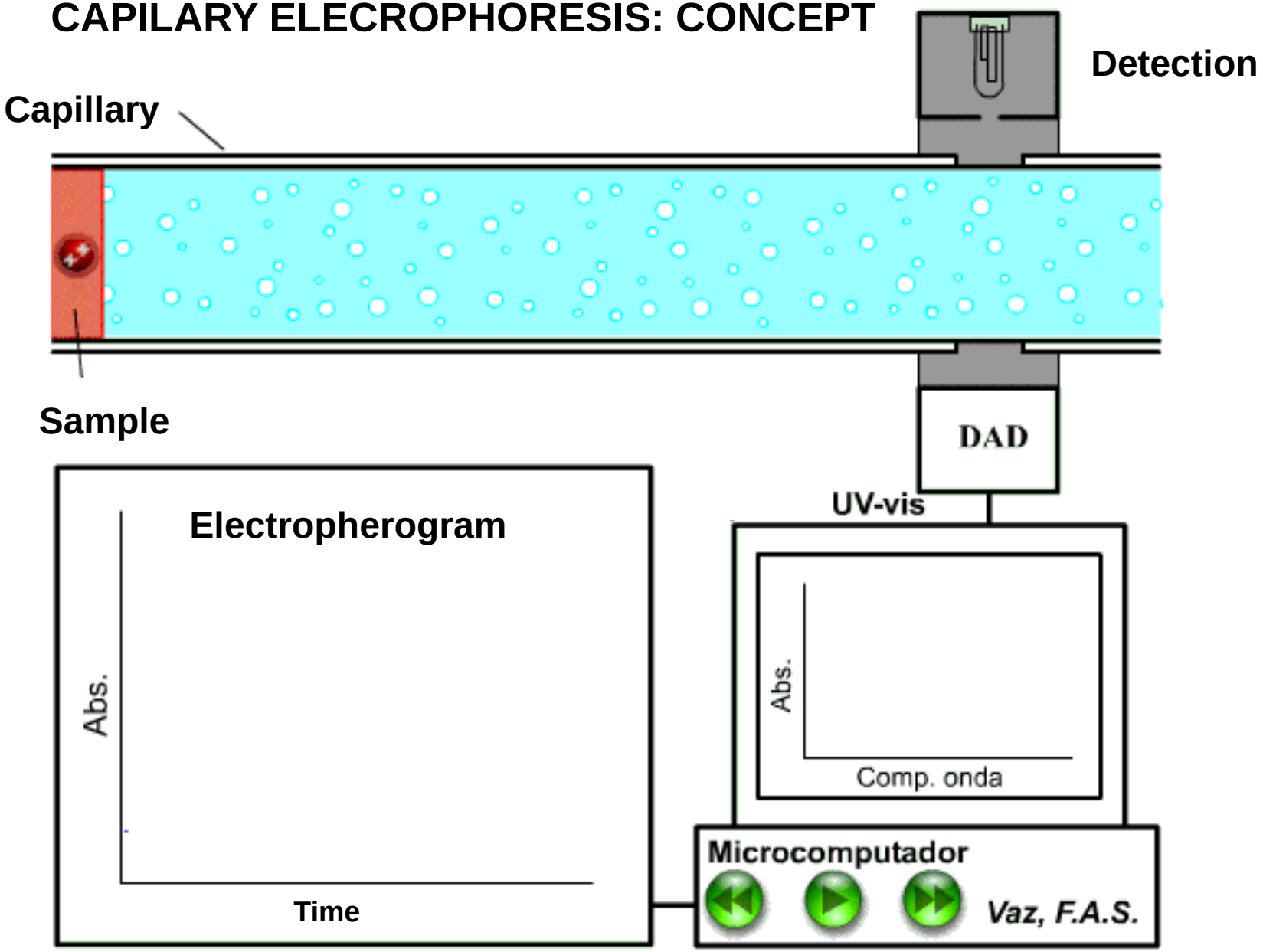
“FEDERAL UNIVERSITY FROM JUDGE OUTSIDE”

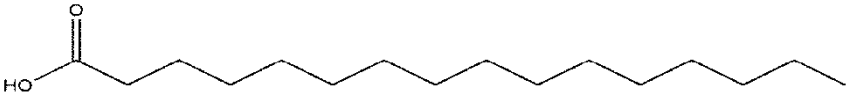
“NOVEL CAPILLARY ELECTROPHORESIS APPLICATIONS FOR ANALYSIS OF FOOD, DRUGS AND BIODIESEL”

Prof. Dr. Marcone Augusto Leal de Oliveira

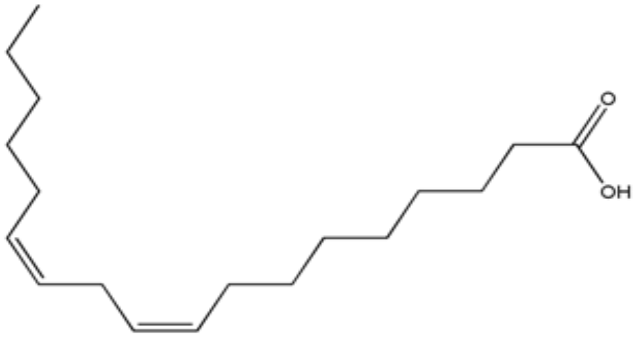
CAPILARY ELECROPHORESIS: CONCEPT



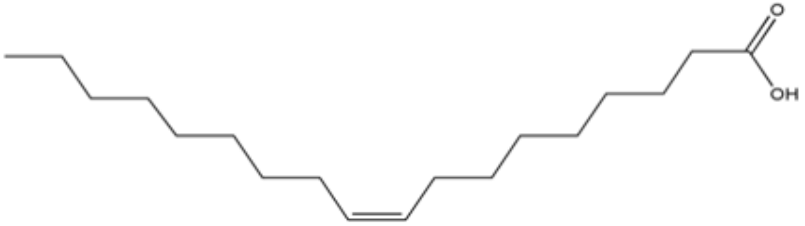
“ANALYSIS OF FATTY ACIDS BY CAPILLARY ELETROFORESIS AND IMPLICATIONS IN DIFFERENT POTENTIALITIES”



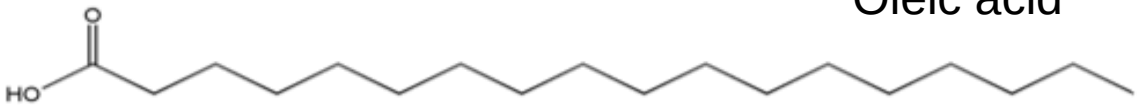
Palmitic acid



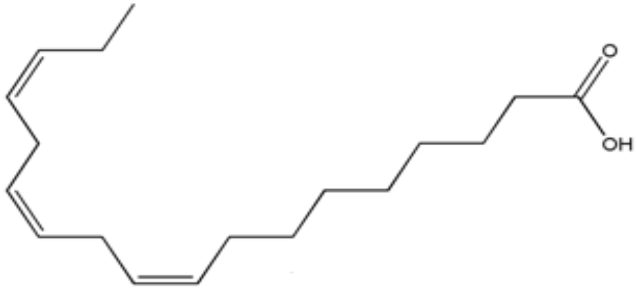
Linoleic acid



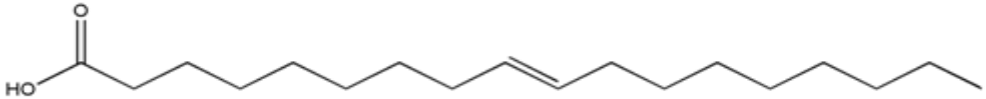
Oleic acid



Stearic acid



Linolenic acid



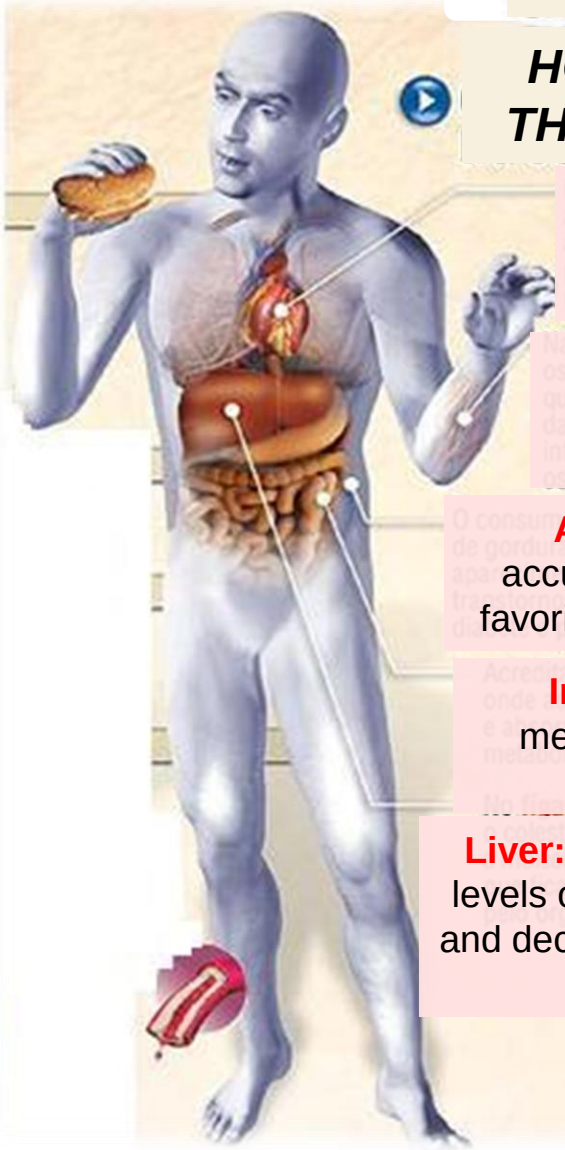
Elaidic acid

Fatty acids

linolenic (omega-3)

linoleic (omega-6)

Essential for human food!



KNOW THE TRANS FAT

HOW IT DOES IN THE HUMAN BODY

Heart: trans fat is deposited in the coronary arteries.

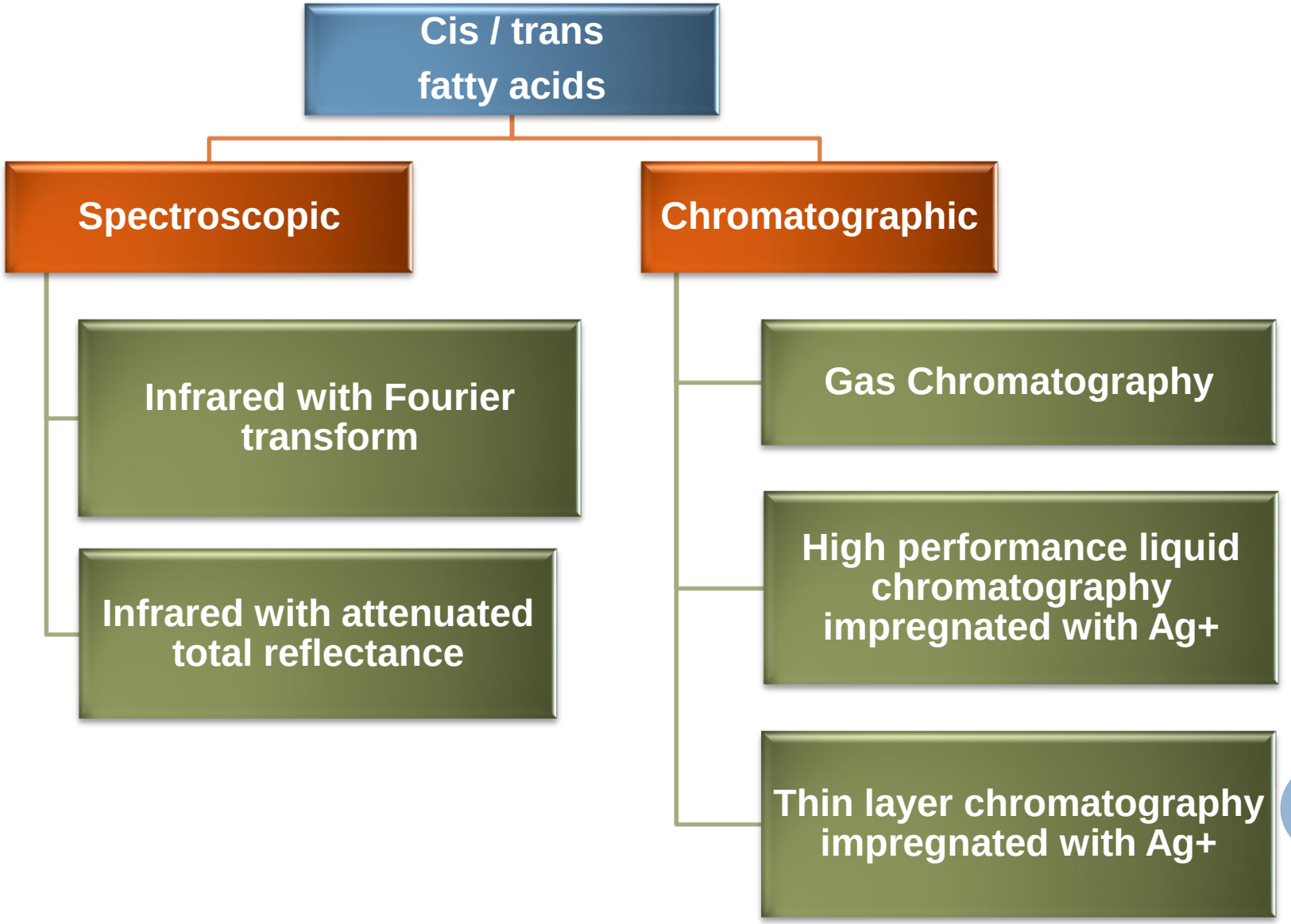
Blood: trans is involved in the formation of atherosclerotic plaques

Abdomen: trans fat accumulated in this region favoring metabolic syndrome

Intestine: trans fat is metabolized with greater difficulty

Liver: trans fat increases the levels of bad cholesterol (LDL) and decreases the level of good (HDL).

Classical analysis methods



ANALYSIS OF FATTY ACIDS BY CAPILLARY ELECTROPHORESIS

pH ≥ 5 (phosphate buffer, tetraborate)
Organic solvents (ACN, 1-octanol)
Selector of cis-trans isomers (Brij 35)

Indirect UV Detection

Direct UV detection

Chromophore agent (SDBS)

Unsaturated

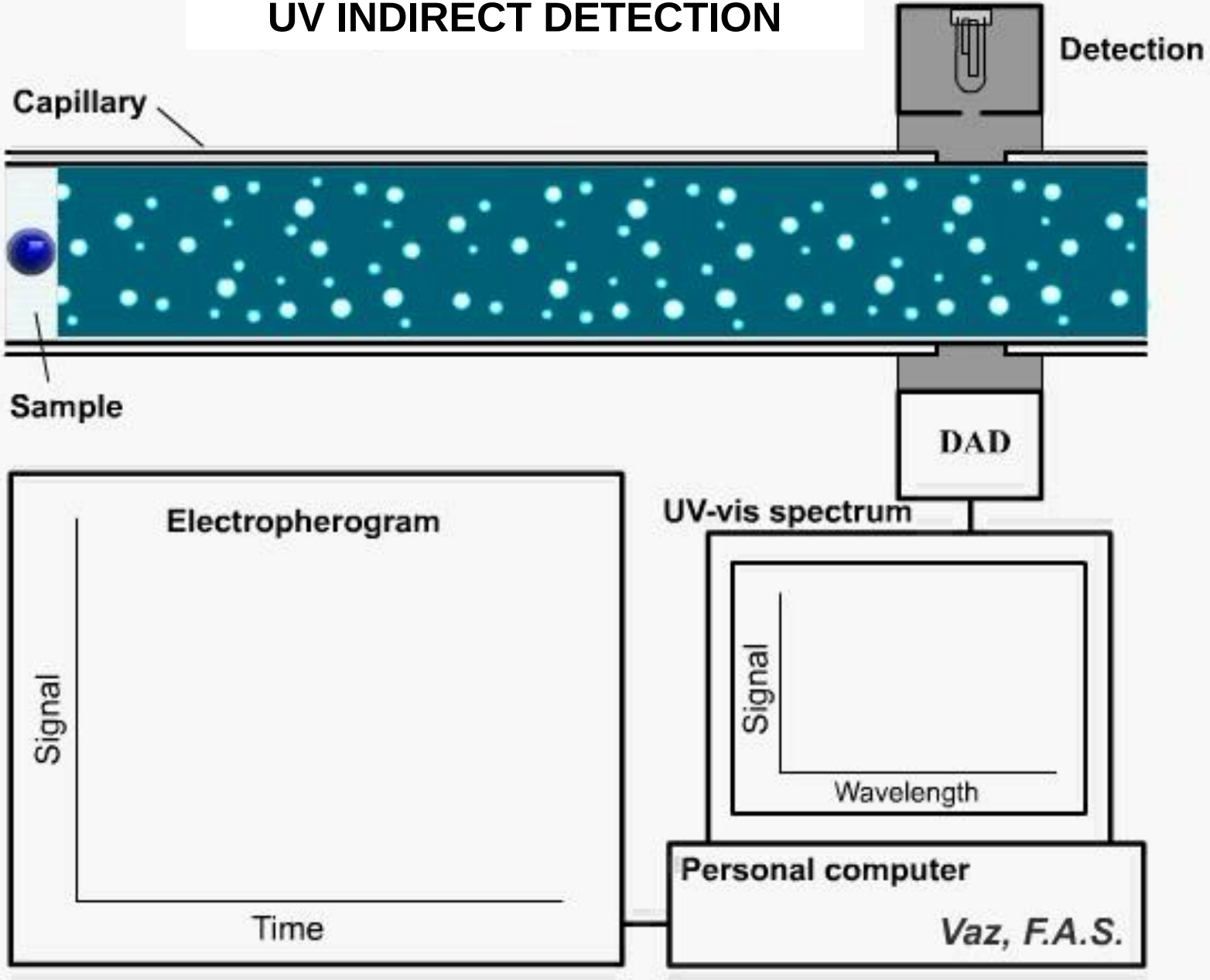
Saturated and unsaturated

Other possibilities:

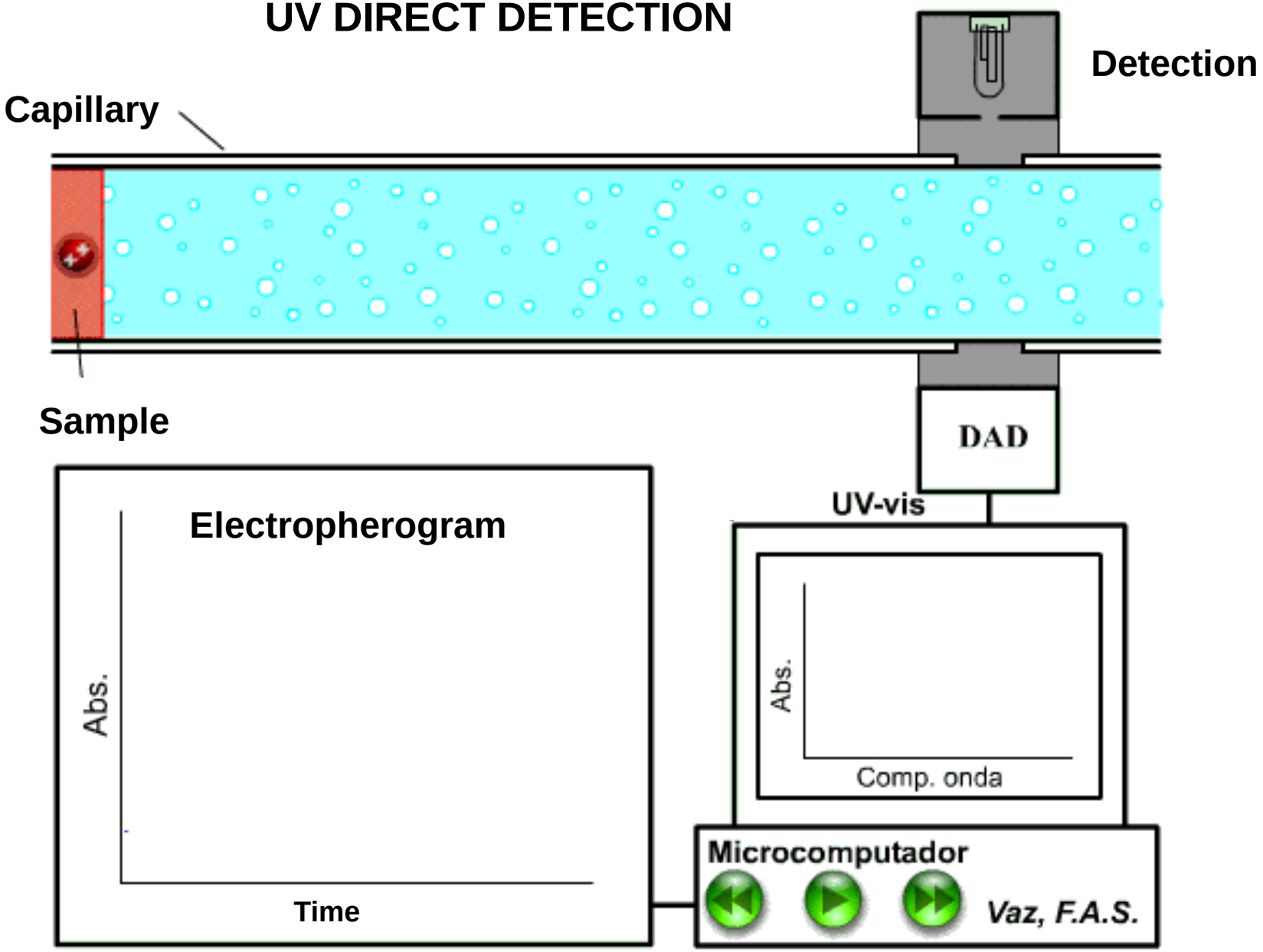
C^4D

Indirect Fluorescence

UV INDIRECT DETECTION



UV DIRECT DETECTION



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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.

2^3CCD WITH TRIPLICATE IN CENTRAL POINT

Issue	Brij	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

Factors and levels

X_1-Brij 35 (mmol L^{-1}):
(-1) 9,0; (0) 10,0; (1) 11,0; (-1,68) 8,3; (1,68) 11,7

X_2-1-octanol (% v/v):
(-1) 1,8; (0) 2,0; (1) 2,2; (-1,68) 1,7; (1,68) 2,3

X_3-ACN (% v/v):
(-1) 44,0; (0) 45,0; (1) 46,0; (-1,68) 43,3; (1,68) 46,7

Fixed parameters

injection 5 s x 12.5 mbar
voltage +19 Kv
temperature 25°C
λ= 224 nm (indirect detection)
15,0 mmol L^{-1} de NaH_2PO_4 / Na_2HPO_4
4,0 mmol L^{-1} de SDBS
Capillary TSH: 48.5 cm full lenght size (40 cm efective, 75 µm d.i e 375 mm d.e.

Table 2: 2^3CCD design results for robustness evaluation

	$R_{C18:1t/C18:1c}$			$R_{C16:0/C18:2cc}$		
Factor	**Coeff.**	**Error**	**p-value**	**Coeff.**	**Error**	**p-value**
Constant	1.080	0.075	0.005*	1.060	0.018	0.000*
X_1	-0.112	0.035	0.088	-0.017	0.009	0.195
X_2	0.001	0.035	0.986	0.014	0.009	0.257
X_3	0.018	0.035	0.652	-0.040	0.009	0.045*
$X_1 X_1$	0.054	0.039	0.298	0.011	0.010	0.382
$X_2 X_2$	-0.032	0.039	0.496	-0.035	0.010	0.066
$X_3 X_3$	-0.009	0.039	0.835	-0.012	0.010	0.326
$X_1 X_2$	0.030	0.046	0.584	0.027	0.011	0.137
$X_1 X_3$	-0.002	0.046	0.962	0.010	0.011	0.472
$X_2 X_3$	0.032	0.046	0.556	0.005	0.011	0.703

*significant effects within 95% confidence interval

Score and loading

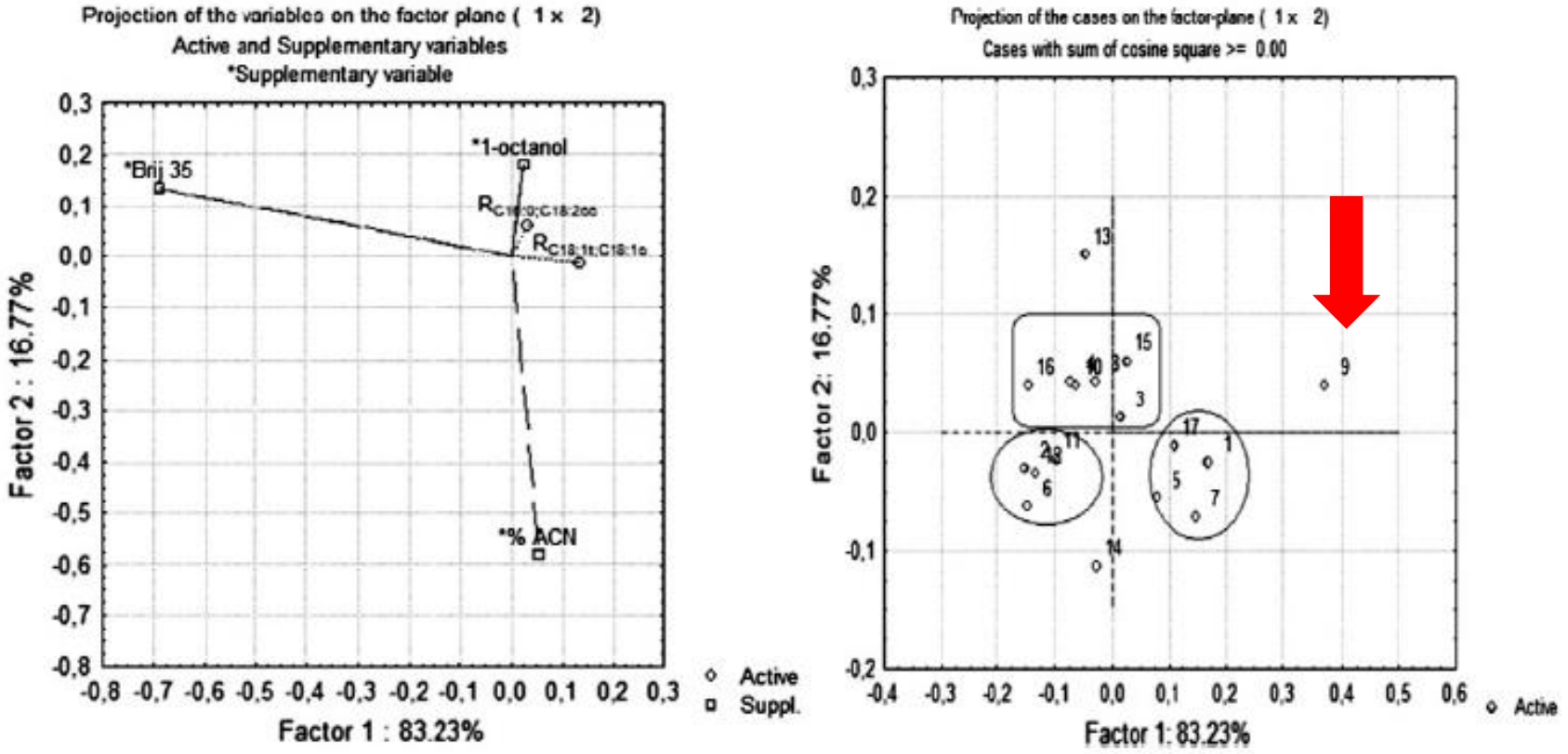
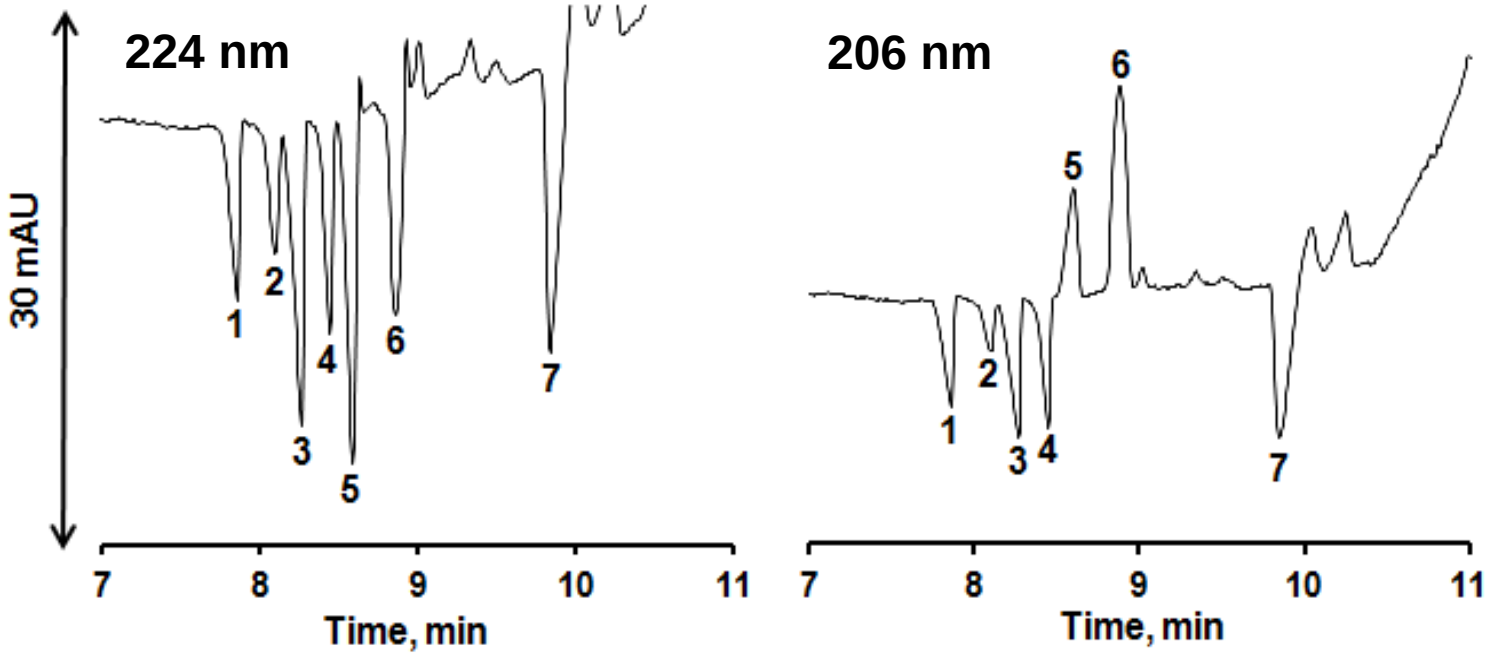


Fig. 2. PCA plots: scores and loading.

1-octanol variation from 2.0 to 2.2%



Optimized condition

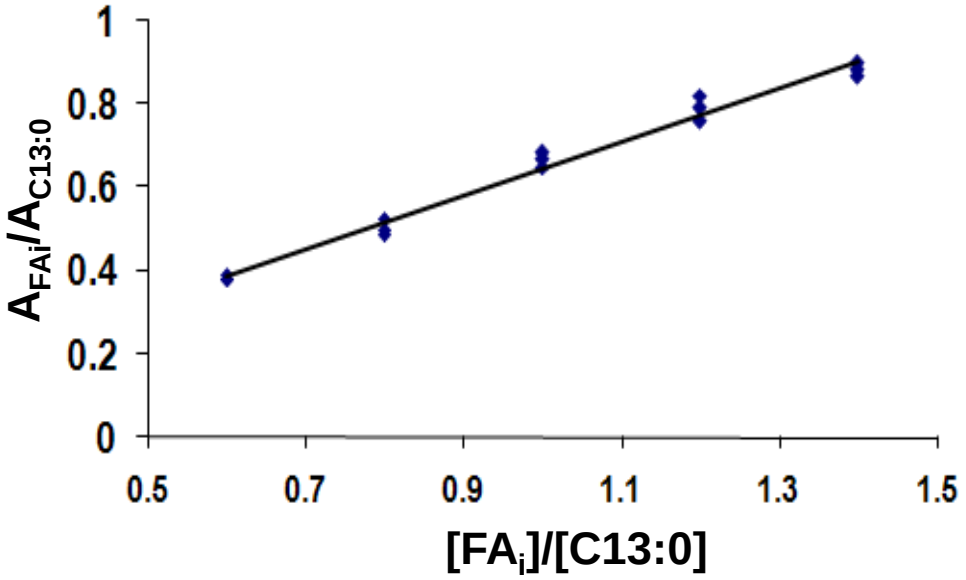
15,0 mmol L^{-1} of NaH_2PO_4 / $Na_2HPO_{4;}$(pH 6,86); 4,0 mmol L^{-1} of SDBS; 8,3 mmol L^{-1} of Brij 35; 45% v/v of acetonitrile , **2,1% de 1-octanol**

1- C18:0; 2- C18:1t; 3- C18:1c; 4- C16:0; 5- C18:2cc; 6- C18:3ccc and 7- C13:0 Standard mixture in fixed concentration of 0,5 mmol L^{-1}

Response factor calculation (R_F)

Analytical curve

- 0,15 mmol L^{-1} a 1,10 mmol L^{-1} for each internal standard of FA
- Internal standard (C13:0) in fixed concentration of 0,50 mmol L^{-1}



Fit of the model

Homocedaticity test ,
Normality and independency

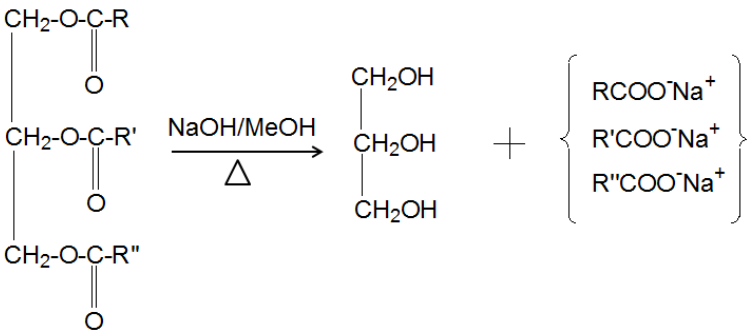
$$F_{calculado} = \frac{s^2_{y,x}}{s^2_y} = \frac{\sum_{i=1}^{p} m_i(\bar{y}_i - \hat{y}_i)^2/(p-2)}{\sum_{i=1}^{p}\sum_{j=h}^{m_i}(y_{ij} - \bar{y}_i)^2/(m-p)}$$

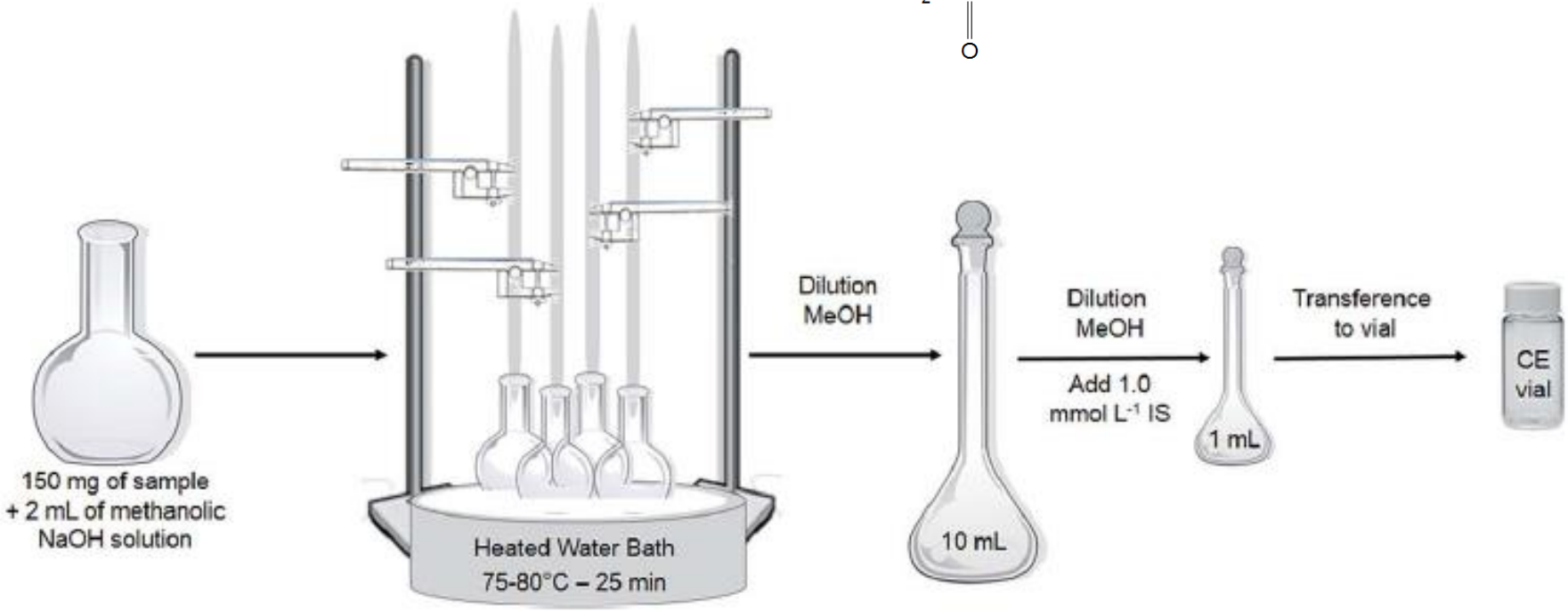
Response factor calculation

$$\frac{A_{FA_i}}{A_{C13:0}} = R_f \frac{[FA_i]}{[C13:0]}$$

Danzer & Currie. Guidelines for Calibration in Analytical Chemistry Part 1: Fundamentals and Single Component Calibration. *International Union of Pure and Applied Chemistry*, 70, 993-1014, 1998.

SAMPLE PREPARATION





$$\frac{A_{AG_i}}{A_{C13:0}} = F_R \frac{[AG_i]}{[C13:0]} \Rightarrow \%AGi = \frac{A_{AGi} \cdot [C13:0] \cdot V \cdot MM_{AGi}}{F_R \cdot A_{C13:0} \cdot m} \cdot 100$$

Statistical results: model fit and calculated R_F for each fatty acid of interest

Statistical results: lack of fit model and R_f calculated for each FA.

FA	F_{calc}	F_{tab}	Slope	Intercept	r
C18:0	0.39	5.41[c]	0.477(±0.021)	−0.018(±0.033)	0.999
C18:1t	2.89	3.84[b]	0.506(±0.018)	−0.103(±0.027)	0.992
C18:1c	2.54	3.84[b]	0.555(±0.021)	−0.046 (±0.021)	0.984
C16:0	2.36	3.84[b]	0.589(±0.022)	−0.028(±0.030)	0.960
C18:2cc	0.43	3.36[a]	0.626(±0.022)	−0.0736(±0.031)	0.999
C18:3ccc	3.04	3.84[b]	0.818(±0.032)	−0.002(±0.042)	0.991

υ_1:numerator freedom degree; υ_2: denominator freedom degree.

[a] $F_{tab}(\upsilon_1 = 4, \upsilon_2 = 11)$.

[b] $F_{tab}(\upsilon_1 = 4, \upsilon_2 = 8)$.

[c] $F_{tab}(\upsilon_1 = 3, \upsilon_2 = 5)$.

ELECTROPHEROGRAM FOR SAMPLES

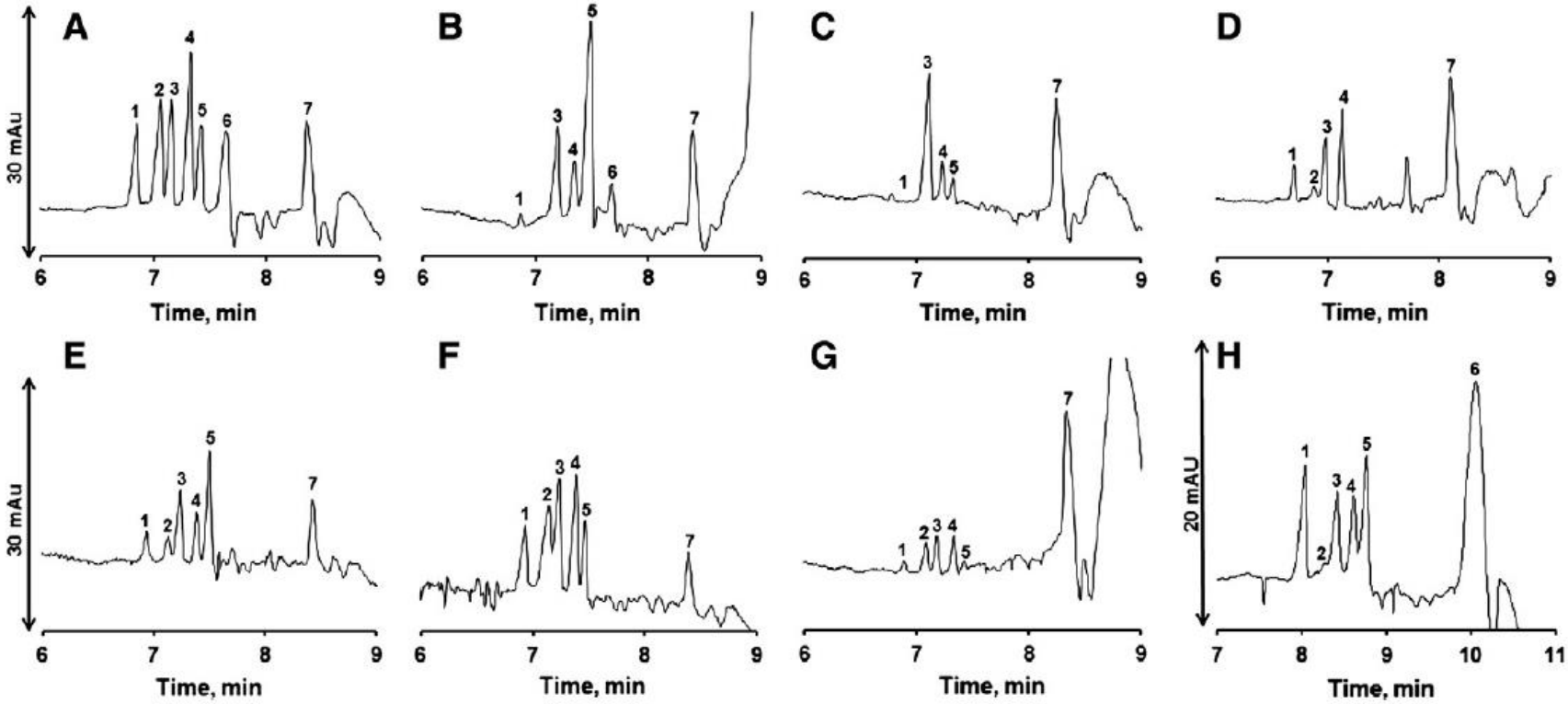


Fig. 4. Standard fatty acid electropherogram of (1) C18:0, (2) C18:1 9t, (3) C18:1 9c, (4) C16:0, (5) C18:2cc, (6) C18:3ccc, (7) C13:0 (PI), all with concentration of 0.50 mmol L^{-1}. B — soy oil, C — olive oil, D — butter, E — margarine, F — filled cookie, G — hydrogenated vegetable fat and H — bovine 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.

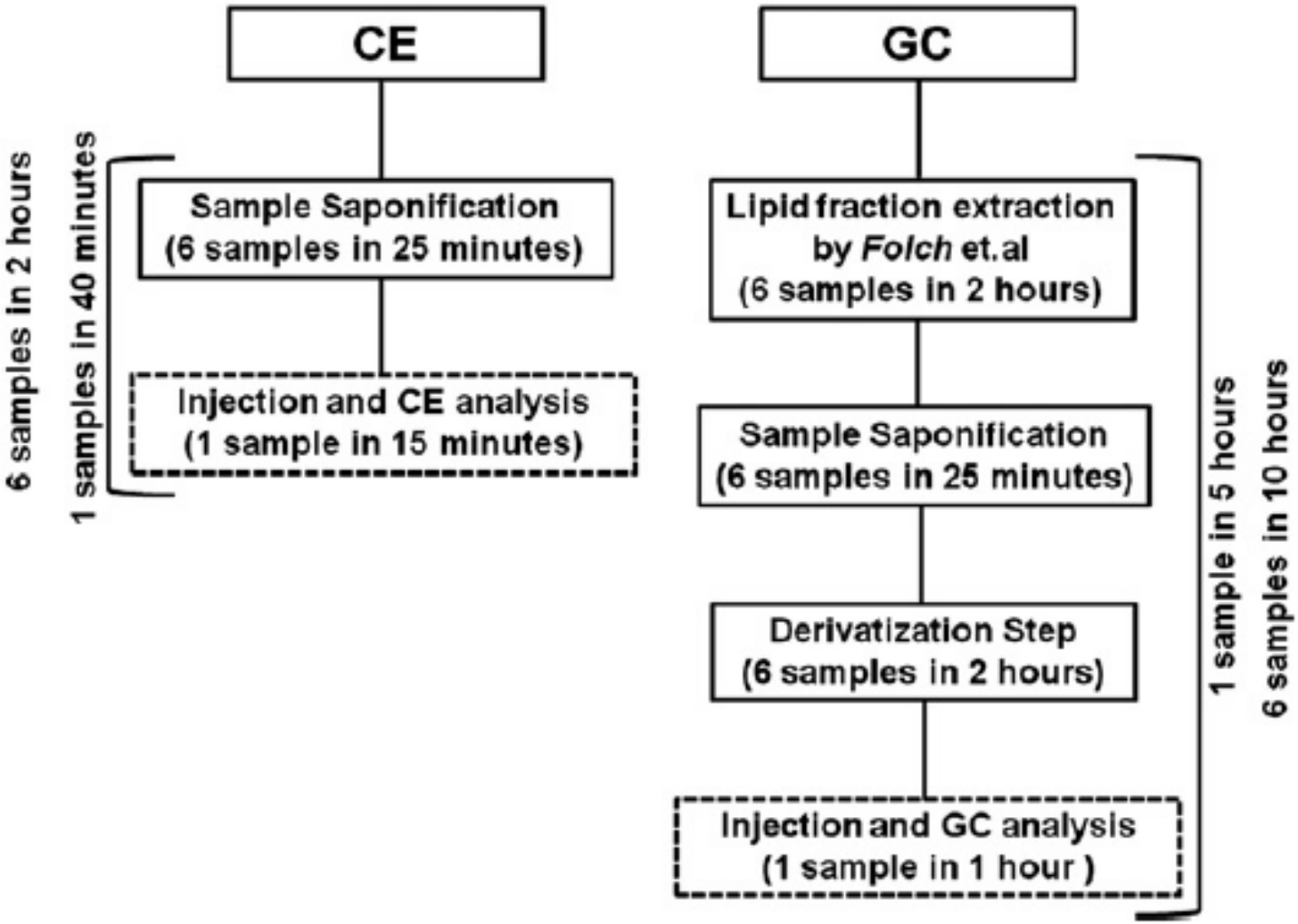


Fig. 6. Analytical throughput comparative scheme between CE and GC methods.

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.

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Article

Study of Fatty Acids Profile in Biological Sample by Capillary Zone Electrophoresis Associate to Chemometric Approach

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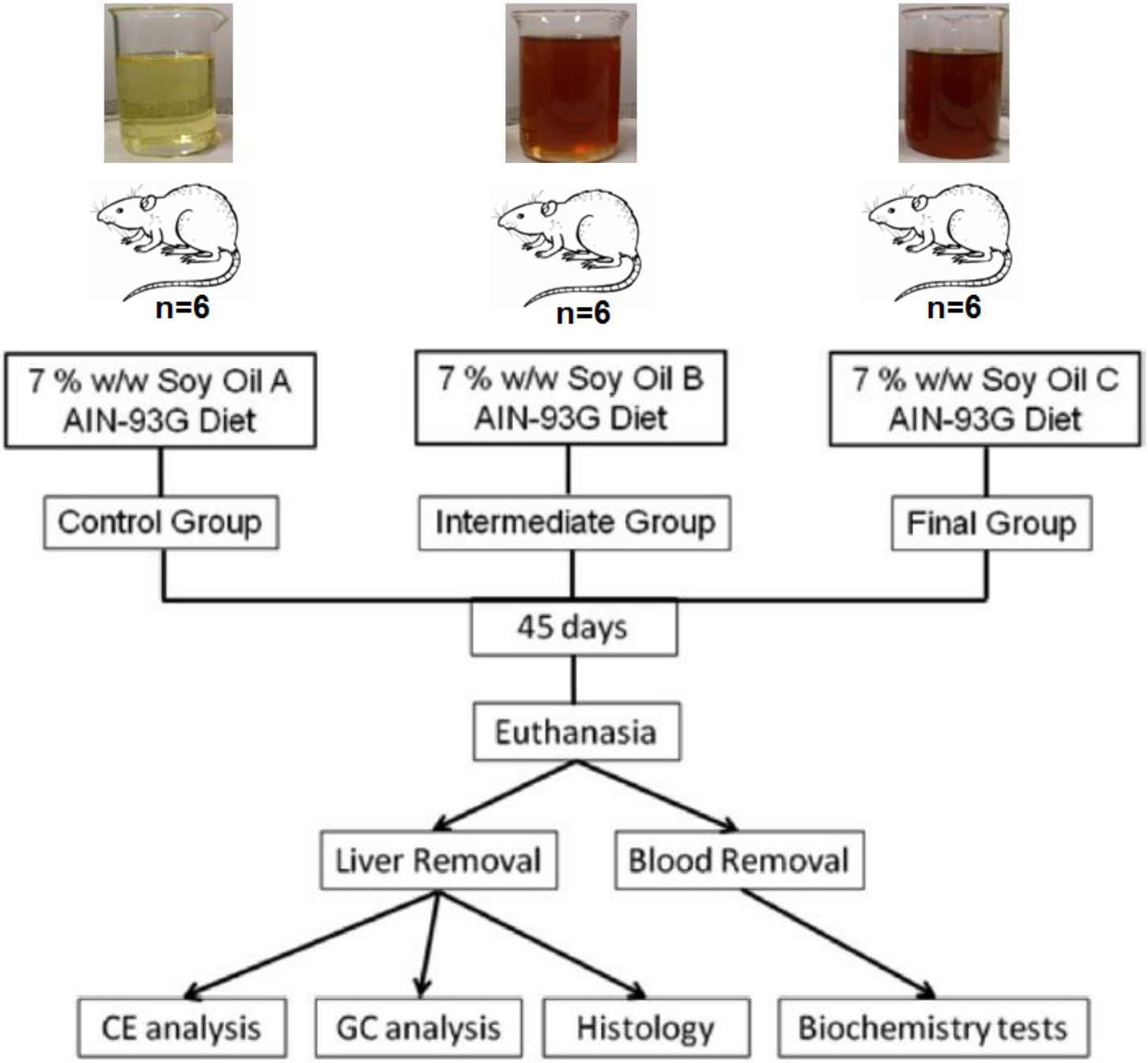
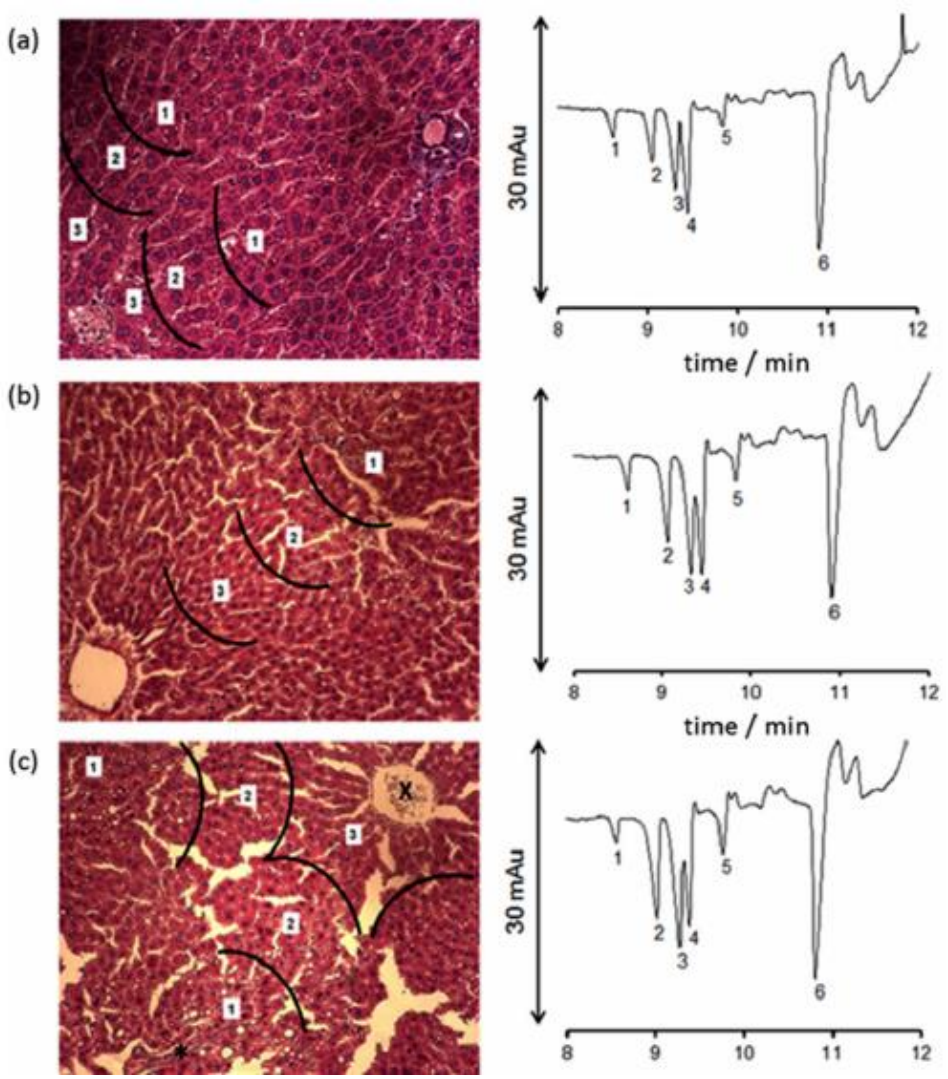
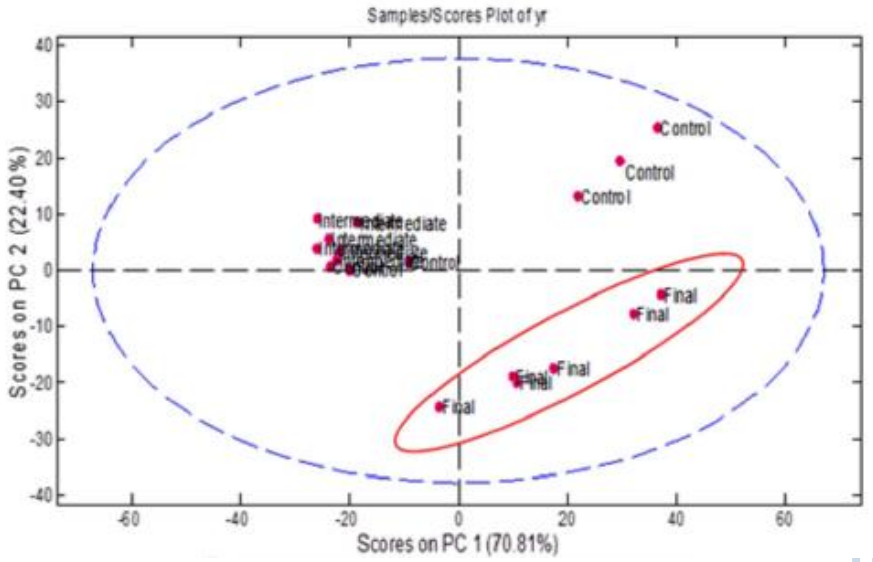
Foi proposta a determinação de ácidos graxos (FA) no fígado de ratos Wistar de três grupos diferentes (seis ratos por grupo) submetidos à dieta (AIN-93G) utilizando a técnica de eletroforese capilar de zona (CZE). Cada grupo recebeu a mesma dieta, sendo o óleo de soja a fração lipídica da dieta (7% m/m). O primeiro grupo foi alimentado com ração contendo óleo de soja fresco, o segundo foi alimentado com a dieta cuja fração lipídica foi óleo de soja utilizado durante 7 dias em processo de fritura por imersão e, finalmente, o terceiro grupo foi alimentado com dieta cuja fração lipídica foi óleo de soja utilizado durante 15 dias em processo de fritura. Após 45 dias consumindo essas dietas, os ratos foram submetidos à eutanásia e o teor de FA no fígado foi monitorado por


n=6
n=6
n=6
7 % w/w Soy Oil A AIN-93G Diet
7 % w/w Soy Oil B AIN-93G Diet
7 % w/w Soy Oil C AIN-93G Diet
Control Group
Intermediate Group
Final Group
45 days
Euthanasia
Liver Removal
Blood Removal
CE analysis
GC analysis
Histology
Biochemistry tests





- Modulate the inflammatory response and immune response.
- They play an important role in platelet aggregation, growth and celular differentiation.

	Animals	C18:0 / (g 100g^{-1})		C18:1 9c / (g 100g^{-1})		C16:0 / (g 100g^{-1})		C18:2 cc / (g 100g^{-1})		C16:1c / (g 100g^{-1})	
		CE	GC	CE	GC	CE	GC	CE	GC	CE	GC
Control group	1	0.77	0.76	1.96	1.95	2.51	2.40	1.56	1.31	0.35	0.32
	2	0.60	0.62	1.68	1.76	1.91	1.85	1.78	1.25	0.27	0.25
	3	0.61	0.64	1.59	1.70	2.19	2.12	1.36	1.11	0.37	0.39
	4	0.54	0.55	1.39	1.52	1.67	1.66	1.25	1.17	0.26	0.25
	5	0.81	0.82	0.85	0.83	1.41	1.24	1.48	1.11	0.16	0.12
	6	0.62	0.65	1.10	1.31	1.59	1.57	1.66	1.37	0.21	0.24
Mean		0.66	0.67	1.43	1.51	1.88	1.81	1.52	1.22	0.27	0.26
Standard deviation		0.11	0.10	0.40	0.40	0.41	0.41	0.20	0.11	0.08	0.09
t-test p-value		0.02[a]		0.05		0.08		0.02[a]		0.72	
Intermediate group	7	0.60	0.61	1.61	1.95	2.02	2.07	1.20	1.20	0.36	0.38
	8	0.65	0.69	1.88	1.95	2.20	2.15	1.24	1.11	0.41	0.40
	9	0.55	0.51	1.28	1.40	1.58	1.51	1.39	1.20	0.22	0.24
	10	0.55	0.52	0.91	0.95	1.17	1.09	1.32	0.87	0.17	0.16
	11	0.54	0.58	0.78	0.75	1.14	0.99	1.03	0.81	0.13	0.10
	12	0.64	0.69	1.17	1.21	1.56	1.51	1.35	1.15	0.24	0.20
Mean		0.59	0.60	1.27	1.37	1.61	1.55	1.25	1.06	0.26	0.25
Standard deviation		0.05	0.08	0.42	0.50	0.43	0.48	0.13	0.17	0.11	0.12
t-test p-value		0.52		0.12		0.02[a]		0.01[a]		0.15	
Final group	13	0.66	0.75	2.10	2.04	2.39	2.20	1.21	0.95	0.42	0.40
	14	0.60	0.69	1.11	1.47	1.30	1.56	1.04	0.95	0.25	0.29
	15	0.61	0.65	1.93	1.98	2.47	2.20	1.22	1.02	0.42	0.41
	16	0.65	0.62	1.23	1.46	1.43	1.44	1.23	1.13	0.21	0.24
	17	0.52	0.55	1.46	1.47	1.65	1.54	0.98	0.84	0.31	0.28
	18	0.47	0.55	1.23	1.25	1.37	1.39	1.16	0.86	0.23	0.20
Mean		0.58	0.64	1.51	1.61	1.77	1.72	1.14	0.96	0.31	0.30
Standard deviation		0.08	0.08	0.41	0.32	0.53	0.38	0.10	0.11	0.10	0.09
t-test p-value		0.11		0.13		0.85		0.01[a]		0.95	
Normality p-value		0.43		0.05		0.22		0.64		0.04[a]	
[b]LOD (mmol L^{-1})		0.0029		0.0033		0.0032		0.0017		0.0021	
[b]LOQ (mmol L^{-1})		0.0089		0.0100		0.0096		0.0052		0.0062	
[b]Linear range (mmol L^{-1})		0.15-1.10		0.15-1.10		0.15-1.10		0.15-1.10		0.05-1.05	

[a]p-value > 0.01 (Interval 99% of confidence); [b]values of CE method; LOD: limit of detection and LOQ: limit of quantification, normality Shapiro-Wilk test.

ANALYTICAL SCIENCES MAY 2011, VOL. 27

Analysis of Omega 3 Fatty Acid in Natural and Enriched Chicken Eggs by Capillary Zone Electrophoresis

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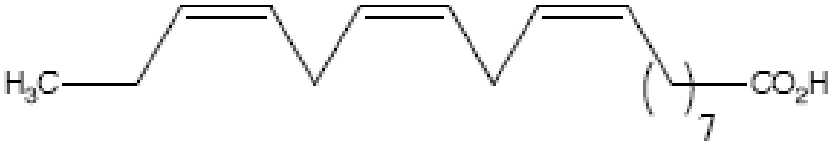
Qualitative differentiation between natural and enriched chicken eggs through omega (ω) 3 fatty acid profiles by capillary zone electrophoresis (CZE) under direct UV detection at 200 nm is proposed. The electrolyte background consisted of 12.0 mmol L^{-1} tetraborate buffer (pH 9.2) mixed with 12.0 mmol L^{-1} Brij 35, 17% acetonitrile (ACN) and 33% methanol (MeOH). Omega 3 fatty acid profile in chicken egg samples were analyzed by CZE system and confirmed by single-quadrupole mass spectrometry with an electrospray ionization probe set to negative ionization mode after sample preparation by the Folch method. The results showed that ω fatty acid profiles analyzed by the CZE approach can be used to chemical markers to monitor fraud, presenting simplicity, short analysis time (10 min) and low cost as advantages.

(Received April 17, 2010; Accepted March 3, 2011; Published May 10, 2011)

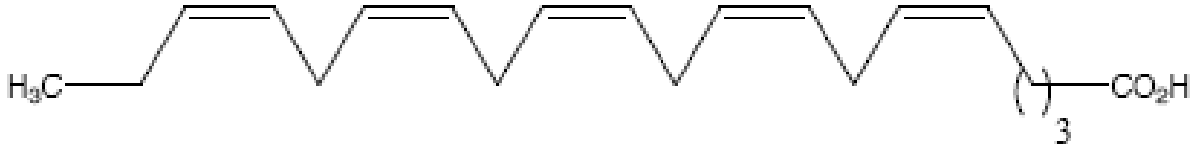
How to differentiate enriched eggs from natural ones?

Oleic acid $M_W = 282.46$

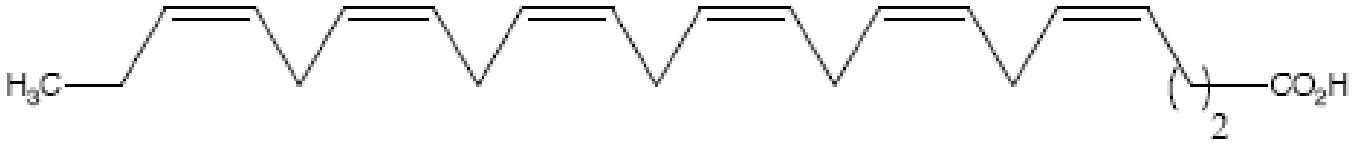
Linoleic acid $M_W = 280.241$



Linolenic acid $M_W = 278.22$



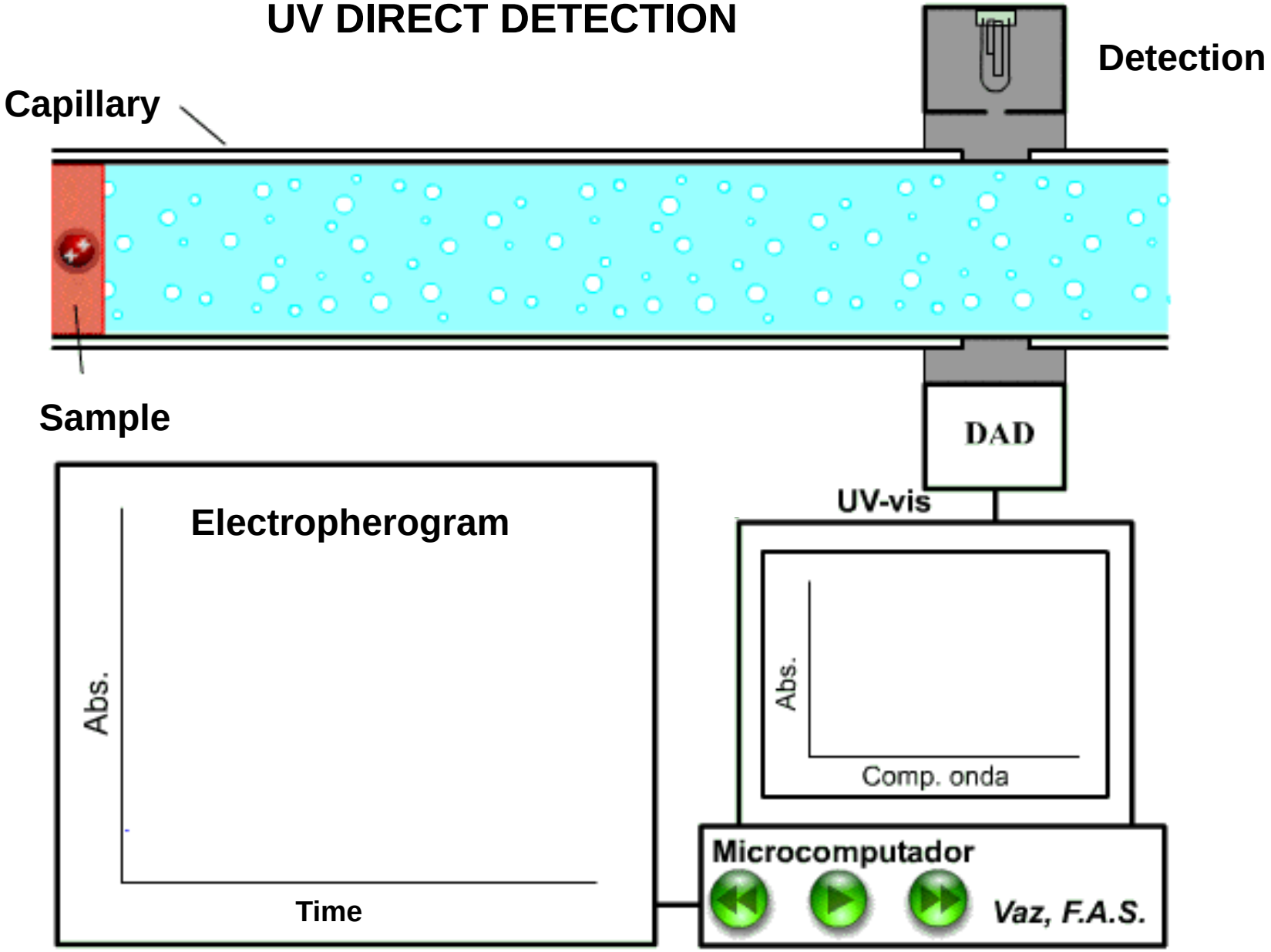
Eicosapentaenoic acid $M_W = 302.22$



Docosahexaenoic acid $M_W = 328.24$

Fig. 1 Omega fatty acids chemical structures.

UV DIRECT DETECTION



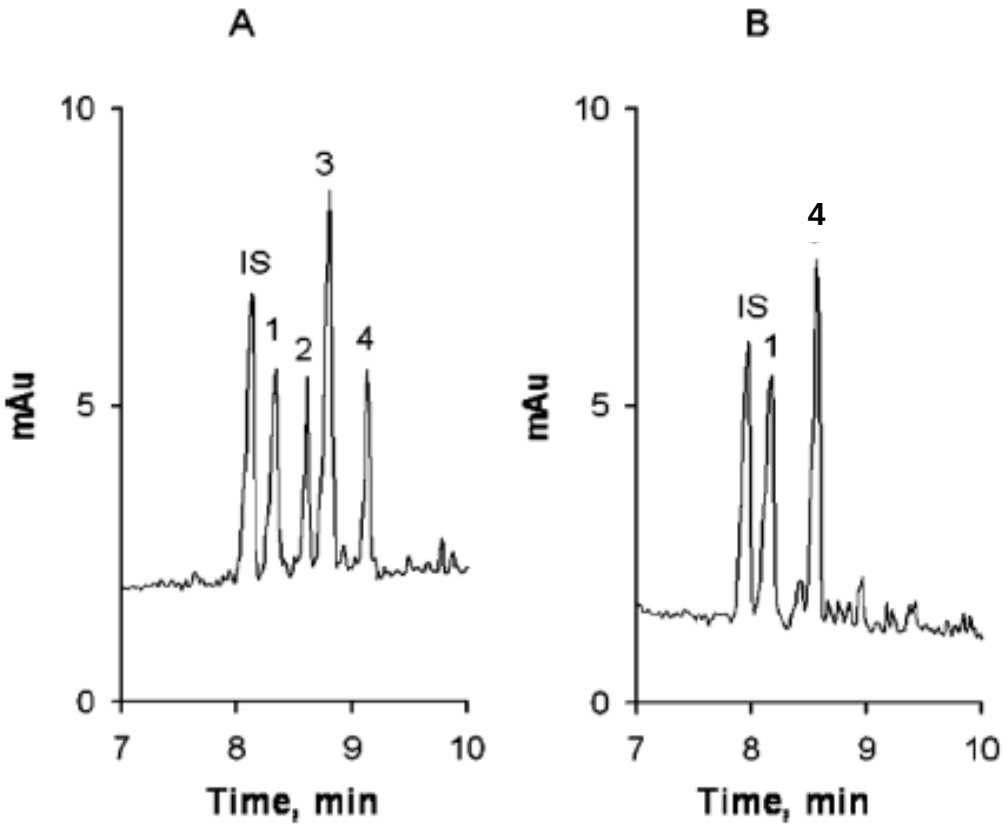


Fig. 4 Electropherograms obtained for (A) enriched and (B) natural egg samples. IS, C18:1t; 1, C18:1c; 2, C18:2cc; 3, C22:6cccccc; 4, C18:3ccc. BGE: 12 mmol L^{-1} of tetraborate buffer pH 9.2 combined with 12.0 mmol L^{-1} of Brij 35, 17% ACN and 33% MeOH. Operational conditions: injection, 25 mBar, 5.0 s; voltage, +27 kV; tenperature, 27°C; $\lambda = 200$ nm. Capillary dimensions: 48.5 cm total (40 cm effective length) × 75 µm i.d. × 375 µm o.d.

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Analytical Methods

Capillary zone electrophoresis for fatty acids with chemometrics for the determination of milk adulteration by whey addition

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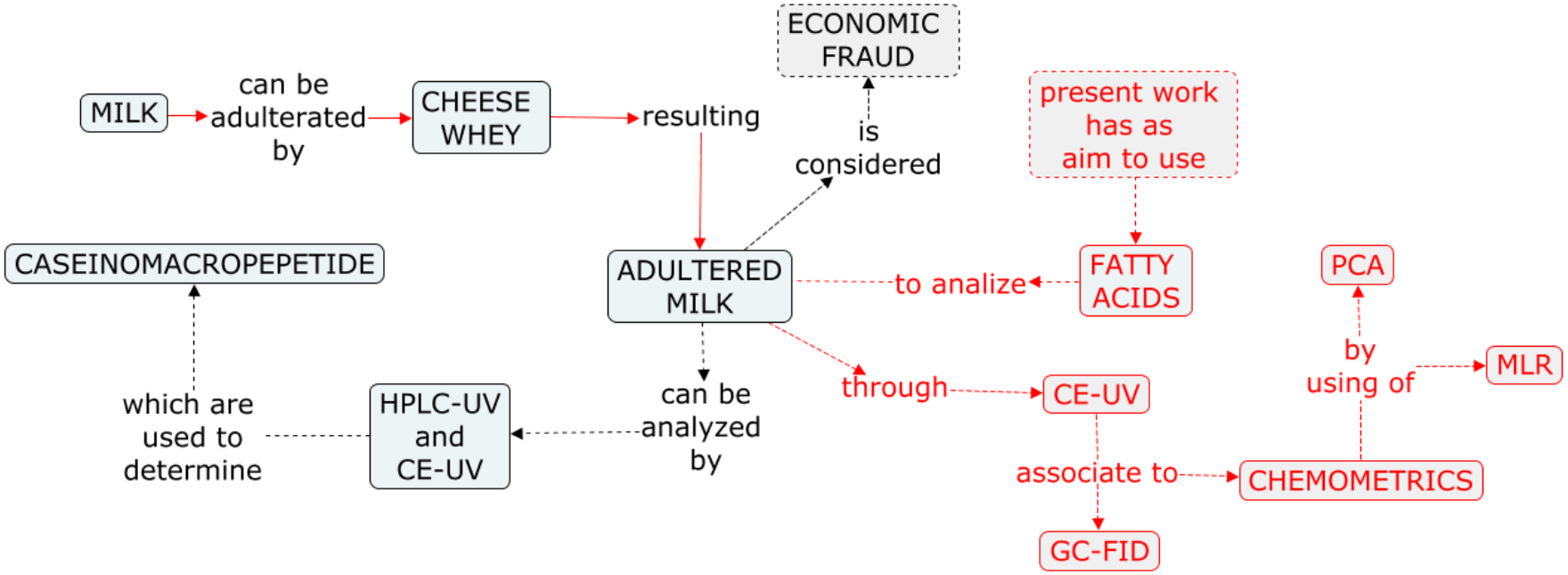
ABSTRACT

Adulteration of milk with whey is difficult to detect because these two have similar physical and chemical characteristics. The traditional methodologies to monitor this fraud are based on the analysis of caseinomacropeptide. The present study proposes a new approach to detect and quantify this fraud using the fatty acid profiles of milk and whey. Fatty acids C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 were selected by gas chromatography associated with discriminant analysis to differentiate milk and whey, as they are present in quite different amounts. These six fatty acids were quantified within a short time by capillary zone electrophoresis in a set of adulterated milk samples. The correlation coefficient between the true values of whey addition and the experimental values obtained by this technique was 0.973. The technique is thus useful for the evaluation of milk adulteration with whey, contributing to the quality control of milk in the dairy industry.

BACKGROUND AND OBJECTIVES



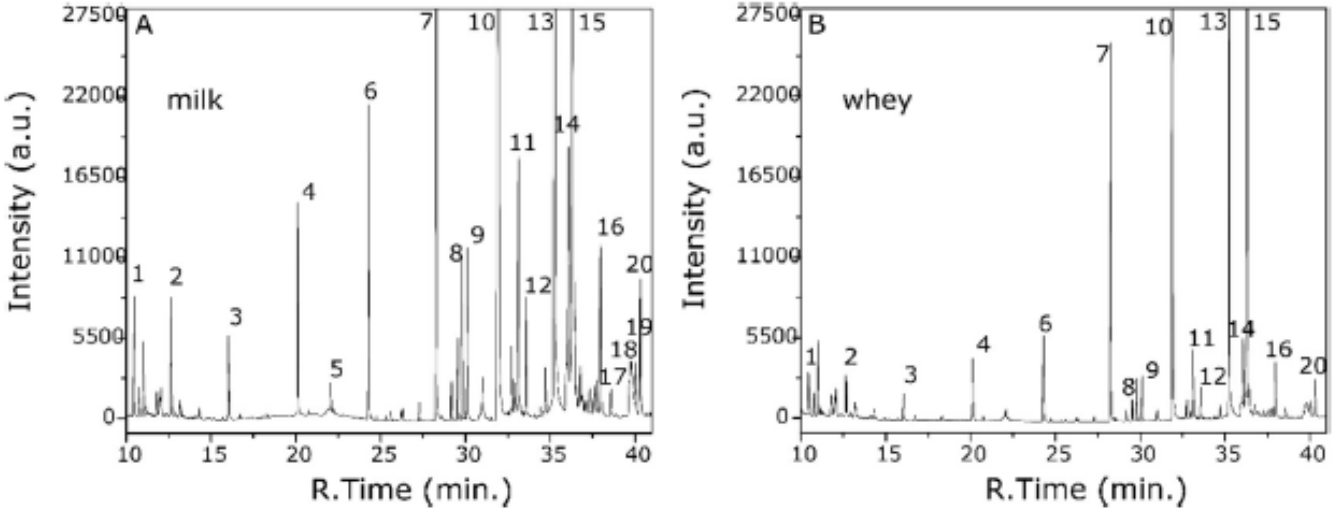


Fig. 1. GC-FID analysis to evaluate FA profile. (A) milk and (B) whey. Various FA peaks were identified in both milk and whey (Figure 1) by comparing with FAME 37 standard mixture: 1 – C4:0; 2 – C6:0; 3 – C8:0; 4 – C10:0; 5 – C11:0; 6 – C12:0; 7 – C14:0; 8 – C14:1; 9 – C15:0; 10 – C16:0; 11 – C16:1; 12 – C17:0; 13 – C18:0; 14 – C18:1t; 15 – C18:1c; 16 – C18:2; 17 – C20:0; 18 – C18:3γccc; 19 – C20:1; 20 – C18:3αccc.

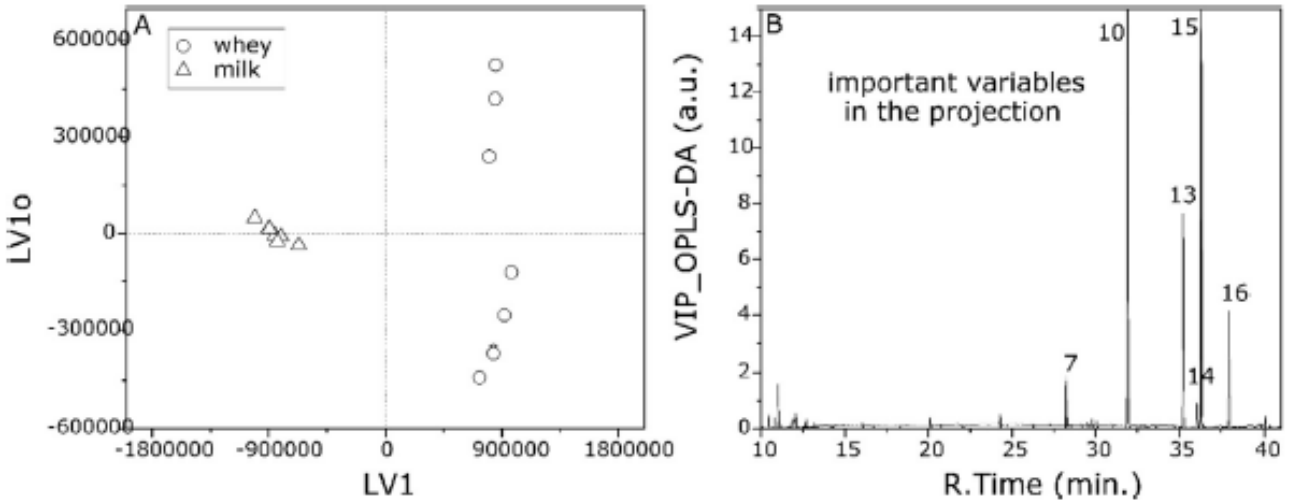
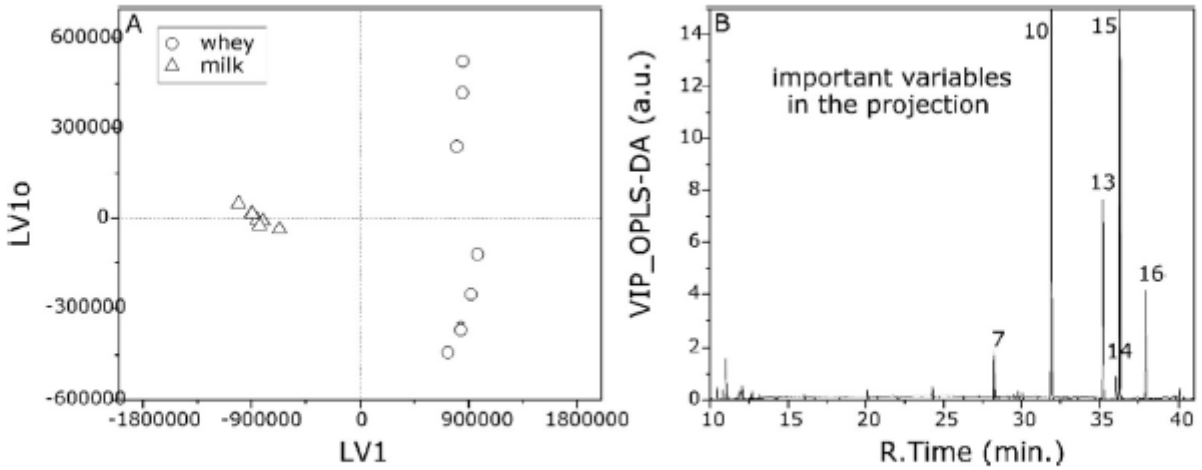


Fig. 2. Multivariate analysis associated to GC-FID results. In (A) is shown the score plot of OPLS-DA model and (B) is the most importance variables to explain the separation between milk and whey samples. Peaks identified by comparison with FAME 37 standards are: 7 – C14:0; 10 – C16:0; 13 – C18:0; 14 – C18:1t; 15 – C18:1c; 16 – C18:2.



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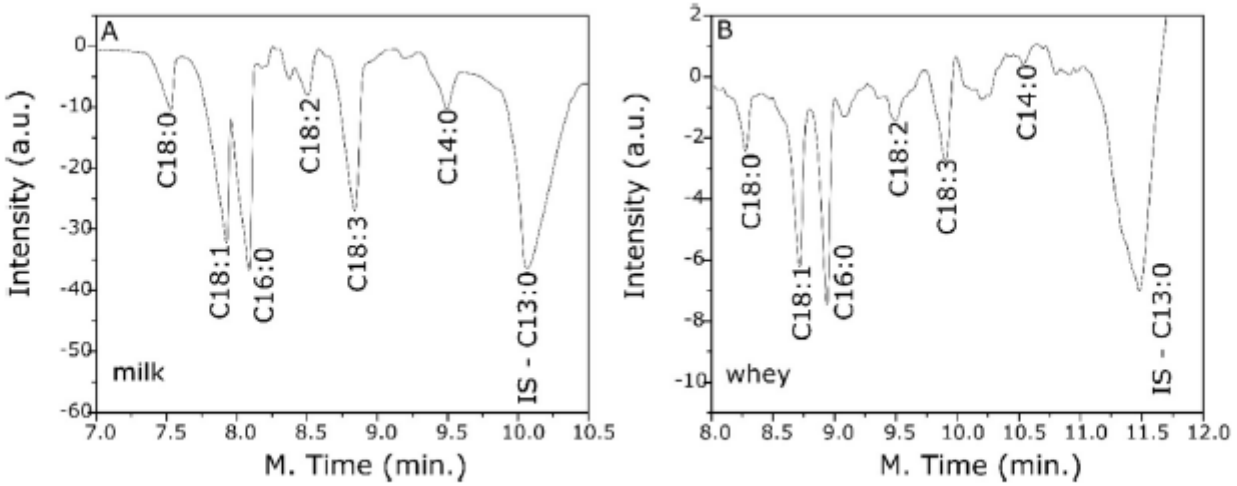


Fig. 3. FA profile analyzed by CZE-UV with indirect detection at 224 nm. Milk (A) and whey (B).

$$\%_{\text{whey addition}} = 26.9 + \frac{1}{A_{IS}}(126.7A_{C18:0} + 18.5A_{C18:1} - 46.9A_{C16:0} - 178.6A_{C18:2} + 29.2A_{C18:3} - 54.2A_{C14:0})$$

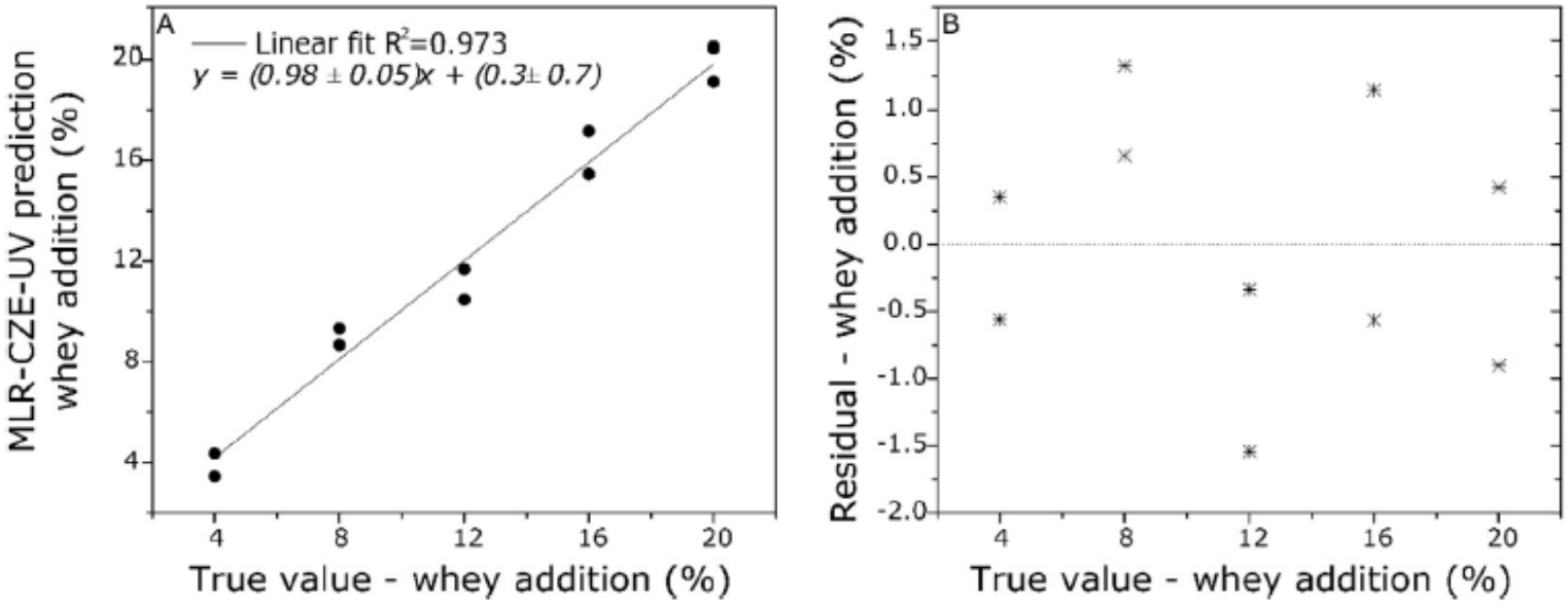


Fig. 5. Correlation graph for whey percentage prediction added to fluid raw milk between the values obtained by MLR and true values.

“Screening method for detection of free trans fatty acid in biodiesel by capillary electrophoresis "

MAIN MOTIVATIONS

- *The acid content in biodiesel is due to the FFA content, which is formed through the hydrolysis of the esters, both during production and in the storage of the product*

- *A high acid content may indicate that production has not been performed properly and is an indicator of the presence of water in the fuel, which can cause corrosion, engine deposits and nozzle nozzles*

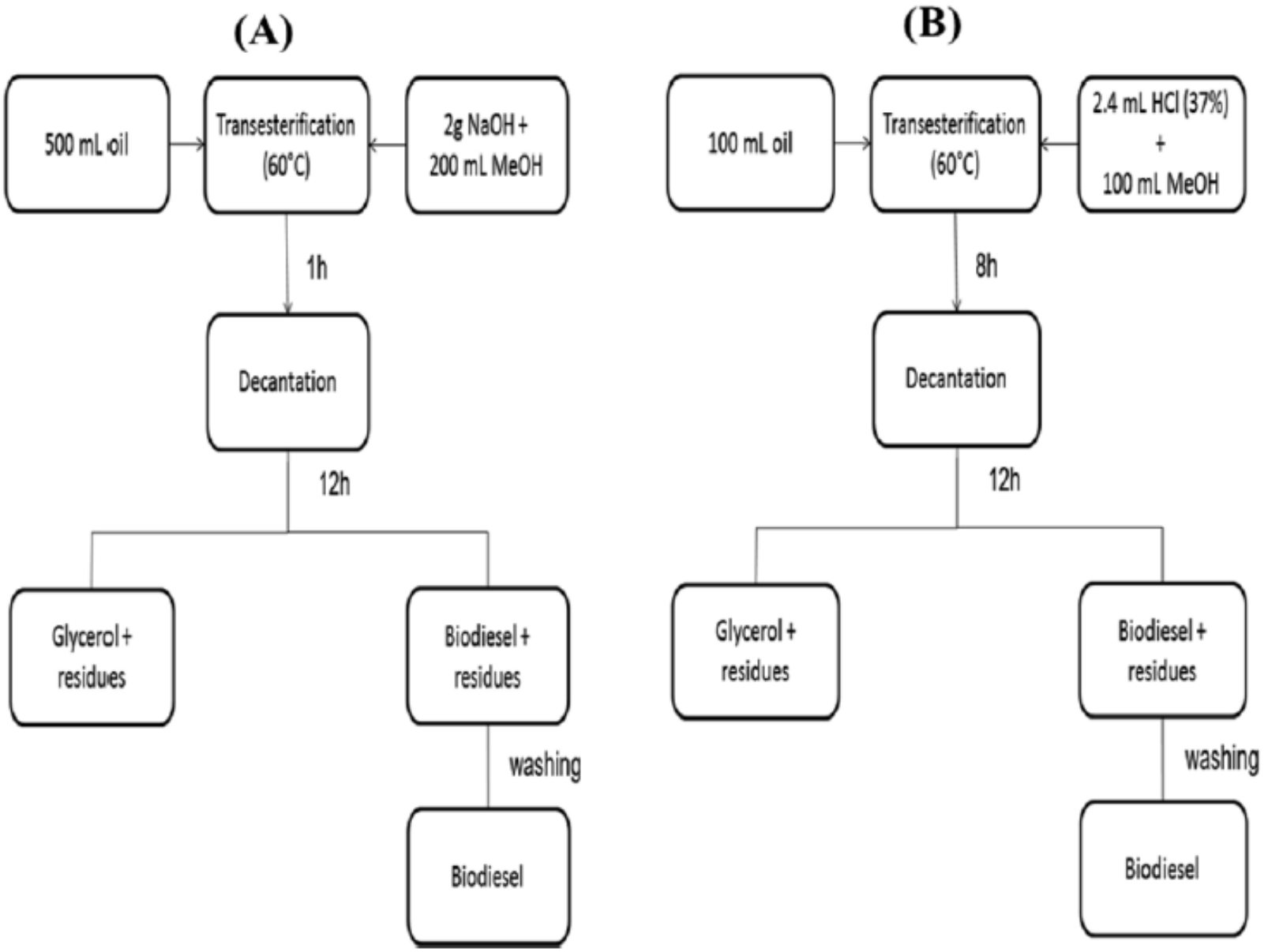
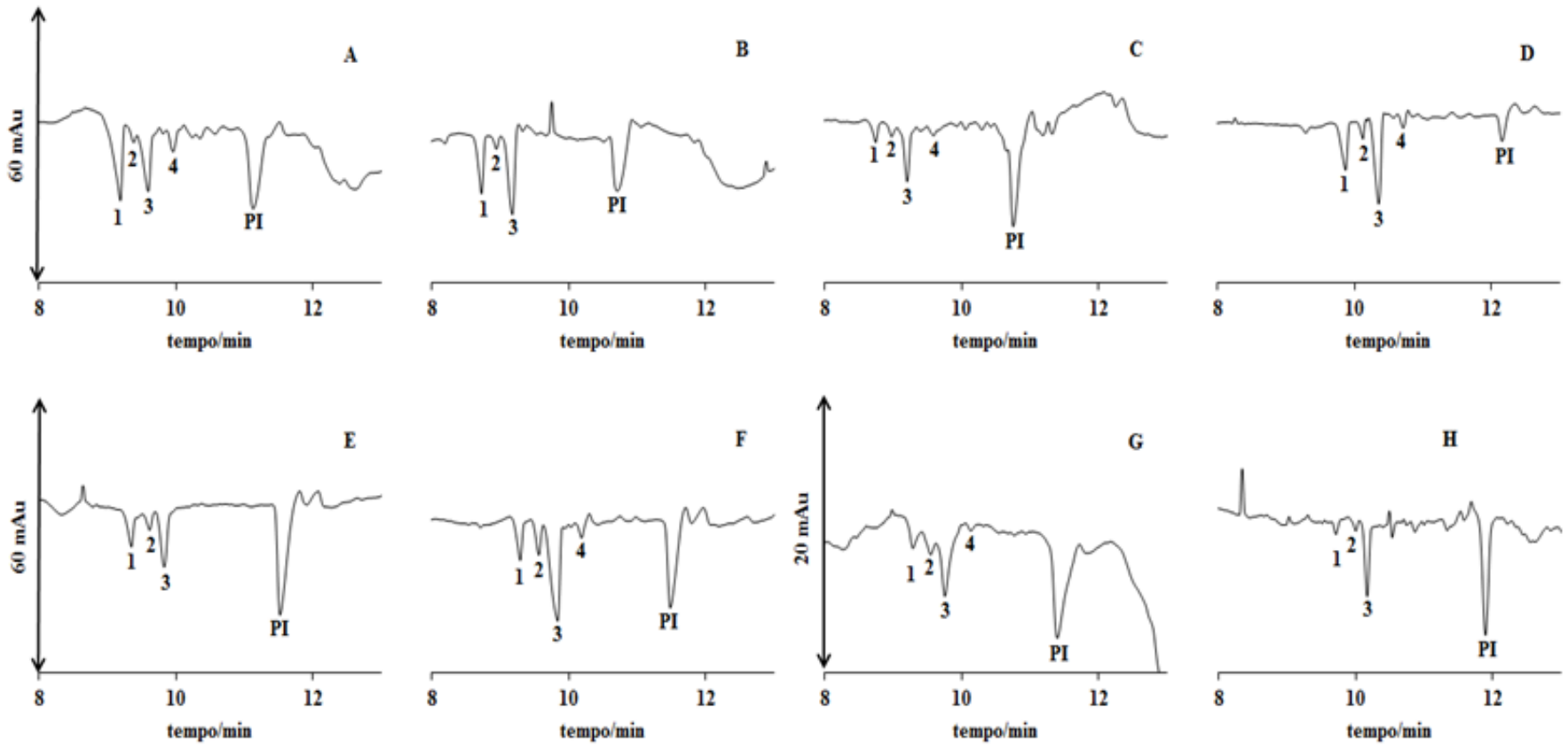


Figure 2. *Biodiesel preparation. (A) by basic catalysis and (B) by acidic*

Biodiesel samples analized



A- canola biodiesel; B-sunflower biodiesel; C-biodiesel from frying oil, D-biodiesel obtained via acid catalysis, E-biodiesel from soybean-SC, F- biodiesel from soybean-GO, G- biodiesel from soybean-SP, H-biodiesel industrial. Peaks: (1) C16:0, (2) C18:1, (3) C18:2, (4) C18:3, (PI) C13:0

Analytical Methods

PAPER

Method optimization for trans fatty acid determination by CZE-UV under direct detection with a simple sample preparation

T. L. A. Prado,[a] B. L. S. Porto[b] and M. A. L. Oliveira*[a]

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www.rsc.org/methods

An alternative method for trans fatty acid (TFA) determination, expressed in elaidic acid, by capillary zone electrophoresis using UV-VIS direct detection (200 nm) with a simple sample preparation is proposed. The method optimization was through the 3^2 full factorial design with triplicate in the central point and the sample preparation comprised only a saponification step. The background electrolyte was composed of 12 mmol L^{-1} of tetraborate buffer at pH 9, 12 mmol L^{-1} of Brij 35, 33% methanol and 17% acetonitrile. A baseline separation of elaidic (C18:1t-9), oleic (C18:1c), linoleic (C18:2cc) and linolenic (C18:3ccc) acids within an analysis time of 4 minutes was achieved, taking into account the statistical approach based on response factor calculation using nonadecanoic acid (C19:1c) as the internal standard (IS). The method potentiality was successfully demonstrated in the determination of TFAs in cake mix, wafer stick, coconut donut and guava paste biscuit samples and presented no significant difference when compared to the gas chromatography classical method within the 95% confidence interval. In addition, the method offered inherent advantages such as no lipid extraction step, higher throughput, lower analytical cost, no need for specific columns and use of small amounts of organic solvents and reagents.

1. Introduction

Significant changes in the societal diet and lifestyle occurred in the last few years and the most worrisome ones were the lessening of physical activity practice and consumption of healthy foods. These issues are among the main determinants of mortality and there is strong scientific evidence that an unhealthy diet and insufficient physical activity are the major risk factors to develop coronary heart disease, cerebrovascular stroke, several forms of cancer, type 2 diabetes, hypertension

polyunsaturated vegetable oils, which are vulnerable to oxidation. The obtained hydrogenated vegetable oils (HVOs) are more stable and consequently, the use of these fats increases products' shelf life and gives them a better texture.[8] Although TFAs from meat, milk and dairy products, reported as vaccenic acid, have been part of the diet for a long time, higher TFA consumption has been observed as a consequence of HVO introduction in dietary habits. Foods containing HVO or fried in them must have EA in some extent and the literature reports that EA is responsible for 80–100% of TFAs in samples.[9,10]

Analytical Methods

CRITICAL REVIEW

Cite this: *Anal. Methods*, 2017, **9**, 2483

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.

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DOI: 10.1039/c7ay00193b

rsc.li/methods

Partial conclusions

- The R_F calculation method positively points to the quantification of FA
- The CZE-UV method in comparison with the official GC-FID one presented no significant differences within 95% confidence interval for the analyzed samples
- In general way, the proposed methods by CE are simple, fast, presents low consumption of reagents and signals positively for the determination of FA in food or biodiesel samples

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Microchemical Journal

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

Optimization of photo-polymerized sol–gel monolithic stationary phases prepared in polyacrylate-coated fused-silica capillaries for capillary electrochromatography

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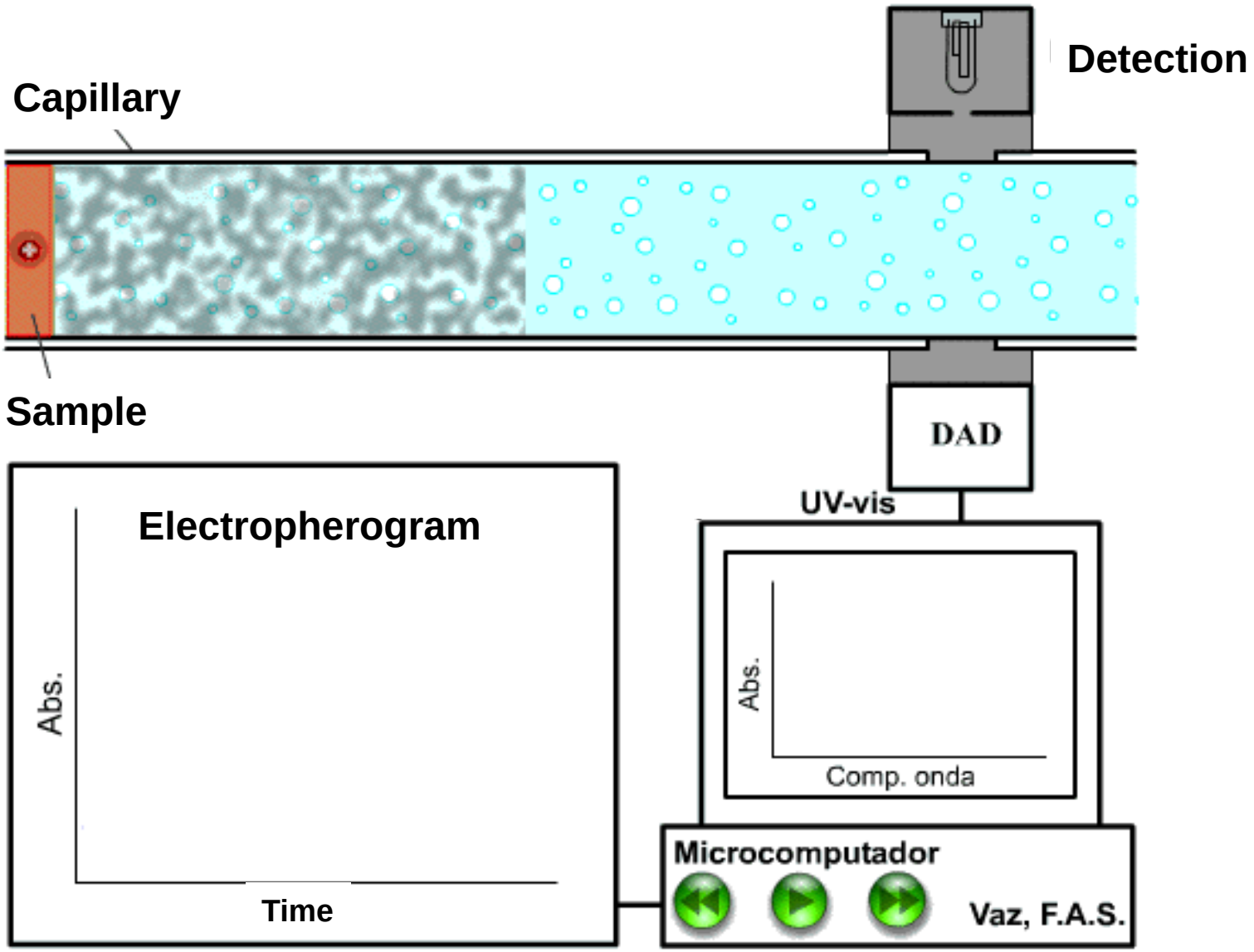
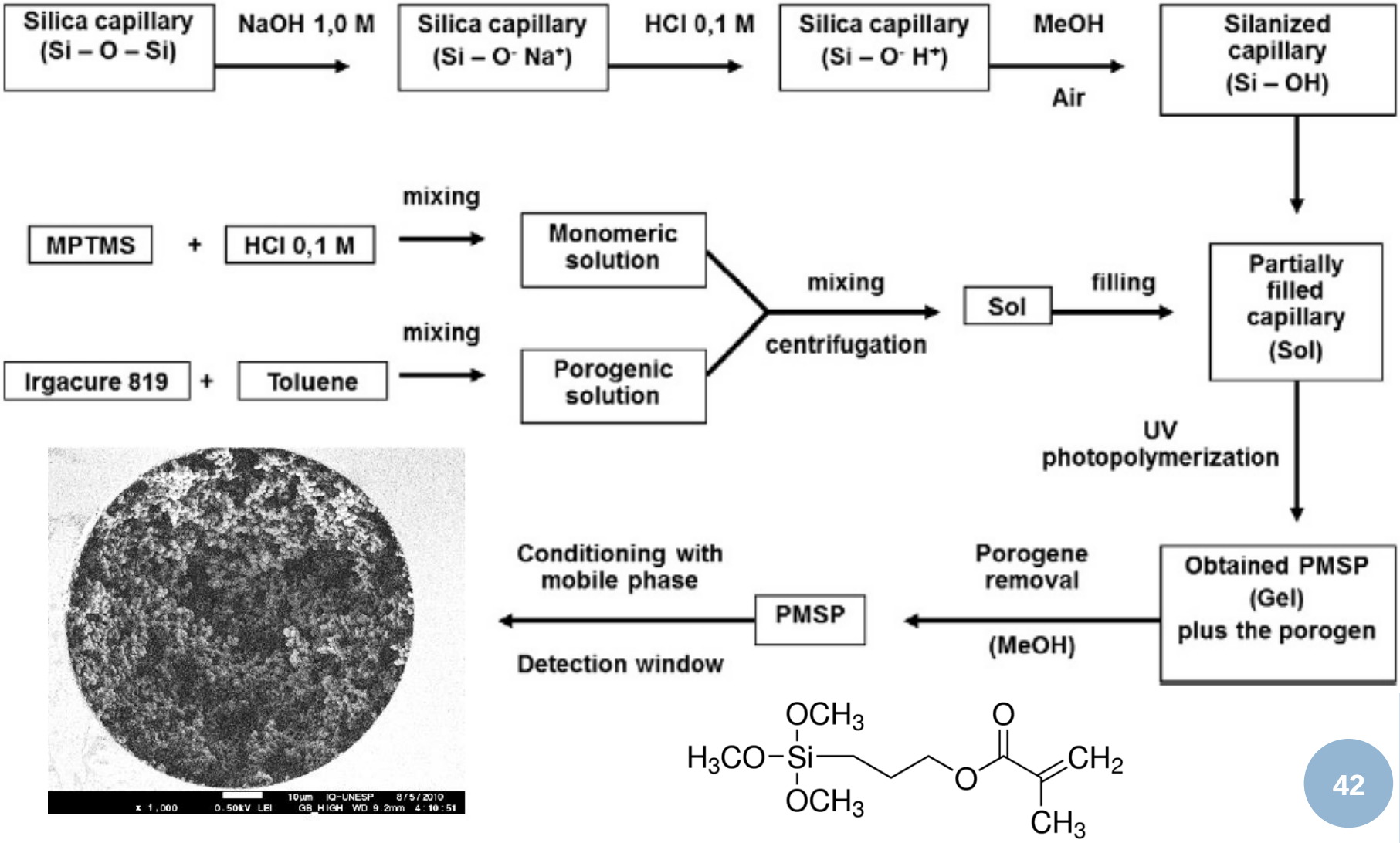
Short-end injection
Polycyclic aromatic hydrocarbons
Polyacrylate-coating fused-silica capillary

ABSTRACT

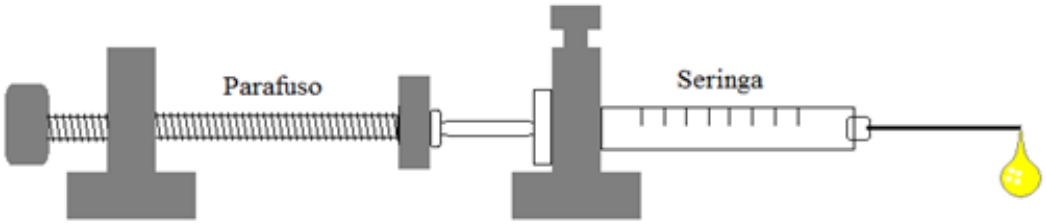
The preparation of photo-polymerized sol–gel monolithic stationary phases (MSP) within 100 μm internal diameter polyacrylate-coated fused-silica capillaries for use in capillary electrochromatography (CEC) was optimized. Eight mixtures containing different amounts of methacryloxypropyltrimethoxysilane (MPTMS) as the polymeric precursor, hydrochloric acid solution as the catalyst, toluene as the porogen and bis(2,4,6-trimethylbenzoyl)-phenylphosphine oxide (Irgacure 819) as the photo-initiator were irradiated at 370 nm inside the capillaries in order to complete the MSP polymerization, according to a fractional factorial experimental design 2^{4-1}_{IV}. All the preparation procedure, from capillary pretreatment until the MSP is ready to use in CEC, were made in less than four hours in mild conditions. A high pressurization injection device (HPID) useful for micro-volume syringes was built in order to achieve practical, controlled and precise injections of sols, solvents and electrolytes in the capillaries. The eight MSP were equally washed, conditioned and submitted to CEC procedures via short-end injection, which showed higher efficiency and peak height taking shorter analysis time. Electrochromatographic behaviors of the MSP were corroborated with morphological characterizations by scanning electron microscopy. The optimum condition, which allowed the separation of standard mixture containing thiourea (marker compound), naphthalene, acenaphthene, fluorene, phenanthrene and anthracene in twelve minutes without external pressure assistance, showed efficiencies up to 51,460 N/m, relative standard deviation from 0.05 to 3.3% for migration/retention time and from 0.14 to 1.6% for relative area (considering thiourea as an internal standard) and also showed no statistical evidence that three MSP prepared at the same condition are different within 95% confidence interval.

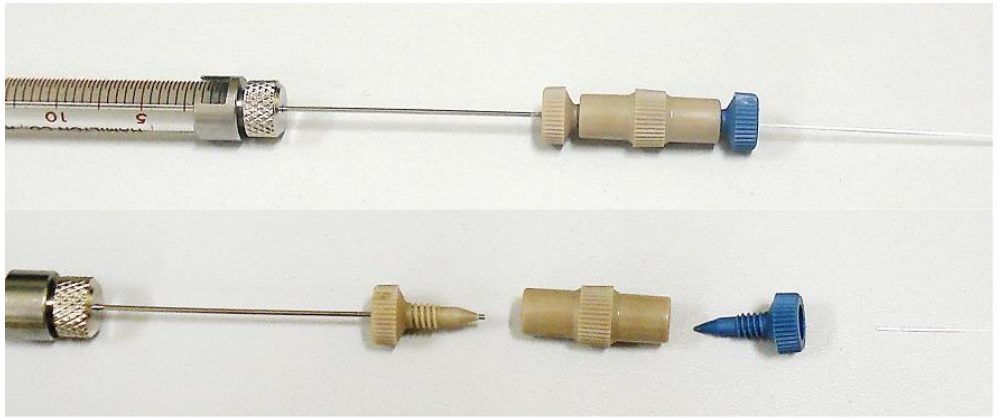
Capillary electrochromatography (CEC)





MPTMS – 3-(Trimethoxysilyl)propyl methacrylate

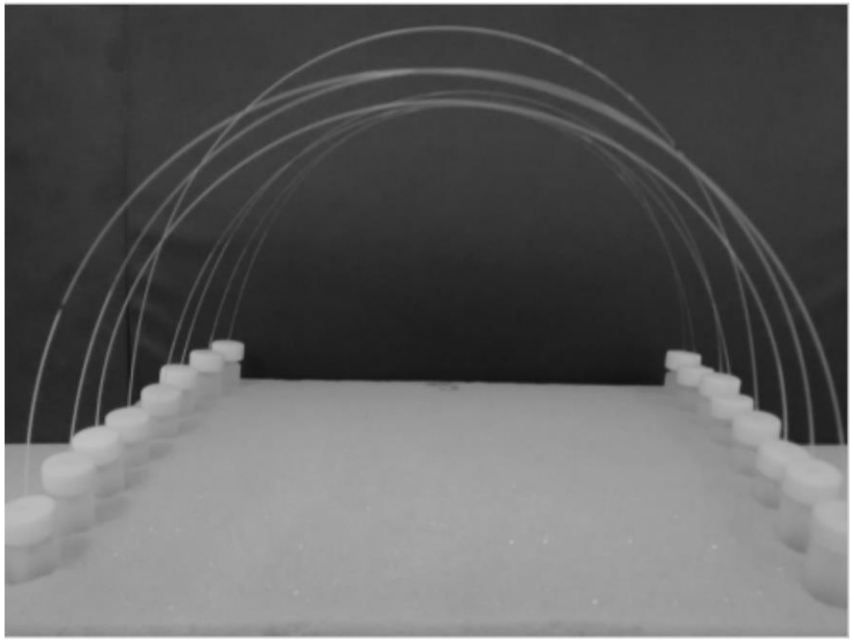

Parafuso
Seringa



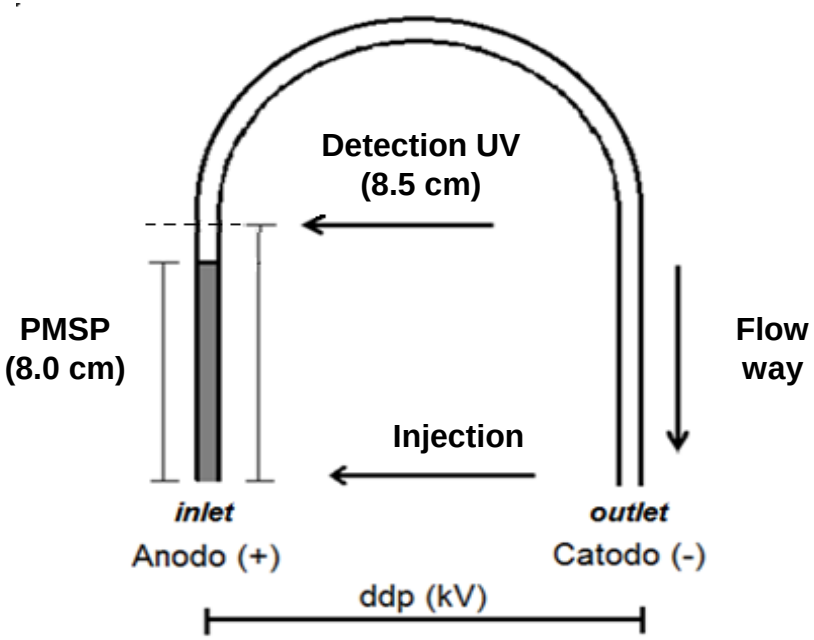

GASTIGHT @ #1705

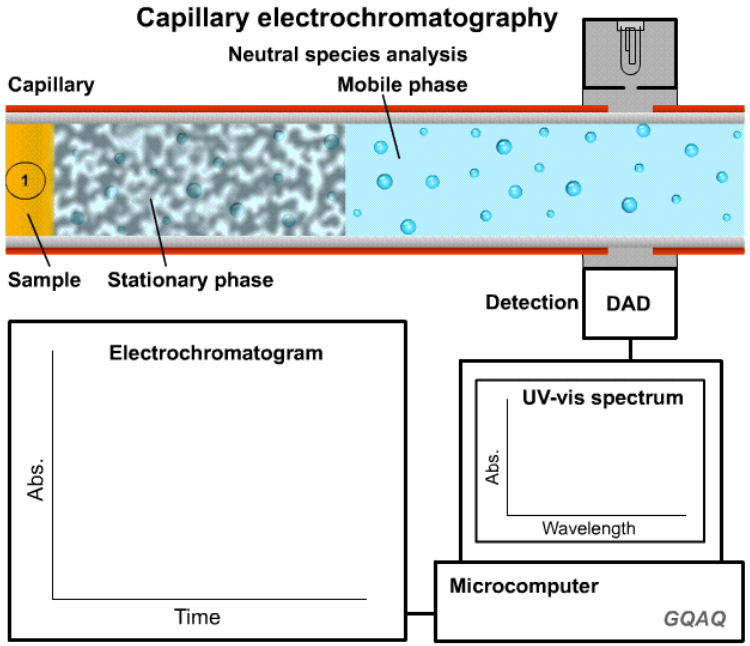
- Photochemical reactor (375 nm) for 20 min

Química Nova, v. 31, n. 8, p. 2156-2158, 2008.



Fused-silica capillaries externally coated with a fluoropolymer (TSU series) with dimensions of 100 µm i.d. and 360 µm o.d.





2^{4-1} fractional factional design with encoded variables and responses.

Experiments	X_1[a]	X_2[b]	X_3[c]	$X_4(123)$[d]	Response (no. of peaks)
1	−	−	−	−	5
2	+	−	−	+	4
3	−	+	−	+	6
4	+	+	−	−	5
5	−	−	+	+	5
6	+	−	+	−	4
7	−	+	+	−	5
8	+	+	+	+	4

[a] % v/v of toluene to monomeric solution: (−): 80, (+): 90.
[b] % m/m of Irgacure 819 to MPTMS: (−): 1.5, (+): 3.5.
[c] mol_{H2O} (from the HCl 0.12 mmol L^{-1} solution) to mol_{MPTMS} ratio: (−): 4, (+): 5.
[d] UV radiation incidence time (minutes): (−): 10, (+): 30.

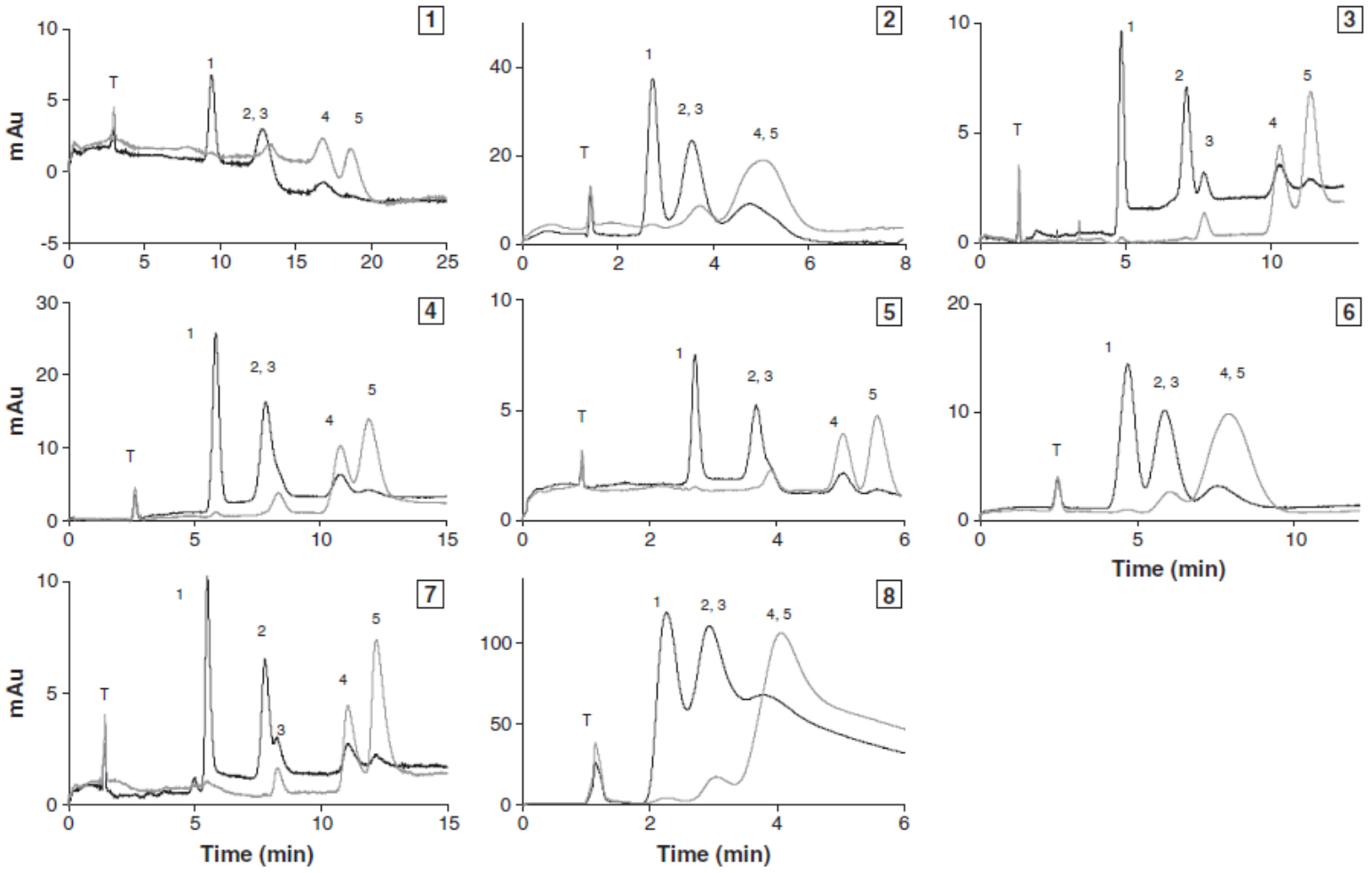


Fig. 4. Electrochromatograms of the 2^{4-1}_{IV} experimental fractional factorial design, obtained through an 8.0 cm MSP within 36 cm (8.5 effective length) 100 μm i.d. polyacrylate-coated fused silica capillaries. Elution order: thiourea (T); naphthalene (1); acenaphthene (2); fluorene (3); phenanthrene (4); anthracene (5), diluted in MeOH (1.0 mmol L^{-1}). MP: NH_4Ac 16.67 mmol L^{-1} pH 7.0 (60%)/ACN (40%), fast-CEC mode conditions: voltage: −20 kV, injection: −25 mbar × 3 s; temperature: 20 °C; detection: 220 nm (black line) and 250 nm (gray line).

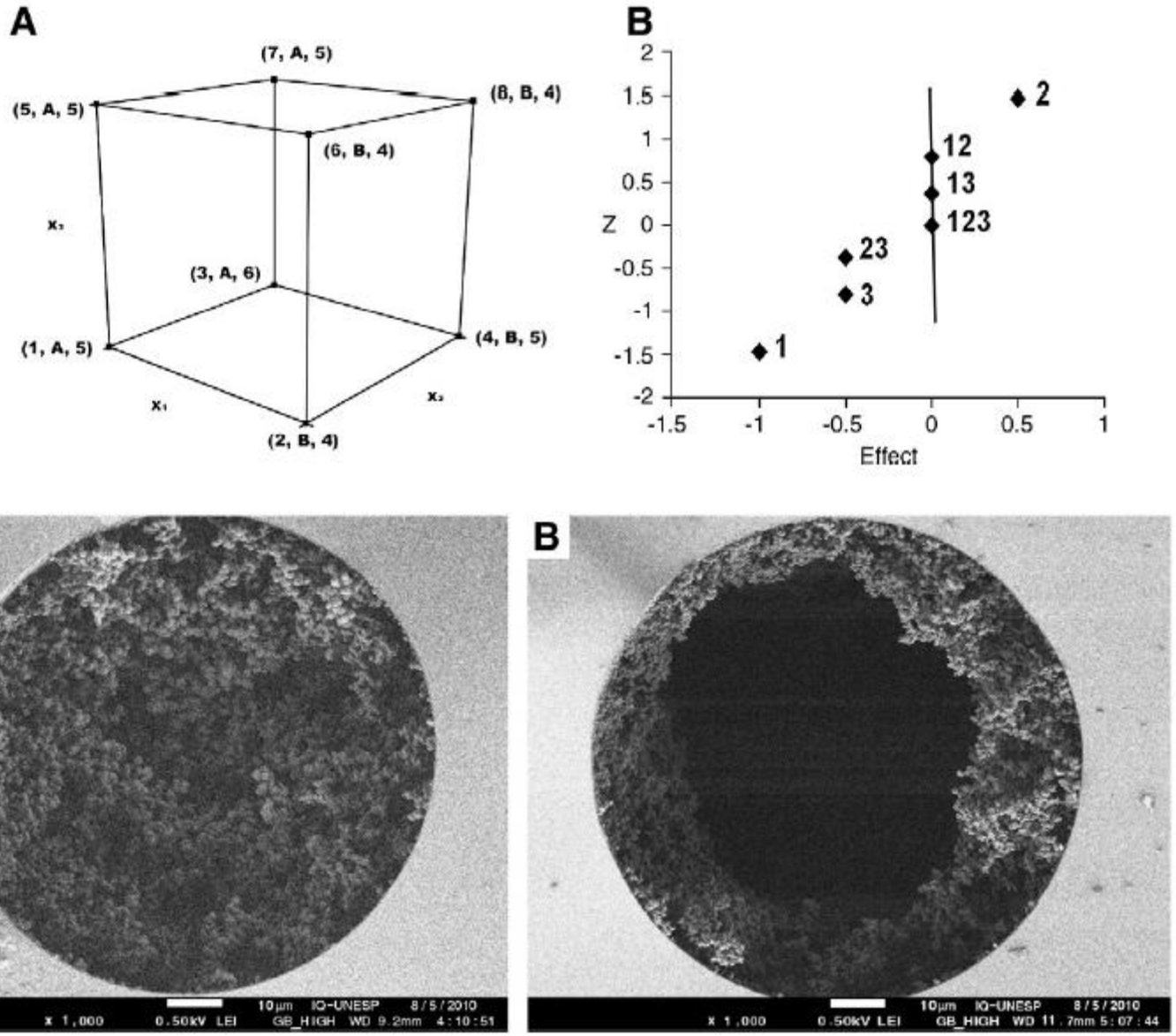


Fig. 6. Scanning electron microscopy images of monoliths of the experiments A) 5 (similar to 1, 3 and 7) and B) 6 (similar to 2, 4 and 8).

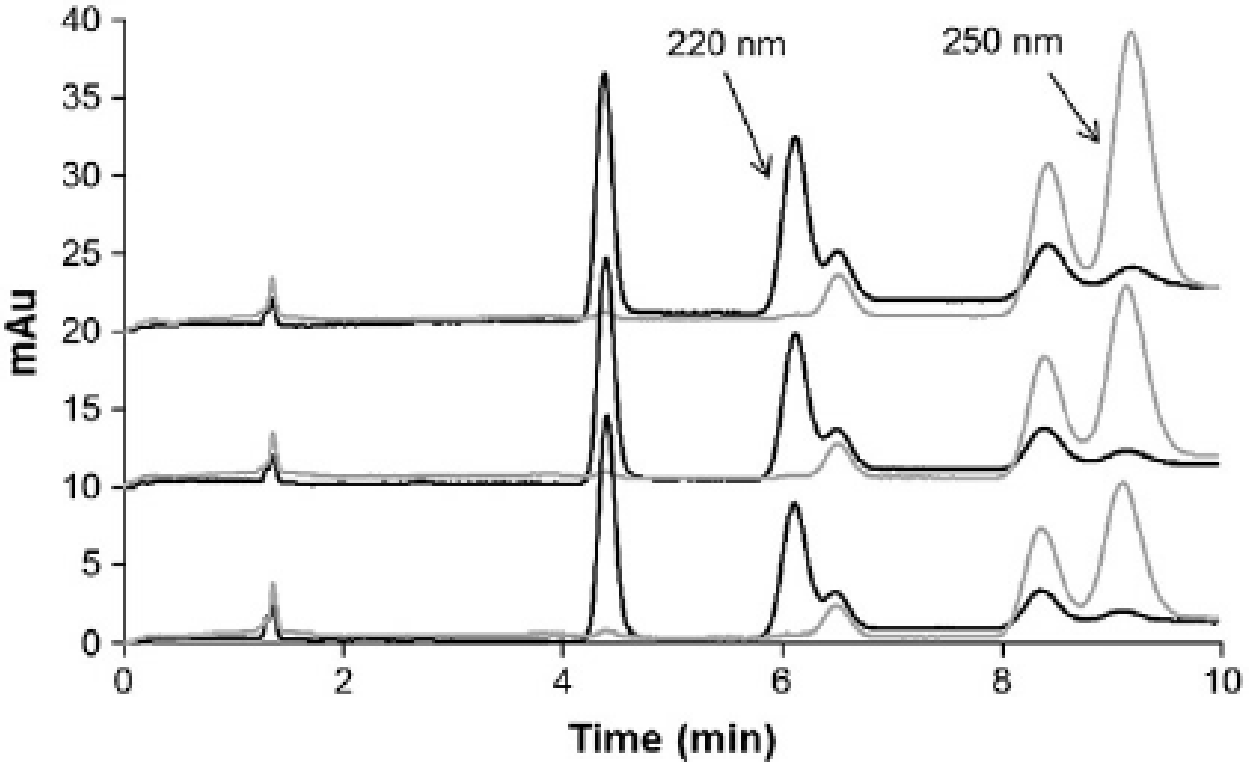


Fig. 8. Electrochromatograms obtained in triplicate in the same column. Elution order: thiourea (T), naphthalene (1), acenaphthene (2), fluorene (3), phenanthrene (4) and anthracene (5), all diluted in MeOH to 1 mmol L^{-1} each. Conditions: Temperature: 20 °C, detection: 220 nm (black line) and 250 nm (gray line); MSP: 8.0 cm long; 100 μm i.d. polyacrylate-coated fused silica capillaries; MP: NH_4Ac 16.67 mmol L^{-1} pH 7.0 (60%)/ACN (40%), fast-CEC mode: voltage: −20 kV, injection: −25 mbar ×3 s.

http://dx.doi.org/10.5935/0103-5053.20150016

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0103 - 5053 $6.00+0.00

Article

Optimized Separation Method for Estriol, 17-β-Estradiol and Progesterone by Capillary Electrochromatography with Monolithic Column and its Application to a Transdermal Emulsion

Rafael Marques,[a] Fernando A. S. Vaz,[a] Hudson C. Polonini[b] and Marcone A. L. de Oliveira[*,a]*

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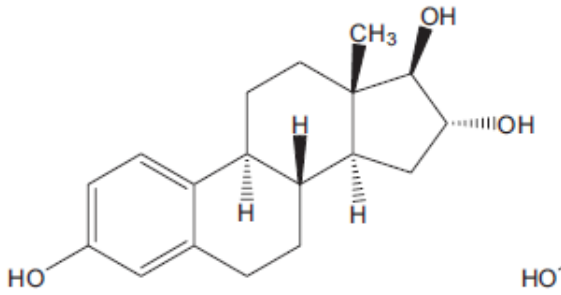
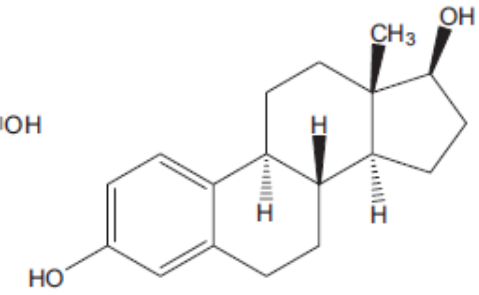
A monolithic stationary phase based on 3-(methacryloxypropyl)trimethoxysilane monomer, prepared within a fused silica capillary externally coated with a UV-transparent fluoropolymer was employed for separation of estriol, 17-β-estradiol and progesterone by capillary electrochromatography in a standard mixture. A 2^3 factorial design was used to optimize the separation system. The optimized condition containing 30% (v/v) of acetonitrile and 10 mmol L^{-1} aqueous ammonium acetate presented a total run time less than 10 min by applying 25 kV. The resolution between adjacent peaks ranged from 1.8 up to 2.9 and the plate numbers *per* column meter in this condition was 1873, 3631 and 3886 for the estriol, 17-β-estradiol and progesterone peaks, respectively. The optimized method was employed in the quantitative analysis of a commercial transdermal emulsion formulation.

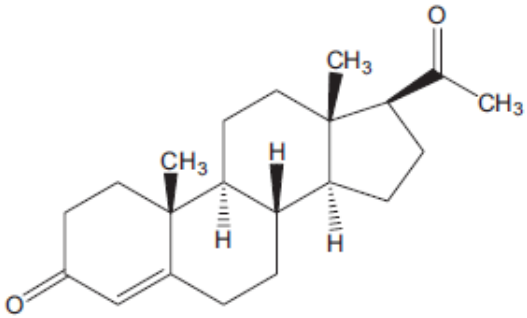
Keywords: capillary electrochromatography, monolithic stationary phase, fluoropolymer-coating fused-silica capillary, steroids, transdermal emulsion



Estriol
pK_a: 10.4



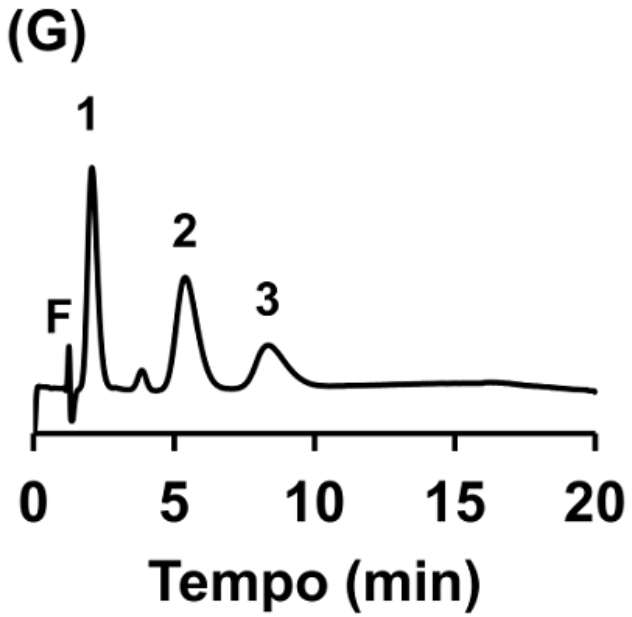
17-β-Estradiol
pK_a: 10.7



Progesterone
pK_a: 18.9

Experiment	Factor		
	Acetonitrile / %[a]	NH_4Ac / (mmol L^{-1})[b]	Voltage / kV
A	50	20	25
B	50	20	20
C	50	10	25
D	50	10	20
E	30	20	25
F	30	20	20
G	30	10	25
H	30	10	20

[a] Percentage relative to the total volume of mobile phase; [b] concentration in the aqueous fraction.



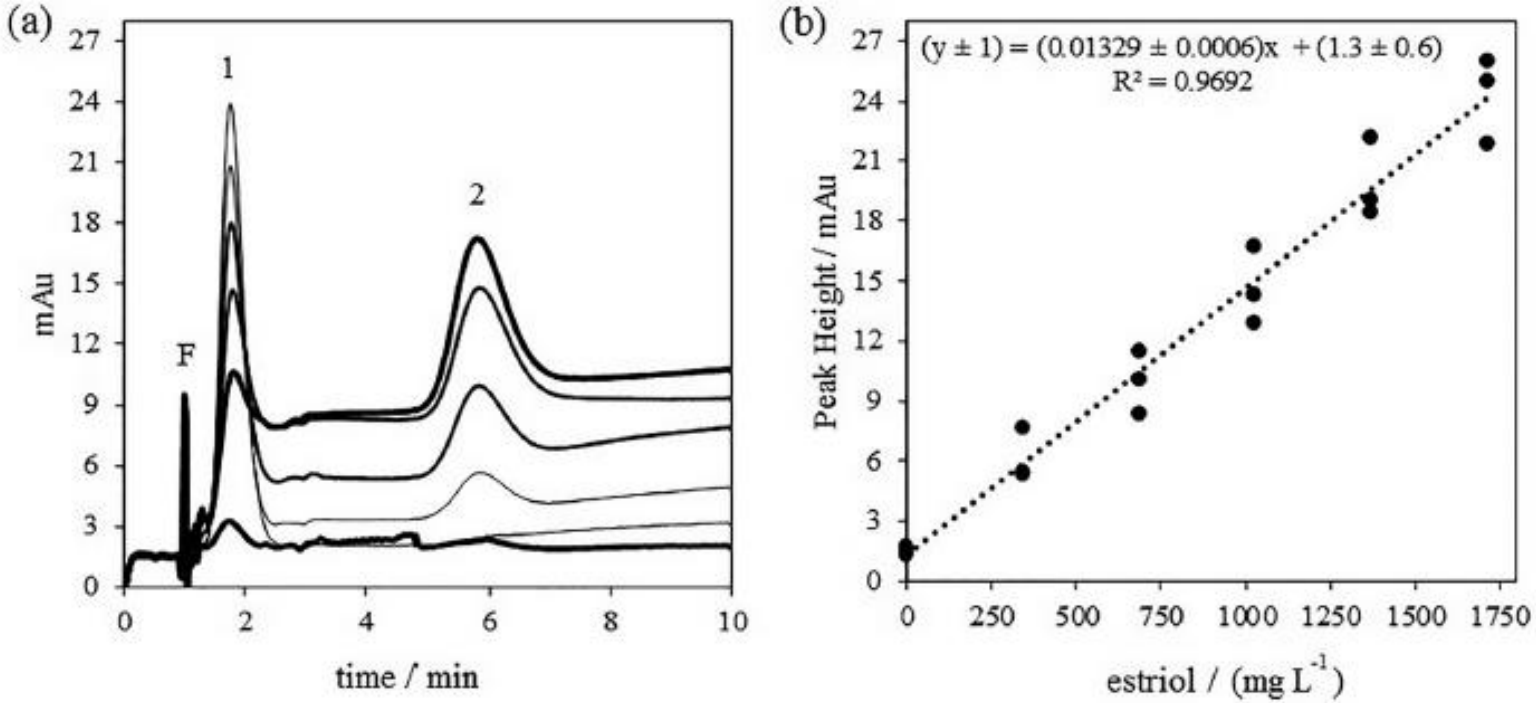


Figure 5. (a) Electrochromatograms of one of the replicates for the standard addition method through condition G; Peaks: (F): EOF, (1): estriol (sample + 0, 1.2, 2.4, 3.6, 4.8 and 5.9 mmol L^{-1}), (2):17-β-estradiol (sample + 5.2, 4.2, 3.1, 2.1, 1.0 and 0 mmol L^{-1}); (b) Standard addition curve (genuine triplicate).

PAPERS PUBLISHED AT 2018…

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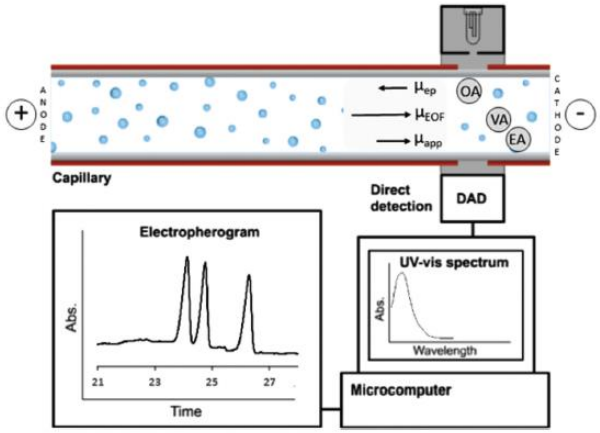
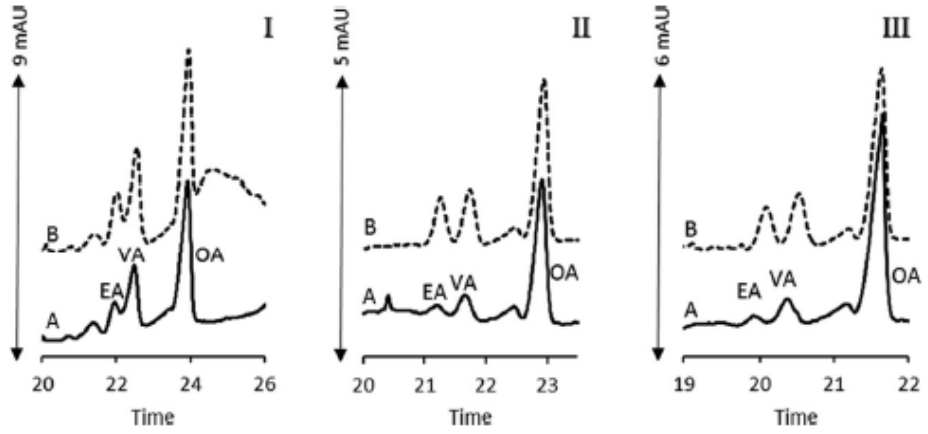
journal homepage: www.elsevier.com/locate/aca

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 Chemistry 258 (2018) 337–342

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Analytical Methods

Lactulose determination in UHT milk by CZE-UV with indirect detection

Leandra Natália de Oliveira Neves[a], Rafael Marques[a], Paulo Henrique Fonseca da Silva[b], Marcone Augusto Leal de Oliveira[c,*]

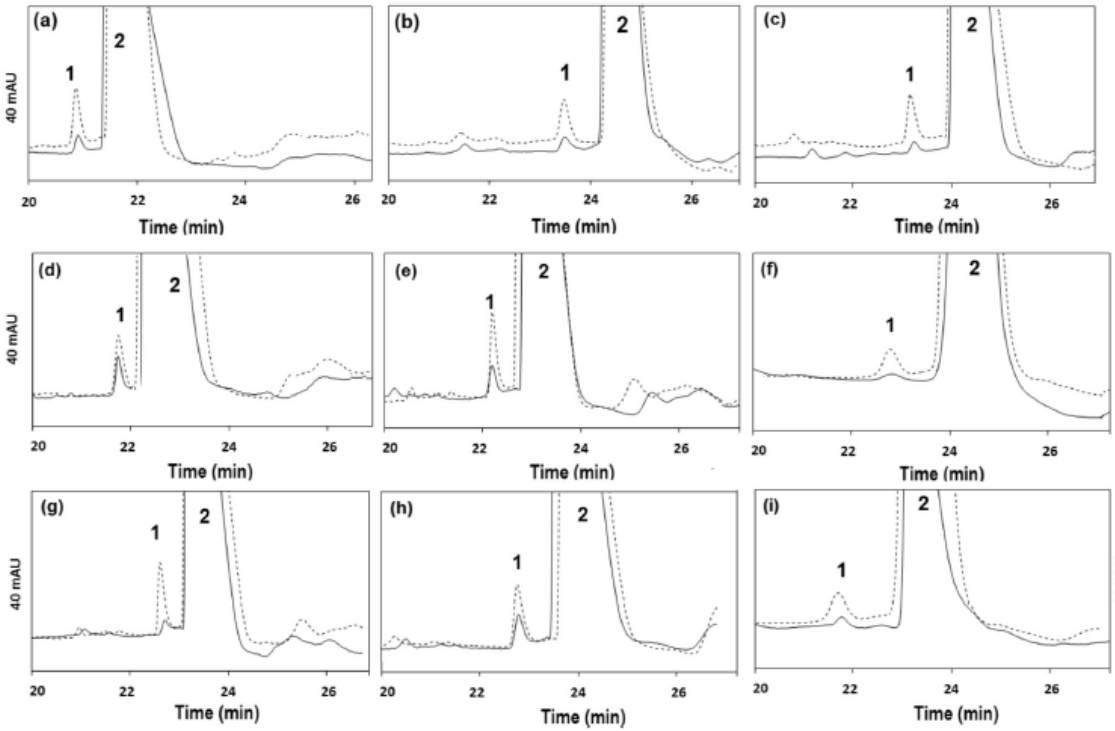


Fig. 2. Electropherograms profile of (a-i) UHT milk samples (solid line) and spiked UHT milk samples (dashed line), where peak 1 is lactulose and peak 2 is lactose. Instrumen conditions as in Fig. 1.

Electrophoresis 2018, *39*, 1399–1409

Lucas Mattos Duarte
Luiz Henrique Cantarino Adriano
Marcone Augusto Leal de Oliveira*

Grupo de Química Analítica e Quimiometria – GQAQ, Department of Chemistry, Institute of Exact Sciences,

Research Article

Capillary electrophoresis in association with chemometrics approach for bitterness hop (*Humulus lupulus L.*) classification

The precursor compounds related to the bitterness of beer are called α-acids. These com-

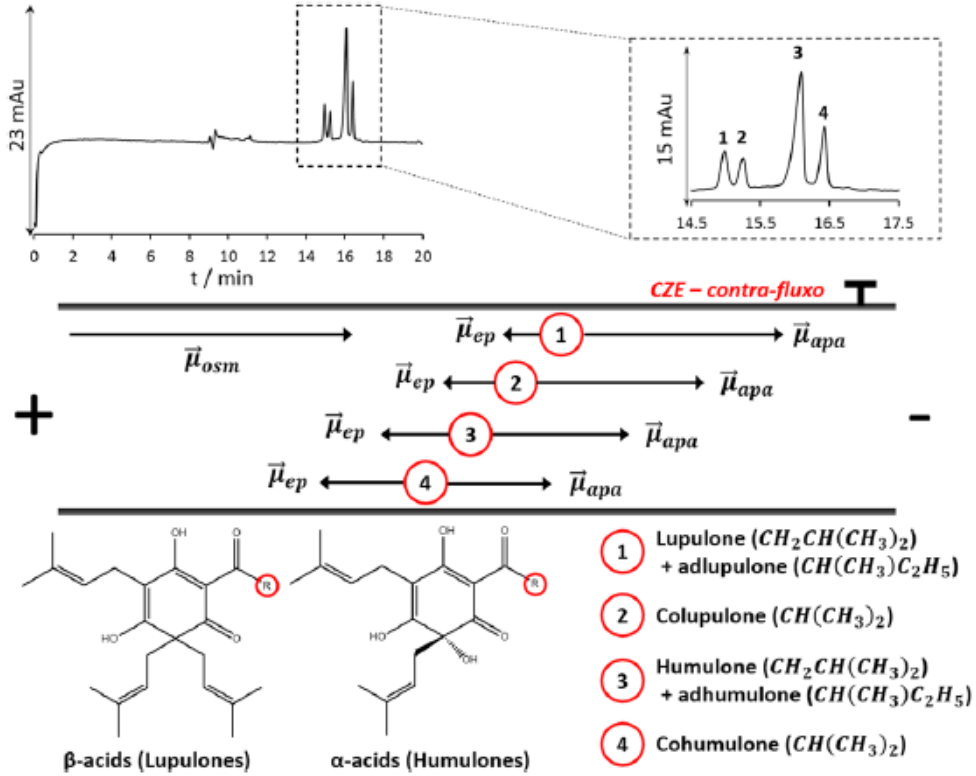


Figure 3. Electropherogram with expansion in the β and α-acids peaks region and a counterflow separation scheme using the electrophoretic and extraction optimized methods, where $\mu_{\rightarrow ep}$ is the electrophoretic mobility, $\mu_{\rightarrow osm}$ is the electroosmotic mobility, and the $\mu_{\rightarrow apa}$ is apparent mobility.

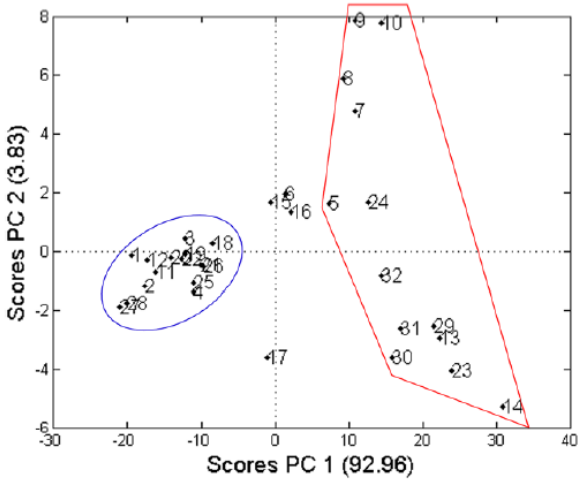


Figure 6. PCA analysis of aligned and meancentering preprocessed electropherograms of 16 hop hydromethanolic extract samples using just the α-acids electropherogram region.

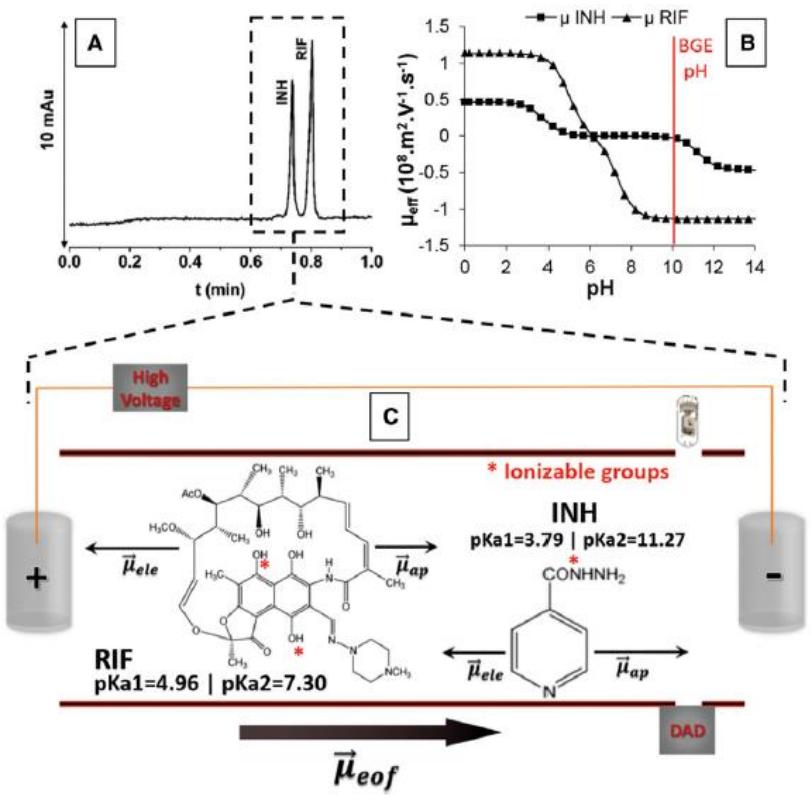
Received: 30 June 2018 | Revised: 11 October 2018 | Accepted: 13 October 2018
DOI: 10.1002/jssc.201800673

RESEARCH ARTICLE

JOURNAL OF SEPARATION SCIENCE

Sub-minute determination of rifampicin and isoniazid in fixed dose combination tablets by capillary zone electrophoresis with ultraviolet absorption detection

Lucas Mattos Duarte[1]* | Tatiane Lima Amorim[1] | Paula Rocha Chellini[2] | Luiz Henrique Cantarino Adriano[1] | Marcone Augusto Leal de Oliveira[1]



Analytical Methods

CRITICAL REVIEW

Cite this: *Anal. Methods*, 2018, **10**, 1103

Simultaneous determination of rifampicin, isoniazid, pyrazinamide and ethambutol in fixed-dose combination antituberculosis pharmaceutical formulations: a review

M. A. L. Oliveira, *[a] P. R. Chellini [b] and T. L. Amorim [a]

According to the World Health Organization, rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride are the first-line drugs used to treat tuberculosis – an infectious disease caused by *Mycobacterium tuberculosis*. Since 2010, tuberculosis has been treated with a fixed-dose combination containing the four drugs in only one tablet, called 4-FDC, which has simplified the treatment, minimized prescription errors and increased patients' adherence to their treatment regime. Within this context, the present review aims to show the evolution of different analytical methods that have been applied to simultaneous 4-FDC content determination in pharmaceutical formulations, such as high performance liquid chromatography, ultrahigh performance liquid chromatography, thin layer chromatography and capillary electrophoresis as separation techniques and molecular spectroscopic techniques associated with chemometric approaches, such as UV, Raman, Fourier transform infrared and near infrared.

Received 20th November 2017
Accepted 8th February 2018

DOI: 10.1039/c7ay02686b

rsc.li/methods

1. Introduction

Tuberculosis (TB) is one of the most widespread, infectious and

there were 10.4 million instances of TB. This rate equals 142 occurrences for every 100 000 people. In addition, TB is one of the 10 prevalent causes of mortality globally and was the cause

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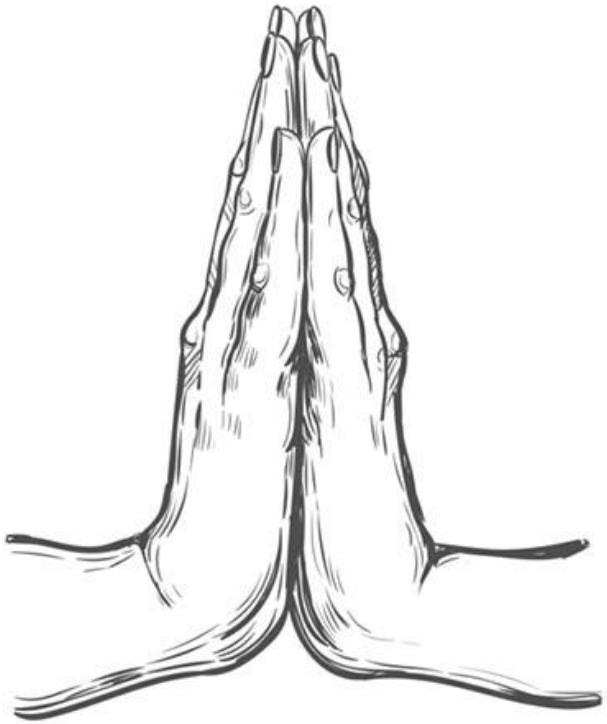

Grupo de Química Analítica e Quimiometria

Prof. Rafael

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conducts research with

SEPARATION METHODS

such as

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CHEMOMETRICS

making use of

factorial design

multivariate methods

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for use in

CEC nano-LC

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such as

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XRF