Grupo de Química Analítica e Quimiometria

UNIVERSIDADE FEDERAL DE JUIZ DE FORA

LVMINA SPARGERE

ANVISA

Agência Nacional de Vigilância Sanitária

Monitoramento quantitativo de gorduras trans em amostras de óleos vegetais e alimentos industrializados

Prof. Dr. MARCONE AUGUSTO LEAL DE OLIVEIRA

Contexto e objetivo do TED nº 1/2022

Instrumento: Termo de Execução Descentralizada (TED) nº 1/2022, firmado entre a Anvisa e a Universidade Federal de Juiz de Fora - UFJF.

Finalidade do projeto: pesquisa para monitorar quantitativamente ácidos graxos trans totais (AGT), expressos em ácido elaídico, em óleos vegetais refinados e alimentos processados, para subsidiar o monitoramento da implementação da RDC nº 632/2022.

Valores repassados: R$ 283.027,59

Escopo amostral e produtos analisados

Local de coleta: redes de supermercados de Juiz de Fora/MG; lotes distintos por unidade. Exclusão de produtos com ingredientes de origem animal para evitar interferência de Ácidos Graxos Trans de Ruminantes - AGTR.

Período da coleta: entre dezembro de 2023 e maio de 2024.

Produtos coletados:

Óleos vegetais refinados (113 amostras)

10 algodão | 31 canola | 24 girassol | 21 milho | 27 soja.

Alimentos processados (200 amostras de 10 categorias de alimentos)

23 macarrão instantâneo | 23 caldos prontos | 16 pipocas micro-ondas | 18 preparados p/ sopas | 16 biscoitos água e sal | 30 salgadinhos | 15 biscoito de polvilho | 24 batatas palha | 15 margarinas | 20 batatas congeladas.

Método analítico (CZE-UV) e parâmetros

Técnica: Eletroforese capilar de zona com detecção UV (CZE-UV)

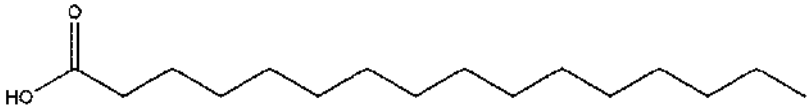
Preparo das amostras e quantificação: triplicata autêntica, hidrólise básica/saponificação; diluição 1:10; adição de padrão (comparação da área da amostra versus amostra enriquecida com ácido elaídico); média das 3 réplicas por unidade.

LOD/LOQ (razão sinal/ruído, convertidos para % m/m):

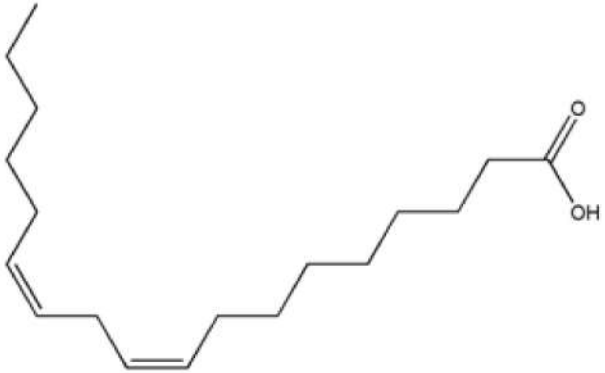
- **Óleos vegetais:** LOD 0,0044 % m/m; LOQ 0,015% m/m (por 100 g de gorduras).
- **Alimentos processados:** LOD 0,045 % m/m; LOQ 0,150 % m/m (por 100 g de produto).

- Limite de AGTI em óleos refinados estabelecido na RDC nº 632/2022: 2 % m/m

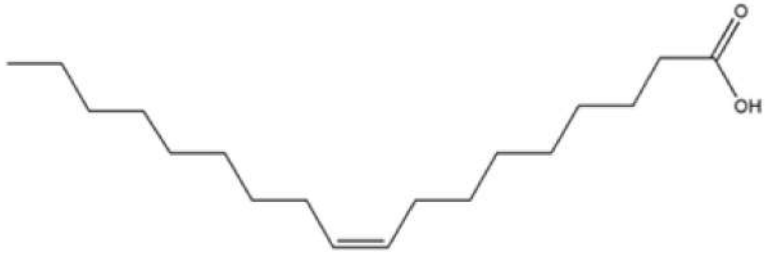
ÁCIDO GRAXOS *CIS-TRANS* MAJORITÁRIOS



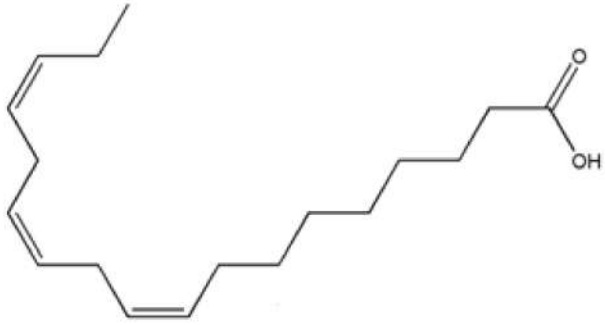
Palmitic acid



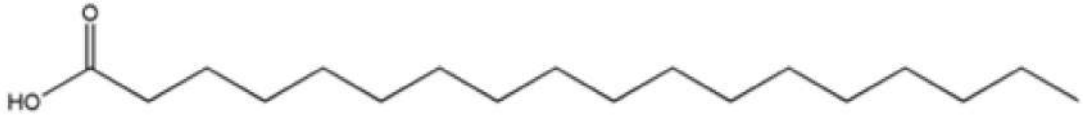
Linoleic acid



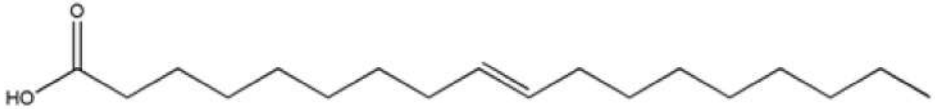
Oleic acid



Linolenic acid

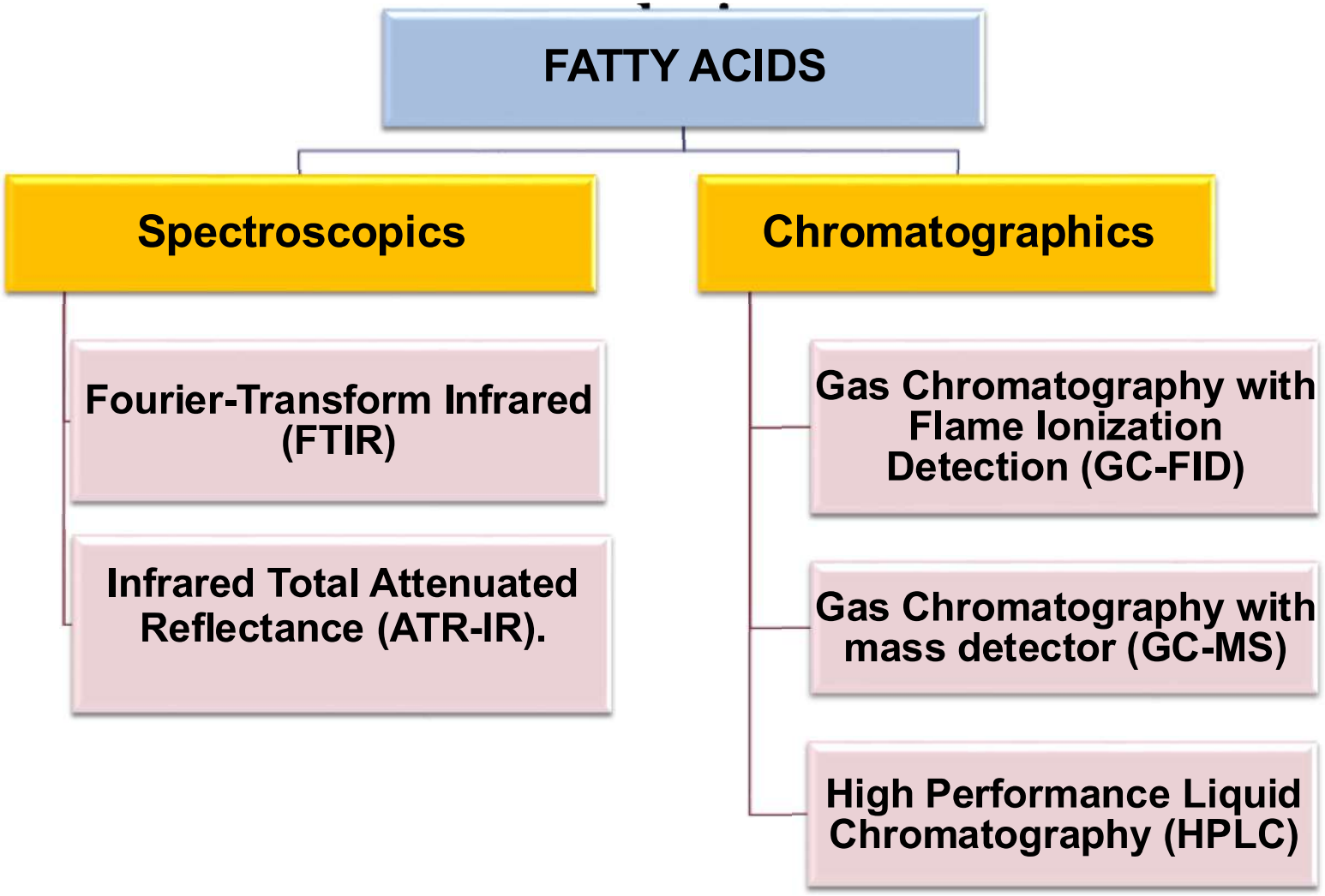


Stearic acid

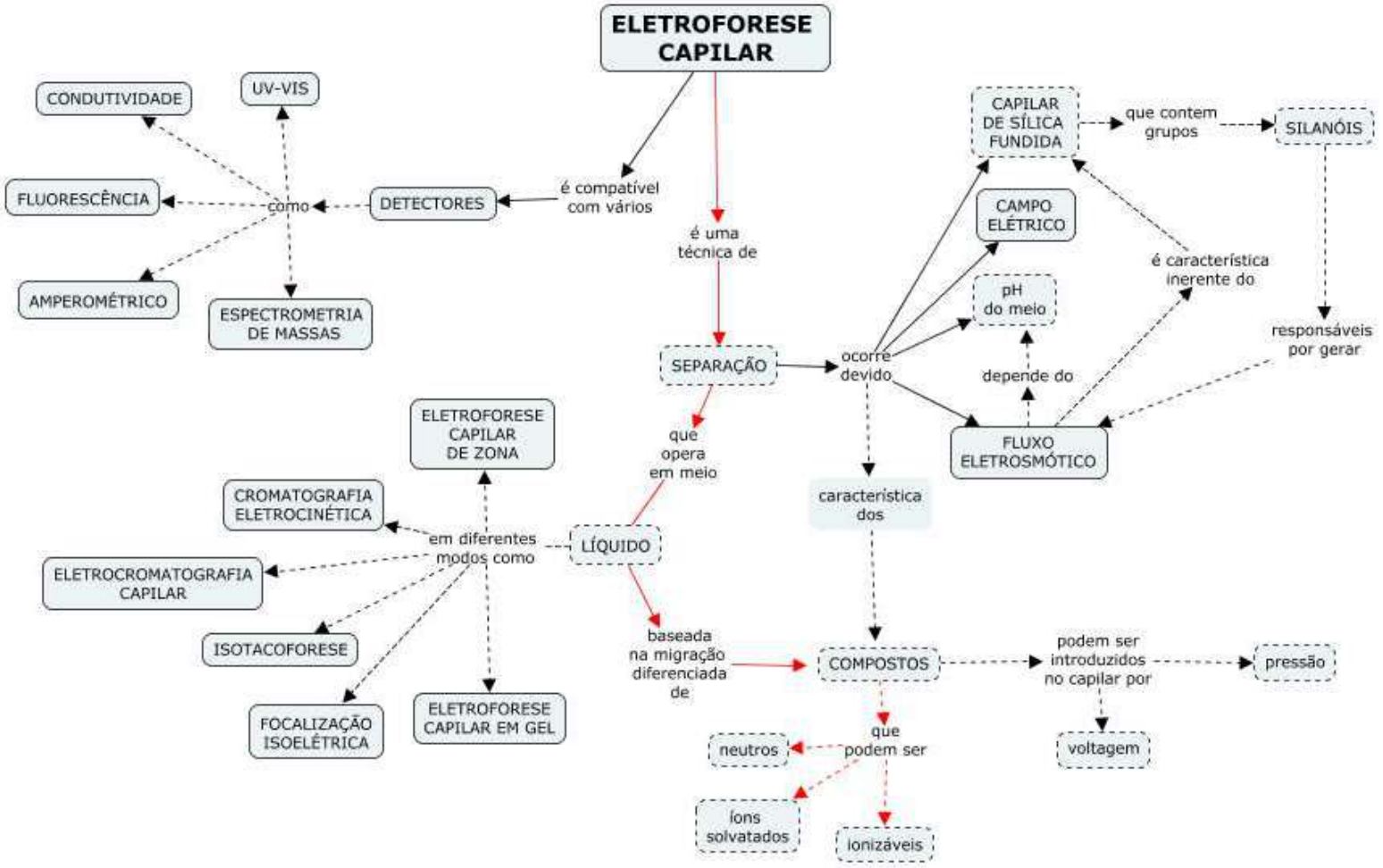


Elaidic acid

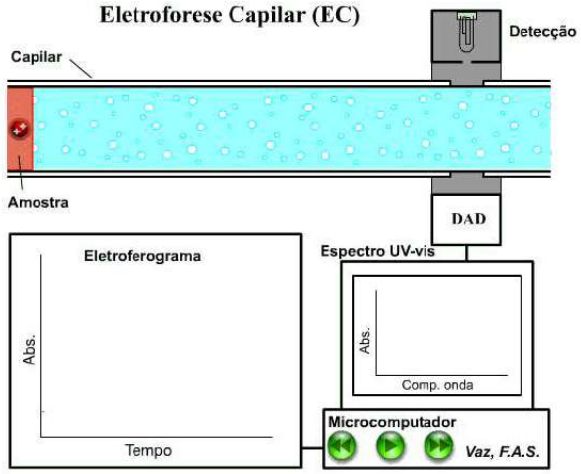
Classical methods to Fatty Acids



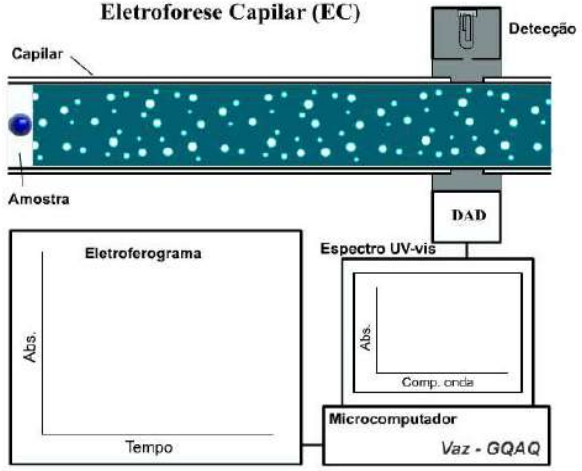
ELETROFORESE CAPILAR: CONSIDERAÇÕES GERAIS

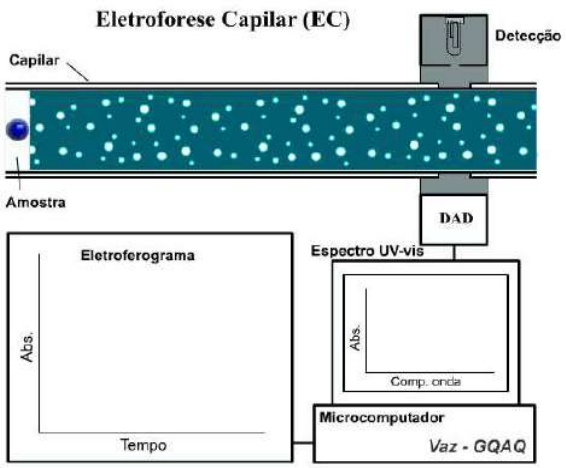


CZE-Detecção direta no UV-Vis



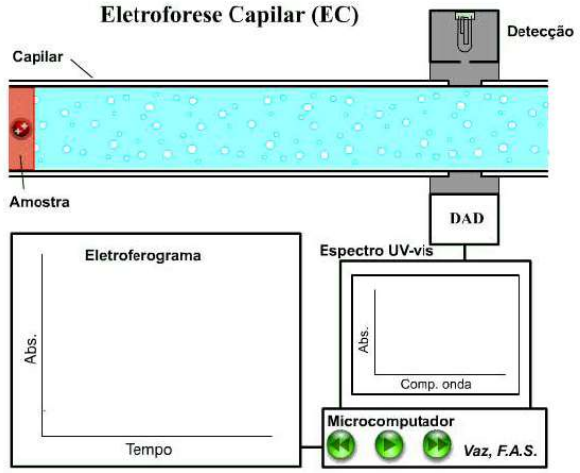
CZE-Detecção indireta no UV-Vis





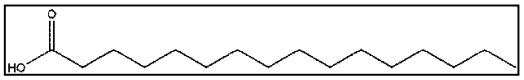
CIS-TRANS FA ANALYSIS BY CZE-UV

FA pKa ~ 5 (buffer solution pH>5)
Organic solvents (ACN, 1-octanol)
Cis-trans isomers selector (Brij 35)



UV indirect detection

UV direct detection

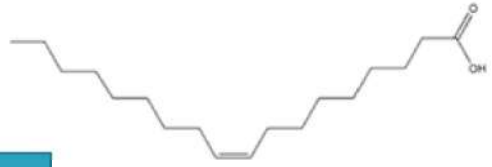


Palmitic acid

Chromophore (SDBS)

Saturated and Unsaturated FA

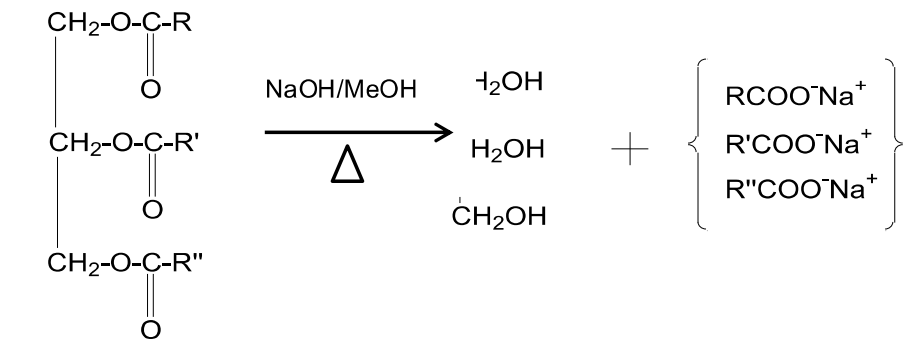
Unsaturated FA



Oleic acid

<u>Other detection possibilities:</u>
- C^4D
- Indirect fluorescence

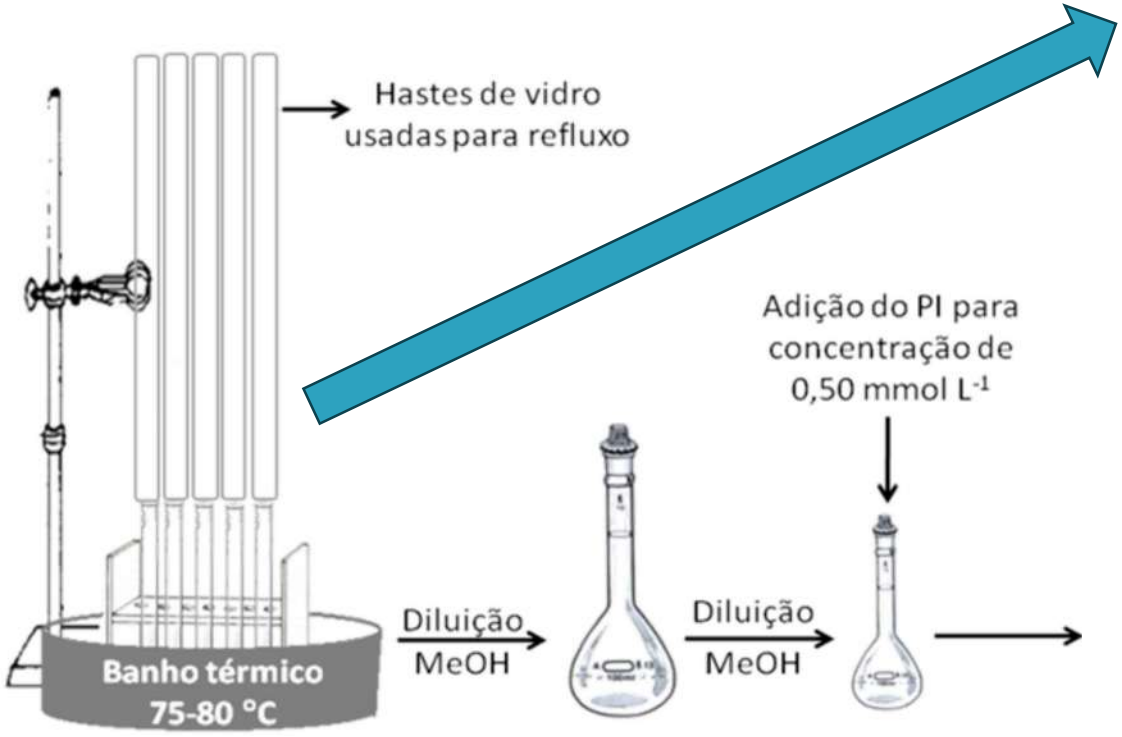
OBTENÇÃO DOS SAIS DE ÁCIDOS GRAXO



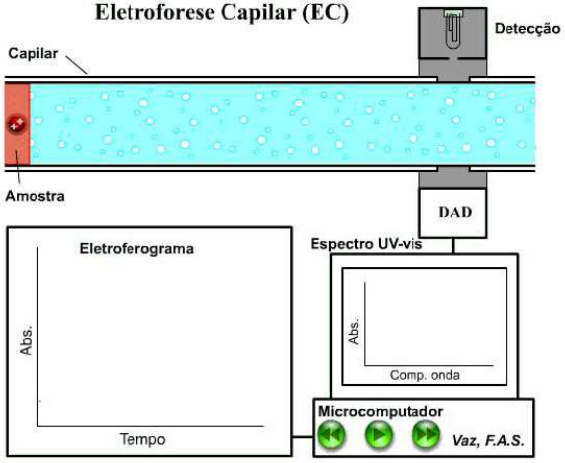
Glicerídeo
(óleos, gorduras, etc...)

Glicerol

Sais de ácidos graxos



150 mg de amostra



Reservatório de 1,0 mL

Eletrólito de corrida

30 mmol/L $Na_2B_4O_7$

12 mmol/L Brij® L23

33 % (*v*/*v*) MeOH

17 % (*v*/*v*) of ACN

Balão volumétrico
de 10 mL

Optimized parameters of the CZE-UV method for EA determination, and injection program for the sample and the sample with EA standard injected by the on-column method.

Parameter	Optimized values
Temperature	27 °C
Wavelength	200 nm
Voltage	+ 19 kV
Generated current (average)	80 µA
Run time	16 min
Injection Program	
Sample	Sample with EA standard addition
Sample: 20 mbar x 3 s	**Sample:** 20 mbar x 3 s
MeOH: 10 mbar x 3 s	**EA 2 mmol L^{-1}:** 10 mbar x 3 s
BGE: 10 mbar x 3 s	**BGE:** 10 mbar x 3 s
Wait: 3 s	**Wait:** 3 s

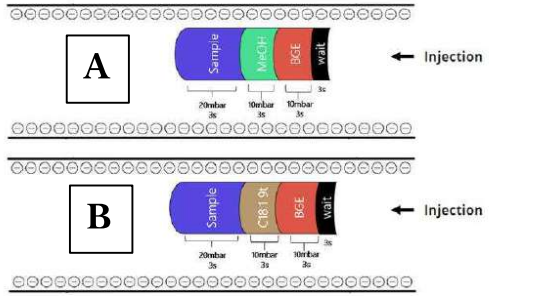
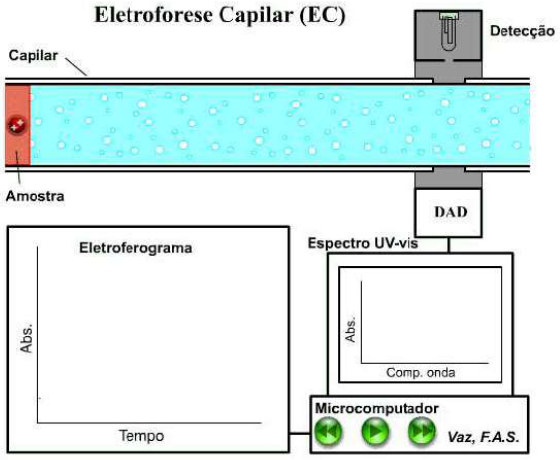


Fig. 1. Schematic representation of the dynamic on column injection program options.



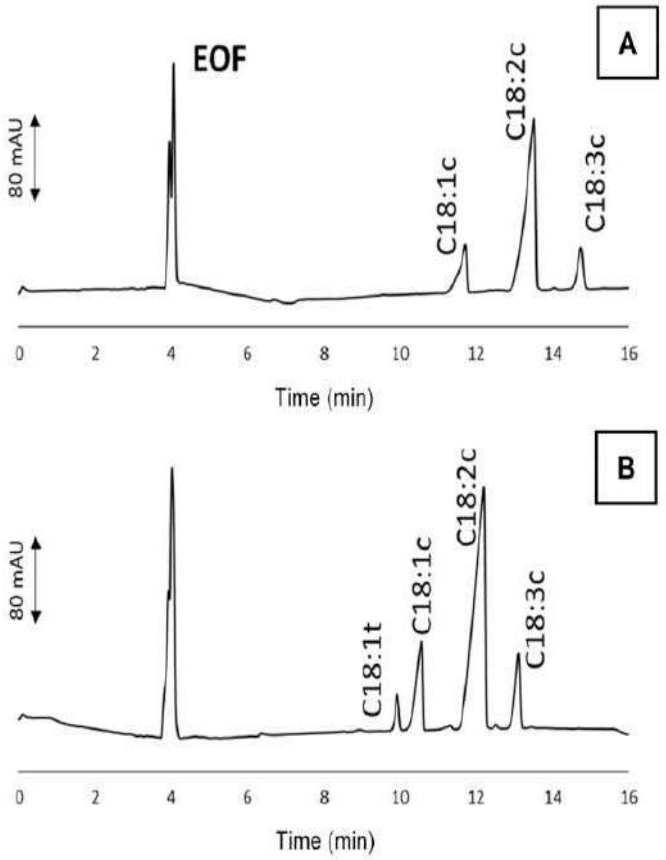


Fig. 2. Electropherograms of a QC of vegetable oils (A), and a QC of vegetable oils fortified with EA (C18:1 t) at a concentration of 2 mmol L^{-1} (B). Electrolyte: 30 mmol L^{-1} of $Na_2B_4O_7$, 12 mmol L^{-1} of Brij® L23 surfactant, 33 % (*v*/*v*) of MeOH, and 17 % (*v*/*v*) of ACN. Other experimental conditions: hydrodynamic injection (25 mbar for 5 s); cartridge temperature: 27 °C; voltage: + 19 kV; λ= detection: 200 nm; and a TSP capillary, 33.5 cm (25 cm effective length), 50 µm i.d., and 375 mm e.d.

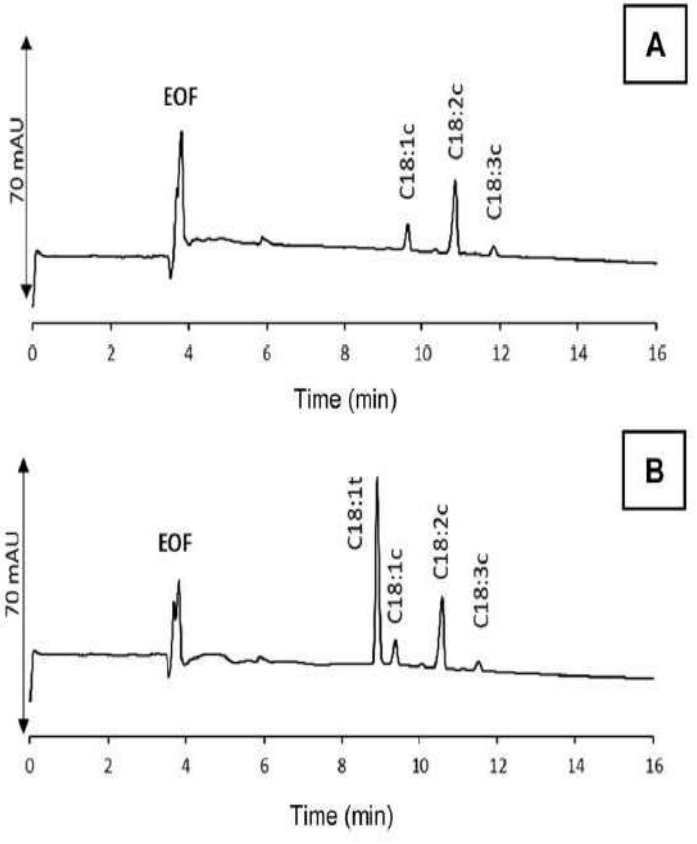


Fig. 3. Electropherograms of a QC of non-diary food products (A), and a QC of non-diary food products fortified with EA (C18:1 t) at a concentration of 2.0 mmol L^{-1} (B). Electrolyte: 30 mmol L^{-1} of $Na_2B_4O_7$, 12 mmol L^{-1} of Brij® L23 surfactant, 33 % (*v*/*v*) of MeOH, and 17 % (*v*/*v*) of ACN. Other experimental conditions: hydrodynamic injection (25 mbar for 5 s); cartridge temperature: 27 °C; voltage: + 19 kV; detection: 200 nm; and a TSP capillary, 33.5 cm (25 cm effective length), 50 µm i.d., and 375 mm e.d.

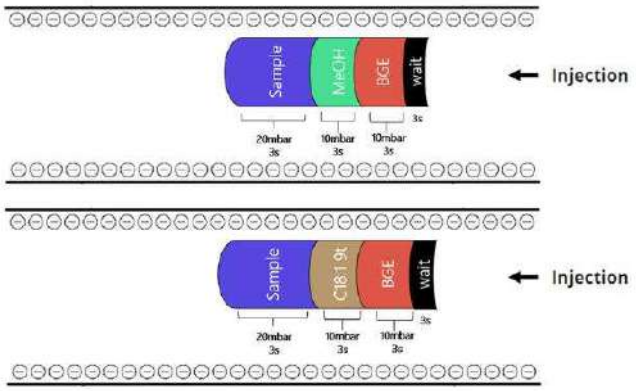
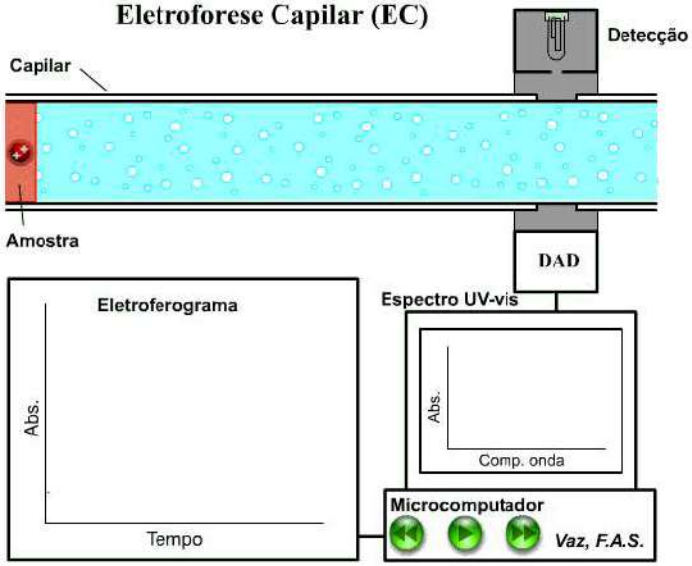


Fig. 1. Schematic representation of the dynamic on column injection program options.



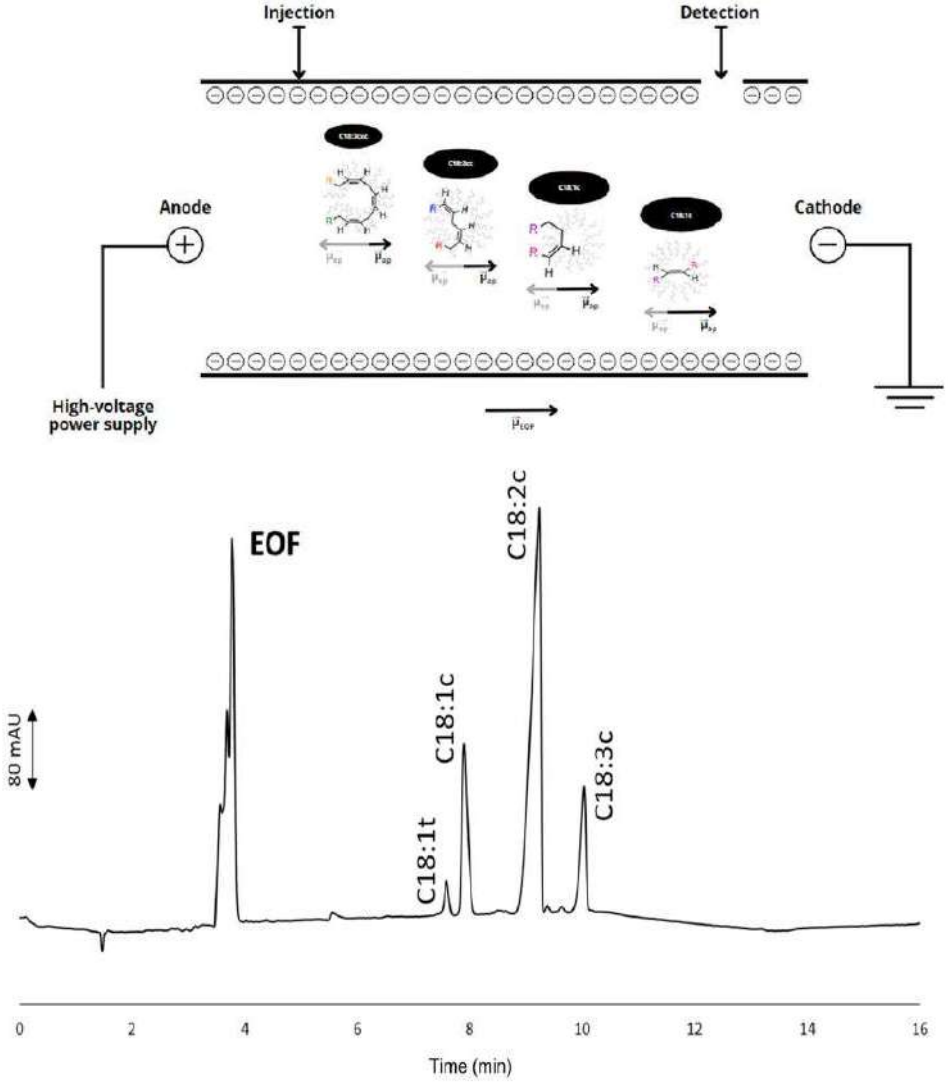


Fig. 5. Electropherogram of a QC of vegetable oil with 2 % of EA (C18:1 t). Electrolyte: 30 mmol L^{-1} of $Na_2B_4O_7$, 12 mmol L^{-1} of Brij® L23 surfactant, 33 % (*v*/*v*) of MeOH, and 17 % (*v*/*v*) of ACN. Other experimental conditions: hydrodynamic injection (25 mbar for 5 s); cartridge temperature: 27 °C; voltage: + 19 kV; detection: 200 nm; and a TSP capillary, 33.5 cm (25 cm effective length), 50 µm i.d., and 375 mm e.d.

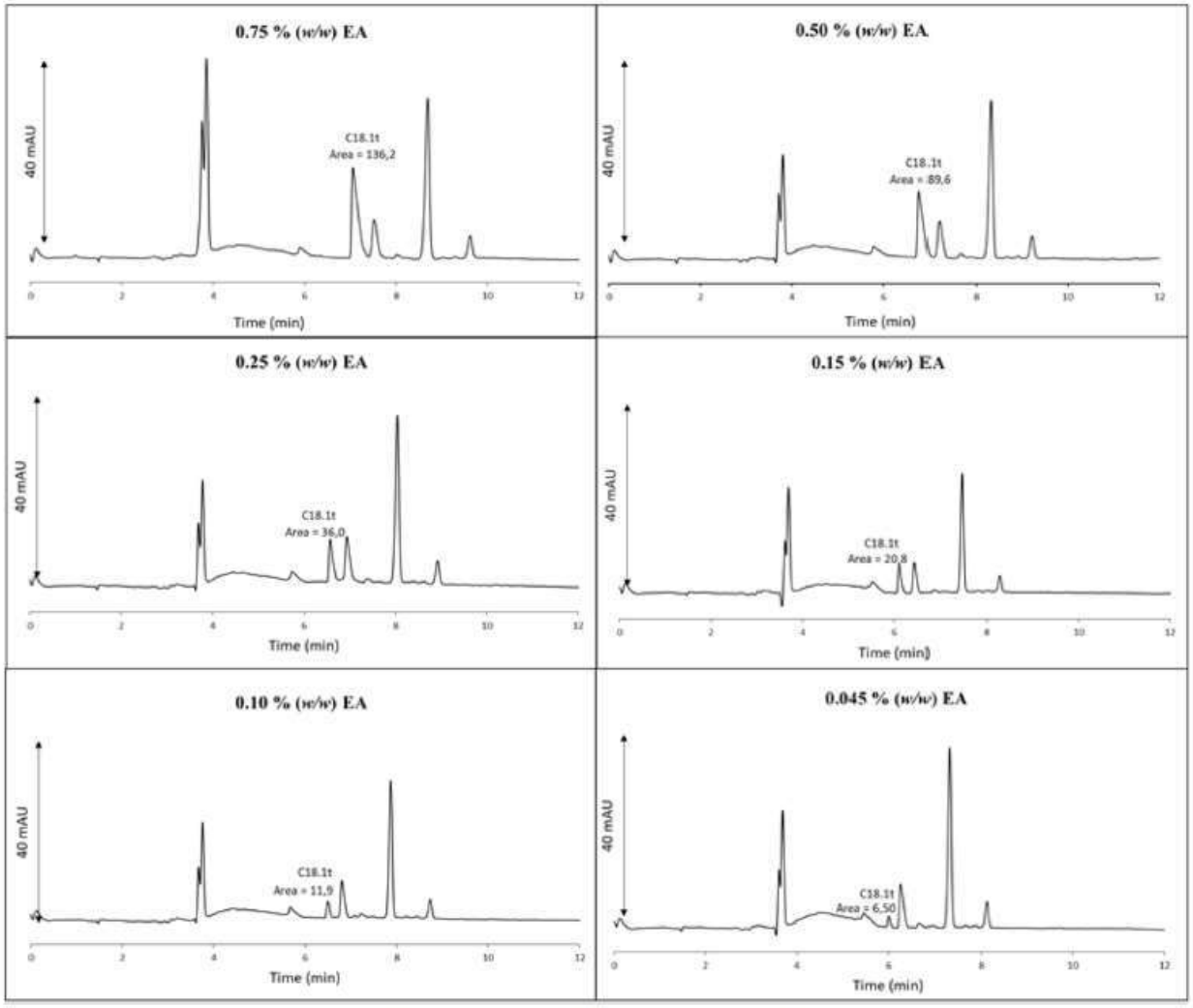


Fig. 6. Electropherograms of successive dilutions until the quantification (0.150 %, w/w) and detection (0.045 %, w/w) limits. Electrolyte: 30 mmol L^{-1} of $Na_2B_4O_7$, 12 mmol L^{-1} of Brij® L23 surfactant, 33 % (v/v) of MeOH, and 17 % (v/v) of ACN. Other experimental conditions: hydrodynamic injection (25 mbar for 5 s); cartridge temperature: 27 °C; voltage: + 19 kV; detection: 200 nm; and a TSP capillary, 33.5 cm (25 cm effective length), 50 µm i.d., and 375 mm e.d.

Concentração mol/L de 2% de trans expresso em ácido elaídico na amostra

mol/L={2.0 g/[(282,46 g/mol) x (0,1L) x (0,919)]}
mol/L=0,077047 x 1000
mmol/L = 77,047

Análise da alíquota da amostra analisada

mol/L={0,15 g/[(282,46 g/mol) x (0,002L) x (0,919)]}
mol/L=0,288927 x 1000
mmol/L = 288,89
Diluindo 1:10 → mmol/L=28,89 mmol/L (~0,75% na amostra)

$$C_x = \frac{(S_1 \times C_S \times V_S)}{((S_2 - S_1) \times V_x)}$$

- S1 é a área do pico de EA na amostra;
- S2 é a área de EA a 2,0 mmol/L na amostra enriquecida;
- CS é a concentração da solução padrão
- VS é o volume da solução padrão
- eVx é o volume da amostra

Fluxograma GC e CE

Food Research International 52 (2013) 33–41

Contents lists available at SciVerse ScienceDirect

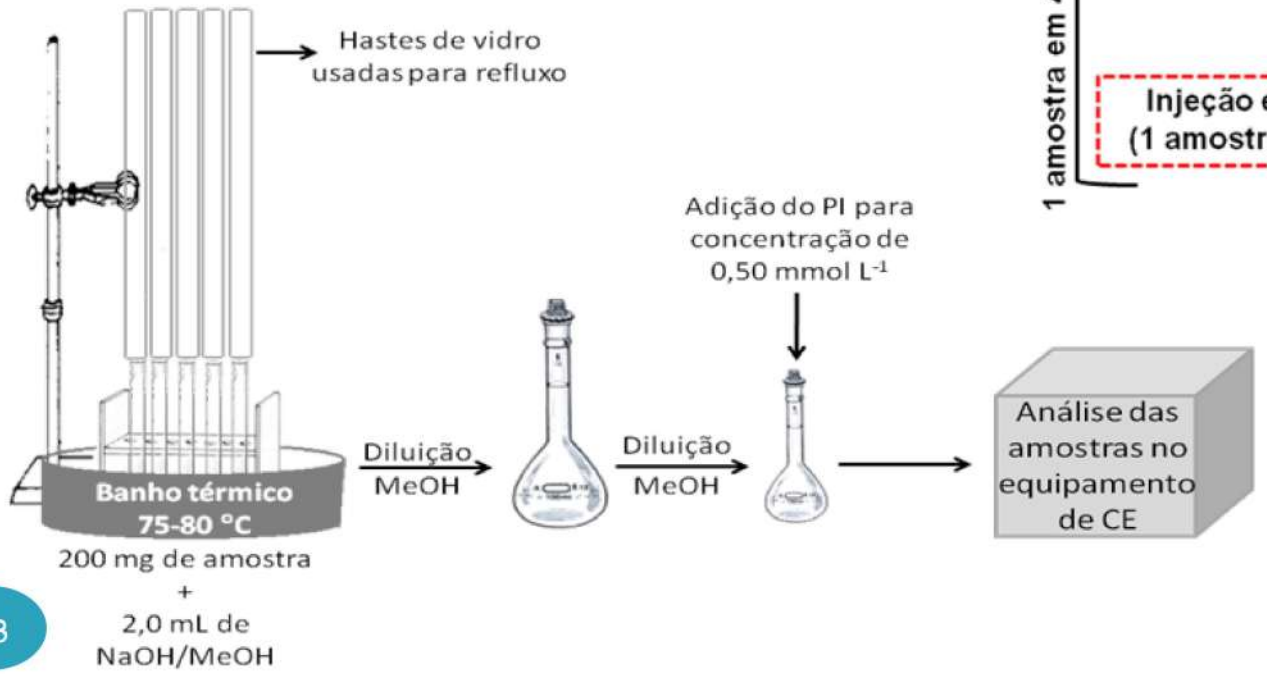
Food Research International

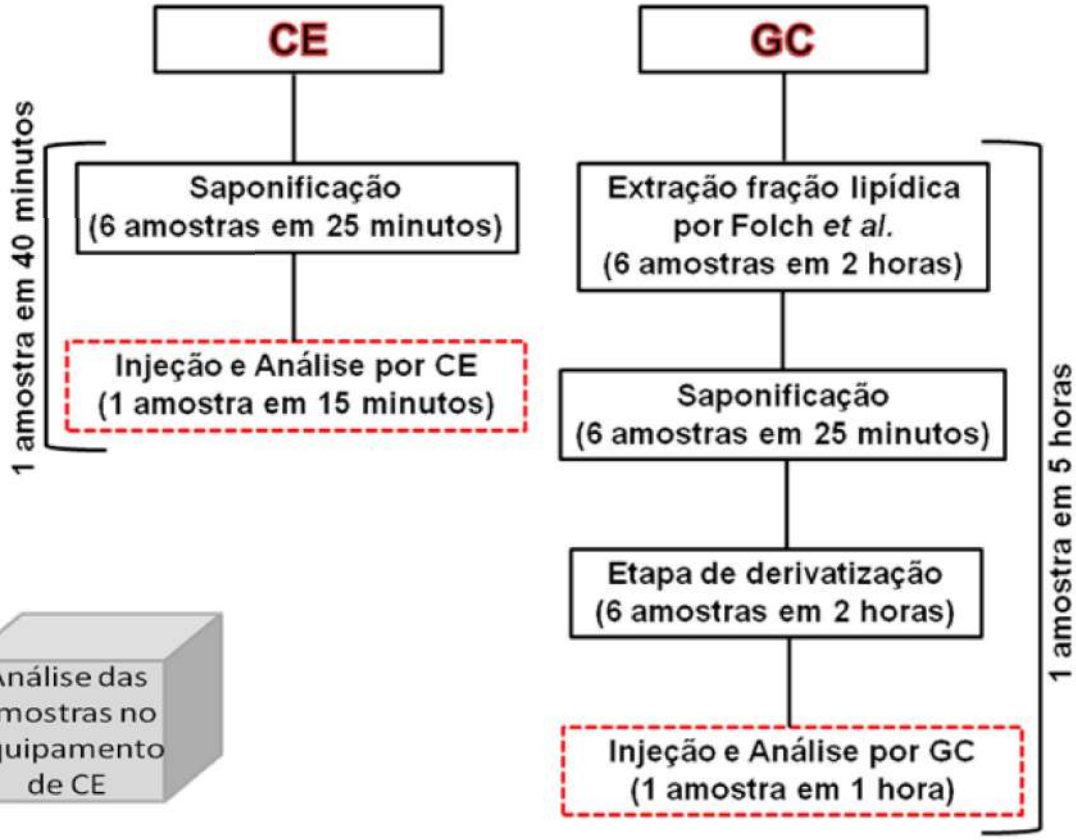
journal homepage: www.elsevier.com/locate/foodres

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

Patrícia Mendonça de Castro Barra [a], Renata de Jesus Coelho Castro [a], Patrícia Lopes de Oliveira [a], Sabria Aued-Pimentel [b], Simone Alves da Silva [b], Marcone Augusto Leal de Oliveira [a,*]

[a] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Cidade Universitária, CEP 36036-330 Juiz de Fora, MG, Brazil
[b] Instituto Adolfo Lutz, Divisão de Bromatologia e Química, São Paulo, SP Brazil





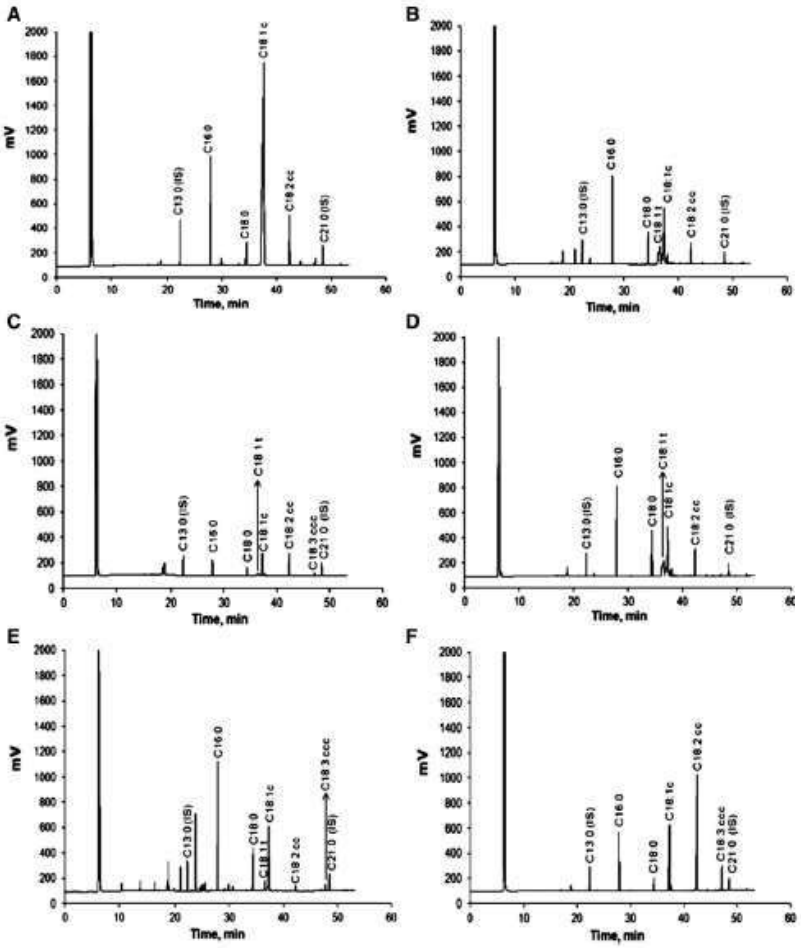


Fig 3. Chromatograms of samples by GC official methodology. A – olive oil, B – hydrogenated vegetable fat, C – margarine, D – filled cookie, E – butter, F – soy oil. Operational conditions: programmed column temperature: 45 °C for 4 min; increase of 12 °C min^{-1} to 175 °C (held for 27 min); then there was an increase of 4 °C min^{-1} to 215 °C (held for 35 min). The temperature of injector and detector was set at 250 °C, the carrier gas was hydrogen and the column pressure was 175 kPa.

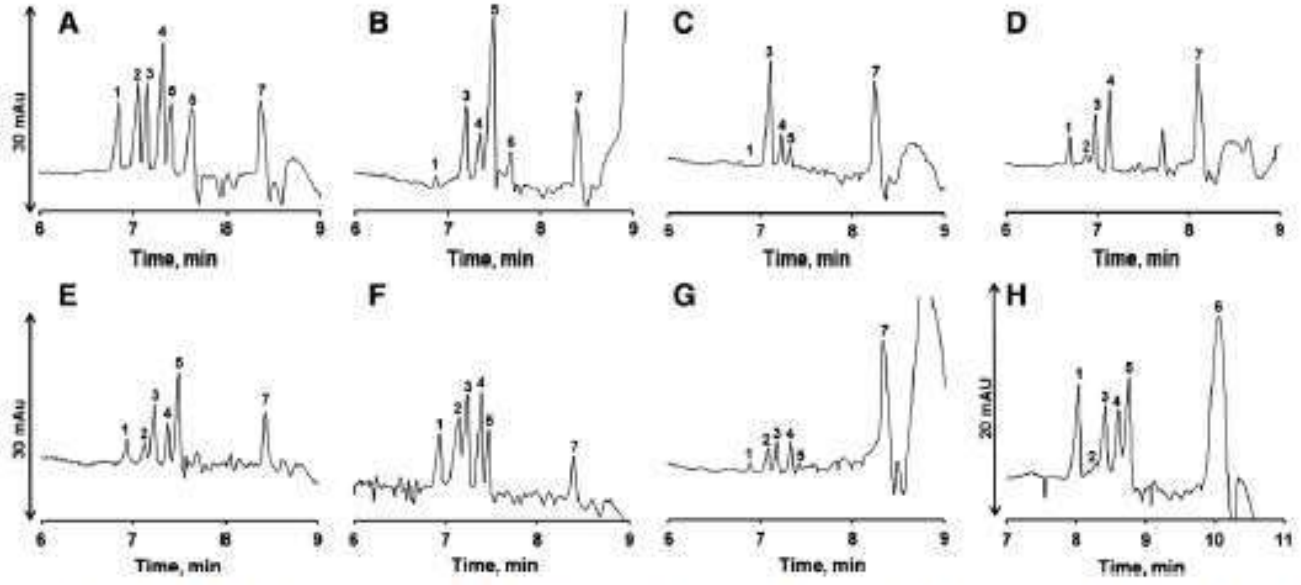
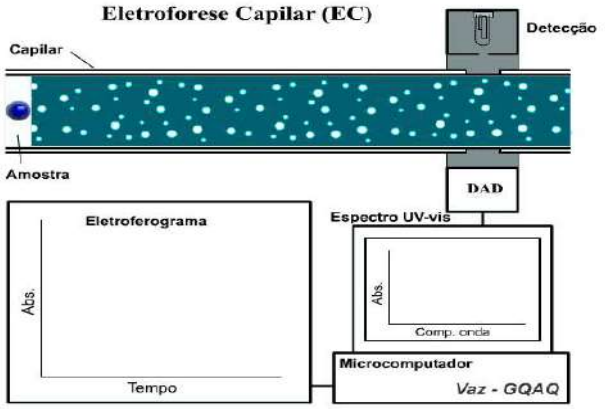


Fig 4. Standard fatty acid electropherograms of (1) C8:0, (2) C18:1 9t, (3) C18:1 9c, (4) C16:0, (5) C18:2cc, (6) C18:3ccc, (7) C13:0 (IS), all with concentration of 0.50 mmol L^{-1}. B – soy oil, C – olive oil, D – butter, E – margarine, F – filled cookie, G – hydrogenated vegetable fat and H – bovine liver. Operational conditions: injection 5 s × 12.5 mbar; + 19 Kv applied voltage, 25 °C cartridge temperature and indirect detection at 400 (±2) nm in sample and 224 (±2) nm in reference (inverted peak), TSH capillary with 48.5 cm long (40 cm effective length) 75 μm I.D and 375 mm O.D. Electrolyte: 15.0 mmol L^{-1} of NaH_2PO_4/Na_2HPO_4 at pH 6.86, 4.0 mmol L^{-1} of SDBS, 8.3 mmol L^{-1} of Brij 35, 45% v/v of ACN and 2.1% of 1-octanol.



FA analysis results by CE and GC.

Samples	C18:0		C18:1t		C18:1c		C16:0		C18:2cc		C18:3ccc	
	CE	GC	CE	GC	CE	GC	CE	GC	CE	GC	CE	GC
SO 1	3.07	3.44	–	–	25.67	25.9	11.15	11.07	46.76	49.03	4.55	5.30
SO 2	3.19	3.19	–	–	25.92	25.3	10.93	11.15	47.19	48.20	4.89	5.26
Mean	3.13	3.32	–	–	25.80	25.60	11.04	11.11	47.98	48.62	4.72	5.28
Standard deviation	0.09	0.18	–	–	0.18	0.46	0.16	0.06	0.31	0.59	0.25	0.03
OO 1	3.62	3.47	–	–	73.66	73.1	10.11	10.22	7.03	6.97	0.65	0.66
OO 2	3.25	3.64	–	–	73.61	72.9	10.13	10.20	6.29	6.91	0.69	0.69
Mean	3.44	3.56	–	–	73.64	73.00	10.12	10.21	6.80	6.94	–	–
Standard deviation	0.26	0.12	–	–	0.04	0.09	0.01	0.02	0.53	0.04	–	–
BT 1	7.66	8.23	2.82	2.86	16.63	16.34	18.57	19.00	0.96	1.06	nq	0.28
BT 2	8.50	8.56	2.80	2.82	17.63	16.10	18.36	18.59	0.98	0.97	nq	0.27
Mean	8.08	8.40	2.81	2.84	17.13	16.22	18.46	18.79	0.99	1.02	–	–
Standard deviation	0.59	0.23	0.01	0.03	0.71	0.17	0.15	0.23	0.01	0.06	–	–
MG 1	3.33	3.44	2.03	2.21	5.659	5.58	2.285	2.43	5.44	5.27	0.69	0.61
MG 2	3.83	3.54	2.71	2.22	5.971	6.18	2.123	2.28	5.32	5.85	0.65	0.63
Mean	3.58	3.49	2.37	2.22	5.82	5.88	2.20	2.36	5.49	5.56	0.67	0.62
Standard deviation	0.36	0.07	0.49	0.01	0.22	0.42	0.11	0.11	0.09	0.41	0.03	0.01
HVF 1	9.06	8.85	26.4	26.65	25.55	26.2	17.14	16.5	5.89	5.69	nd	0.07
HVF 2	8.61	9.18	26.7	25.47	26.64	26.8	16.64	17.3	5.57	6.17	nd	0.07
Mean	8.84	9.02	26.55	26.06	26.09	26.53	16.89	16.88	5.85	5.93	–	–
Standard deviation	0.32	0.23	0.24	0.83	0.77	0.45	0.35	0.60	0.23	0.34	–	–
FC 1	2.99	3.02	5.51	5.56	5.822	5.36	4.387	4.41	2.42	2.03	nd	0.12
FC 2	3.28	3.46	5.27	5.38	5.711	5.4	4.065	4.09	2.41	2.08	nd	0.13
Mean	3.14	3.24	5.39	5.47	5.77	5.38	4.23	4.25	2.11	2.02	–	–
Standard deviation	0.20	0.31	0.17	0.13	0.08	0.03	0.23	0.23	0.50	0.01	–	–
Shapiro–Wilk test[a]	0.547		0.011		0.679		0.163		0.022		0.047	
t test[a]	0.090		0.484		0.128		0.288		0.317		0.529	
Pearson correlation	0.995		0.999		0.999		0.998		0.999		0.999	

–: absent in the sample.
nd: lower of limit of detection.
nq: lower of limit of quantification.
Note: All FA content by CE and GC was expressed in g/100 g of sample.
SO: soy oil, OO: olive oil, BT: butter, MG: margarine, HVF: hydrogenated vegetable fat, FC: filled cookie.
[a] p-values.

Resultados (síntese dos relatórios)

Execução e entregas: relatório de cumprimento de metas do TED enviado, com registro de execução orçamentária/financeira e execução física (coletas, análises por CZE-UV).

Não foram identificados AGT nas amostras de óleos refinados e de alimentos processados analisadas.

Conclusões

- Desenvolvimento de estratégias analíticas alternativas, aplicáveis ao controle de qualidade de alimentos é muito relevante.

- Disponibilização de um método de triagem por CZE-UV para a análise quantitativa de gordura trans, expressa em ácido elaídico, em óleos vegetais e produtos alimentícios não lácteos.

- *O uso do programa de injeção em coluna por CZE-UV e um método de adição de padrão de ponto único para quantificação, apesar de considerar a determinação de ácidos graxos trans totais expressos em AE, é muito útil e interessante devido à etapa simples de preparação da amostra, é automatizado, apresenta maior rendimento analítico, apresenta custo reduzido e evoca mais os princípios da Química Verde, quando comparado ao método de referência GC-FID.*

- *Foi entregue o seguinte produto para a unidade descentralizadora: Relatório contendo a quantificação de ácidos graxos trans totais para cada produto selecionado!*

Food Research International 52 (2013) 33–41

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

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

Patrícia Mendonça de Castro Barra [a], Renata de Jesus Coelho Castro [a], Patrícia Lopes de Oliveira [a], Sabria Aued-Pimentel [b], Simone Alves da Silva [b], Marcone Augusto Leal de Oliveira [a,*]

[a] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Cidade Universitária, CEP 36036-330 Juiz de Fora, MG, Brazil
[b] Instituto Adolfo Lutz, Divisão de Bromatologia e Química, São Paulo, SP Brazil

Journal of Chromatography A 1613 (2020) 460641

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Fast capillary electrophoresis method for determination of docosahexaenoic and eicosapentaenoic acids in marine oils omega-3 supplements

Tatiane Lima Amorim [a,b], Lucas Mattos Duarte [b], Miguel Angel de la Fuente [a], Marcone Augusto Leal de Oliveira [b,*], Pilar Gómez-Cortés [a]

Electrophoresis 2003, *24*, 1641–1647 1641

Method development for the analysis of *trans*-fatty acids in hydrogenated oils by capillary electrophoresis

Marcone A. L. de Oliveira[1]
Victor E. S. Solis[2]
Luiz A. Gioielli[2]
Bronislaw Polakiewicz[2]
Marina F. M. Tavares[1]

[1]Instituto de Química
[2]Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brazil

A novel capillary electrophoresis methodology using UV indirect detection (224 nm) for the analysis of *trans*-fatty acids in hydrogenated oils was proposed. The electrolyte consisted of a pH 7 phosphate buffer at 15 mmol·L^{-1} concentration containing 4 mmol·L^{-1} sodium dodecylbenzenesulfonate, 10 mmol·L^{-1} polyoxyethylene 23 lauryl ether (Brij 35), 2% 1-octanol and 45% acetonitrile. Under the optimized conditions, ten fatty acids, C12:0, C13:0 (internal standard), C14:0, C16:0, C18:0, C18:1c, C18:1t, C18:2cc, C18:2tt and C18:3ccc were baseline-separated in less than 12 min. The proposed methodology was applied to monitor the formation of *trans*-fatty acids during hydrogenation of Brazilnut oil. A crude oil sample (42.1% linoleic acid, 37.3%

Journal of Food Composition and Analysis 148 (2025) 108341

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Determination of trans-fatty acids in non-dairy foods using enhanced CZE-UV: Implications for food health assessment

Marcone Augusto Leal de Oliveira [a,b,*], Jéssica Cordeiro Queiroz de Souza [a], Maria Patrícia do Nascimento [a], Olívia Brito de Oliveira Moreira [a], Patrícia Abranches Geraldo [a]

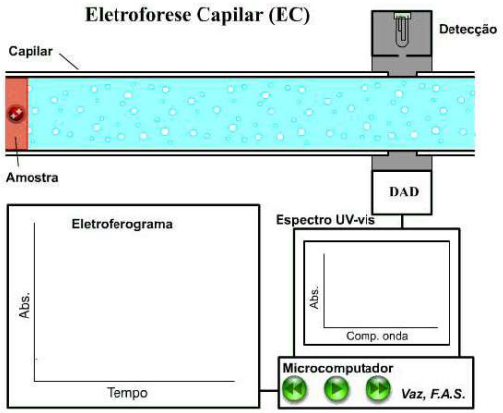
[a] Grupo de Química Analítica e Quimiometria - GQAQ, Chemistry Department, Institute of Exact Sciences, Federal University of Juiz de Fora (UFJF), Juiz de Fora, MG 36036-900, Brazil
[b] National Institute of Science and Technology for Bioanalytics – INCTBio, Institute of Chemistry, University of Campinas (UNICAMP), Campinas, SP 13083-970, Brazil

ARTICLE INFO

Keywords:
Vegetable oils
Non-dairy food
Fatty acids
Capillary electrophoresis
Elaidic acid

ABSTRACT

The consumption of industrial trans-fatty acids is knowingly associated with cardiovascular diseases, obesity, cholesterol increase, and other disorders, which turn into a public health issue. An initiative organized by the World Health Organization foresees the reduction of the consumption of these products worldwide. Therefore, labeling regulations, restrictions, or even prohibition of industrial trans-fat addition in foodstuffs have been defined by health regulatory agencies in several countries. In this context, a novel screening quantitative method by CZE-UV, aiming at enhancing routine analysis, is a cost-effective and viable option to determine elaidic acid as a marker for industrial trans-fatty acids. The new method combines the CZE-UV with an on-column injection program and a single-point standard addition method for quantification, which provides reliable quantitative options with less laborious laboratory steps, high throughput, and a reduction of analysis costs. In order to attend Resolution - RDC No. 632 preconized by ANVISA, about 300 samples, including vegetable oils and non-dairy foods, from different precedency, batches, and brands were successfully analyzed. The method improves upon existing methods by providing adequate results, especially where current food labeling relies on general estimations or ingredient list checks.



Analytical Methods

CRITICAL REVIEW

Trans fatty acid determination by capillary zone electrophoresis: the state of the art and applications

T. L. A. Prado and M. A. L. Oliveira *

A review taking into account literature reports covering alternative methods for *trans* fatty acid (TFA) determination, expressed in elaidic acid (EA), using capillary zone electrophoresis is presented. The manuscript presents ultraviolet-visible and capacitively coupled contactless conductivity detection systems. TFAs were separated by indirect detection, with the use of a chromophore agent in a background electrolyte as well by direct detection, without the use of the chromophore agent. In sum, the present review evidences that capillary electrophoresis (CE) is a very interesting analytical separation technique for TFA quantification in different matrices, which offers many advantages such as short analysis time, simple sample preparation, and use of short and non-specific columns and small amounts of organic solvents and reagents to perform experiments. These compensations make CE approaching very attractive to attend demand of governmental agencies and industries facing the global concern in respect of the harmful health effects caused by increasing intake of TFAs, as EA.

Received 20th January 2017
Accepted 18th March 2017
DOI: 10.1039/c7ay00195b
rsc.li/methods

Analytica Chimica Acta 1068 (2019) 130–137

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Screening method for simultaneous detection of elaidic and vaccenic trans fatty acid isomers by capillary zone electrophoresis

Tatiane Lima Amorim [a], Lucas Mattos Duarte [a], Hélio F. Dos Santos [b], Marcone Augusto Leal de Oliveira [a,*]

Food Analytical Methods
https://doi.org/10.1007/s12161-020-01772-w

Advances in Lipid Capillary Electromigration Methods to Food Analysis Within the 2010s Decade

Tatiane Lima Amorim[1] · Marcone Augusto Leal de Oliveira[1]

Received: 29 February 2020 / Accepted: 10 May 2020

Abstract

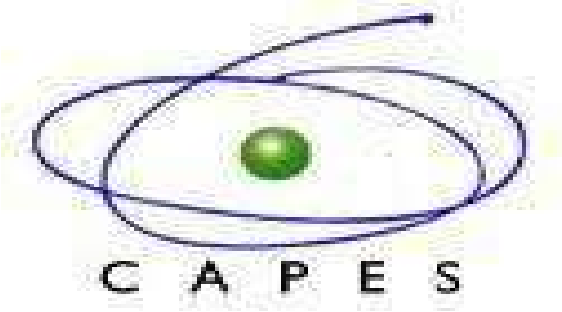
Grupo de Química Analítica e Quimiometria

QGAQ

https://instagram.com/gqaq_ufjf?utm_medium=copy_link


ufjf
UNIVERSIDADE
FEDERAL DE JUIZ DE FORA


inct
bioanalítica


CAPES


CNPq
Conselho Nacional de Desenvolvimento
Científico e Tecnológico


FAPEMIG


Fadepe


INSTITUTO ADOLFO LUTZ


GQAQ
Grupo de Química Analítica e Quimiometria


ANVISA
Agência Nacional de Vigilância Sanitária

MUITA GRATIDÃO
PELA ATENÇÃO!