective length) 75 μm I.D and 375 mm O.D.