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

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Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential omega-3 fatty acids (FA) in human nutrition. Dietary supplements containing these FA are available in the market and there is a need to assess their quality to prevent frauds. The purpose of this work was to optimize and validate a fast capillary electrophoresis (CE) method to determine EPA and DHA content in marine oils omega-3 supplements. Sample preparation comprised only a saponification step and analysis time was 8 min. Validation was performed according to limits of detection and quantification, linearity, accuracy and repeatability. Ten real samples of marine oil supplements were analyzed by CE and compared to conventional gas chromatography method. No significant differences between both methods were found within 95 % confidence interval. Overall, CE method was successful to FA quantification and it could be used for fast FA monitoring during omega-3 supplement manufacture, final product quality assurance and labelling.

Key-words: n-3 fatty acid; capillary electrophoresis; ultraviolet detector; krill oil; fish oil.

1. Introduction

Omega-3 essential fatty acids (FA) are needed to maintain cell membranes structure, brain function, transmission of nerve impulse and metabolic processes. They are called essential, because our organism does not synthesize them and should be included in the diet. Although α -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are all omega-3 FA, plant derived ALA would not exert the same beneficial health effects as long chain omega-3 FA, such as EPA and DHA, available in marine species [1–3]. Dietary EPA and DHA supplementation has been associated with a decreased risk of developing coronary heart disease (CHD) [4–6], maintenance and enhancement of brain health, and prevention of neurological diseases [7]. DHA intake is also involved in nervous and visual tissue formation of the fetus and the newborn [8] and it has been related to gestation duration and infant size [9].

In the last decade, consumption of marine oils have increased exponentially due to their benefits in consumers health [10,11]. Omega-3 lipid supplements containing fish and/or krill oils are available in the market under different formats and formulations. Most of these supplements show the nutritional claim of “high in omega-3 FA” in their labels to attract the consumer and offer to reach the recommended daily intake of 250 mg of EPA + DHA to prevent CHD [12]. The increasing demand of omega-3 supplements together with their high-market value raises the need of severe manufacturing controls and high quality assurance standards. In this sense, the development of fast, simple and accurate methods to determine EPA and DHA in marine oils during and after product manufacture is of vital importance.

Gas chromatography coupled to a flame ionization detector (GC-FID) has been the most widely used technique for omega-3 FA quantification in marine oils. GC-FID gives a detailed FA composition, but requires saponification and methylation of FA into volatile fatty acid methyl ester (FAME) derivatives. Then, FAME are analyzed in specific capillary columns with run times of 60 min or more [13–15]. These chromatographic analyses are laborious, time consuming and require a significant

analytical expertise in order to evaluate overlaps and assign peaks correctly, which is especially complex in marine oils containing more than 100 FAME peaks. Besides, if appropriate precautions are omitted, lipid oxidation may occur in the derivatization step, generating a loss of polyunsaturated fatty acids (PUFA) and short-chain volatile FA [16].

Capillary Electrophoresis (CE) offers the advantages of reducing the sample preparation times requiring only a saponification reaction, in connection with shorter run times. Oliveira *et al.* [17] and more recent literature [18,19] describe the potential of CE on FA analysis. CE has proven to overcome some GC limitations in the analysis of geometrical and positional C18:1 FA isomers, involving ultraviolet (UV) direct detection [20], UV indirect detection [21] and capacitively coupled contactless conductivity detection [22]. However, to the best of our knowledge, only two articles have analyzed omega-3 FA by CE. On one hand, a qualitative differentiation between natural and enriched chicken eggs by omega-3 FA profiling [23] and, on the other hand, a quantitative approach applied to beef and plant oils [24] with a total analysis time of 40 min. CE has not yet been applied to EPA and DHA quantification in marine oils and it can be a useful tool for FA monitoring during supplement manufacture, final product quality assurance and labelling. Within this context, the proposal of this work was to optimize a novel, simple and fast procedure employing Capillary Zone Electrophoresis with direct absorption UV detection (CZE-UV) to determine EPA and DHA levels in omega-3 supplements and to compare it with the conventional GC-FID method.

2. Materials and Methods

2.1 Samples and Reagents

Ten commercial samples of omega-3 supplements from marine origin, containing fish oil or krill oil, were acquired from local supermarkets (Juiz de Fora, MG, Brazil). All reagents used were analytical grade and water was purified by deionization (Milli-Q system; Millipore, Bedford, MA, USA). Acetonitrile (ACN), methanol (MeOH), sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$) and sodium hydroxide (NaOH) were purchased from Vetec (Rio de Janeiro, RJ, Brazil). Polyoxyethylene 23 lauryl ether (Brij 35) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexane, potassium hydroxide (KOH) and sodium bisulphate (NaHSO_4) were purchased from Panreac Química SA (Madrid, Spain). Standard FA (C18:1 9c, C18:1 9t, C18:2 n-6, C18:3 n-3, C20:5 n-3, C22:6 n-3) for CE analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA) and FA standard solutions were prepared using MeOH. Standard FAME (C20:5 n-3, C22:6 n-3 and mixtures GLC 80, 403, 409, 411, 603, 642, 643) for GC analyses were acquired from Nu-Chek Prep Inc. (Elysian, MN, USA) and FAME standard solutions were prepared using hexane. All standard solutions were stored at $-20\text{ }^\circ\text{C}$.

Brij 35 stock solution (100.0 mmol L^{-1}) and NaOH solution (1.0 mol L^{-1}) used for capillary conditioning were prepared in deionized water. NaOH solution (0.5 mol L^{-1}) used for saponification and KOH solution (2 mol L^{-1}) used for methylation were prepared in MeOH. The optimized background electrolyte (BGE) was composed by $30\text{ mmol L}^{-1}\text{ Na}_2\text{B}_4\text{O}_7$, 12 mmol L^{-1} Brij 35, 33 % v/v MeOH and 17 % v/v ACN. Before its use, BGE was sonicated for 20 min to eliminate air bubbles.

2.2. Sample Preparation

2.2.1 Sample Preparation for CE analysis

Omega-3 oils were withdrawn from the capsules with a glass syringe and approximately 150 mg of each sample were saponified with 2.0 mL of a methanolic NaOH solution (0.5 mol L^{-1}) using reflux

tubes for 25 min in a heated water bath (75–80 °C). The saponification content was transferred to a 10.00 mL volumetric flask and volume was completed with MeOH. Before CE injection, samples were diluted in MeOH to a final concentration of 5 % v/v in a 1.0 mL volumetric flask containing 0.5 mol L⁻¹ of elaidic acid (C18:1 *trans*-9), used as internal standard (IS) and transferred to vials.

2.2.2 Sample Preparation for GC analysis

Samples were prepared using basic methylation prior GC analysis according to ISO (ISO-IDF, 2002). 25 mg of sample was weighed in a tube and 200 µL of hexane were added. After agitation, 50 µL of a methanolic KOH (2 mol L⁻¹) solution were added and the solution was agitated in vortex for 1 min. Then, it was rested for 5 min and 125 mg NaHSO₄ were added to the tube, which was centrifuged for 5 min at 0.56 g and at 0°C. The solution was transferred to a GC vial and diluted in a proportion of 1:2 with hexane. FA in samples were quantified by GC employing external calibration. No IS was added to samples in sample preparation step.

2.3 CE and GC analyses

2.3.1 CE Instrumentation, identification and quantification of FA

An Agilent 7100 CE instrument (Palo Alto, CA, USA) equipped with DAD set at 200.0 nm (± 4.0 nm with peak width at 2.5 Hz) was used to carry out all CE experiments. Data acquisition and treatment software used was HP Chem Station, rev A.06.01. Cartridge temperature was maintained at 27 °C, samples were hydrodynamically injected applying 25 mbar during 5.0 s and the electrophoretic system was maintained at 27 kV. A fused-silica capillary with polyimide outer coating (Polymicro Technologies, Phoenix, AZ, USA) with 33.5 cm total length, 25 cm of effective length, 50 µm internal diameter (i.d) and 375 µm outer diameter (o.d) was used in the analyses. First conditioning of capillary was carried out by a flush of NaOH solution at 1.0 mol L⁻¹ (30 min), deionized water (30 min) and BGE

(30 min). Capillary was flushed with 1.0 mol L⁻¹ NaOH solution (2 min), deionized water (2 min) and BGE (2 min) between runs.

Identification of FA was carried out by spiking samples with analytical standards and quantification of C20:5 n-3 and C22:6 n-3 was performed by external calibration (five levels in genuine triplicates: 0.100, 0.275, 0.450, 0.625 and 0.774 mmol L⁻¹, with fixed IS concentration: 0.5 mmol L⁻¹).

2.3.2 GC Instrumentation, identification and quantification of FAME

An Agilent 6890N GC instrument (Palo Alto, CA, USA) equipped with FID detector, auto sampler and a CP-SIL 88 column (100 m, 0.25 mm x 0.2 µm, Varian, Middelburg, The Netherlands) was used to quantify FAME. Chromatographic conditions were: injection volume of 1.0 µL, split 1:100, detector and injector temperatures at 250 °C. Helium was the carrier gas with a flow rate of 1.0 mL min⁻¹. The temperature program was as follows: initial oven temperature at 45 °C; after 4 min, oven temperature was raised at 13 °C min⁻¹ to 165 °C and held for 35 min, then increased to 215 °C at 4 °C min⁻¹ and kept for 30 min [15]. Identification of C20:5 n-3 and C22:6 n-3 was mainly carried out in GC-FID by comparison with the retention times of FAME individual standards. Other FAME were identified by comparison with the retention times of FAME mixtures (GLC 80, 403, 409, 411, 603, 642, 643). Confirmation of peaks was performed on an Agilent chromatograph (model 7890A) with a MS detector (5975C inert MSD). EPA and DHA mass spectra can be visualized in Supplementary Material S1 and S2. The filament trap current was 400 µA at 70 eV. Ion source and interface temperatures were 150 and 230°C respectively. Mass interval was from 30 to 400. Injections were under data system control with an auto-injector. Chromatographic conditions were similar to those described by GC-FID but with a split ratio of 1:20. The injection volume was 1.0 µL. Wiley 275 and NIST 05 libraries were used to confirm the mass spectra and percentages of similarity were higher than 90%.

Quantification of C20:5 n-3 and C22:6 n-3 by GC-FID was performed by external calibration (five levels in genuine triplicates - 0.100, 0.275, 0.450, 0.625, 0.774 % v/v for DHA and 0.100, 0.325, 0.550, 0.775, 1.000 % v/v for EPA).

2.4 Statistical Analysis and Validation

Linear regression models building was carried out by ordinary least squares regression using Excel. Normality test was verified by the Ryan-Joiner test, within 95 % of confidence and homoscedasticity was checked by Levene's test (95 % of confidence), using Minitab 14 software.

FA contents calculated from CE and GC methods were compared using parametric paired t-tests, since normality test was not violated. Excel was employed for paired t-tests calculations. Precision of the CZE-UV method was evaluated with an inter-day repeatability test in quintuplicate, considering the relative standard deviation (RSD) of concentration percentages and migration time of both FA.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated considering the signal-to-noise ratio approach according to equations 1 and 2, where s_b is the standard deviation of the baseline, C_{FA} is the concentration of FA and H is the peak height [25].

$$LOD = \frac{3s_b C_{FA}}{H} \text{ (Equation 1)}$$

$$LOQ = \frac{10s_b C_{FA}}{H} \text{ (Equation 2)}$$

3. Results and Discussion

3.1 Instrumental conditions and BGE optimization

Fish and krill oils are very complex food matrices with more than one hundred FA in their composition. The use of direct UV absorption detection for EPA and DHA quantification is very promising for these marine oils quality assurance, since the double bonds of the molecular structures makes possible a direct detection and no chromophore agent needs to be considered when optimizing BGE [26]. In addition, UV absorption detection eliminates saturated FA interferences because these FA do not absorb in the UV range, which would increase the selectivity of the method. Two methods available in the literature employed this approach to detect EPA and DHA in complex food matrix [23,24]. Soliman *et al.* employed cyclodextrin-modified micellar electrokinetic chromatography and Porto *et al.* used CZE.

A fair separation of 15 omega-3 and omega-6 FA, including EPA and DHA, was reached by Soliman *et al.* and excellent analytical figures of merit (LOD, LOQ, repeatability and linearity) were reported. However, analysis time was very long (40 min) to be applied routinely. BGE in the method of Porto *et al.* was composed by 12 mmol L⁻¹ Na₂B₄O₇, 12 mmol L⁻¹ Brij 35, 33 % MeOH and 17 % ACN v/v. The first advantage of this method was the use of surfactants instead of the costly cyclodextrins in the BGE. Besides, analysis time was substantially shorter (10 min) than in Soliman *et al.* method, making the approach of Porto *et al.* more attractive for routine application. Furthermore, the same BGE was applied in *trans* FA determination of processed foods [27,28], which indicates a great versatility and reproducibility of the BGE for FA direct detection. As a result, the initial purpose of this work was to optimize the analytical conditions described in the method of Porto *et al.* to be applied routinely in marine oil analysis.

Figure 1-I shows the electropherogram of a mixture of standards injected at 0.1 mmol L⁻¹ following the method of Porto *et al.* It can be noticed a co-migration between EPA and ALA in the

standard mixture. Thus, some improvements of the method conditions were carried out. Firstly, it was verified the influence of the buffer concentration to observe ionic strength influence in separation. (Figure 1). The resolution of the critical pair EPA and ALA was enhanced with the buffer concentration of 24 mmol L⁻¹ (Fig 1-II) in comparison to 12 mmol L⁻¹ (Fig 1-I). At 36 mmol L⁻¹ (Fig 1-IV) instability in the current was observed, run time was higher, DHA was not separated from C18:2 n6 and EPA was not resolved from ALA. In contrast, the intermediate buffer concentration at 30 mmol L⁻¹ (Fig 1-III) resolved all the unsaturated FA of the standard mixture within 31.5 min and it was selected for the next optimization steps.

In order to reduce the time of analysis, capillary length was shortened from 48.5 to 33.5 cm, the minimum length possible for the cartridge used. Additionally, injection time was evaluated for further improvement of the method (Figure 2). As it can be observed in the electropherograms, reducing capillary length reduced the time of analysis to approximately 10 min in the standard mixture, maintaining the good resolution for critical pairs. Concerning to injection time, 3 s of injection provided peaks with low absorbance (Figure 2-A), but 5 s (Figure 2-B) and 7 s (Figure 2-C) showed sufficient absorbance for all peaks. The criteria of selection between both conditions (5 and 7 s) was the resolution between the critical pair. In electropherogram B a resolution of 1.05 was obtained for the critical pair EPA and ALA while it was 0.97 in electropherogram C. For this reason, injection time of 5 s was preferred and used in the following experiments. Besides, some perturbation of the baseline was detected at 7 s of injection due to the high quantity of sample injected, which was not observed with the other conditions.

As summary, optimized conditions of the CZE-UV method were fixed as follows: BGE composed by 30 mmol L⁻¹ Na₂B₄O₇, 12.0 mmol L⁻¹ Brij 35, 33 % v/v MeOH and 17 % v/v ACN; cartridge temperature maintained at 27 °C, positive applied voltage of 27 kV, hydrodynamic injection of 25 mbar

for 5 s and employment of a fused silica capillary of 33.5 cm total length (25 cm effective length, 50 μ m i.d. and 375 μ m o.d).

3.2 Omega-3 supplements analyses and method validation

After BGE and instrumental parameters optimization, 10 samples of commercial omega-3 marine supplements were analyzed. Quantification of EPA and DHA in the samples was carried out using C18:1 *trans*-9 as IS, which is highly recommended to improve quantitative performance of CE methods [29]. C18:1 *trans*-9 is commonly found in dairy fat and hydrogenated vegetable oils [26], but not in oils of marine origin. In this line, it is important to highlight that none of the 10 commercial samples analyzed contained C18:1 *trans*-9.

Figure 3 shows the electropherograms for BGE signal, a marine oil sample (solid line) and the same sample spiked with C18:1 *trans*-9, EPA and DHA (dotted line), analyzed in CZE-UV optimized conditions. It illustrates the suitability of the IS selected, corroborates the assignment of EPA and DHA in the electropherogram and shows the absence of BGE interferences for EPA and DHA quantification. It is also worth mentioning that, when comparing the injection of the standard mixture with real samples, migration time slightly changed due to matrix effects. As a result, analysis time of marine oil samples was reduced to approximately 8 min.

LOD and LOQ were calculated before calibration curve construction using analytical EPA and DHA standards in CZE-UV optimized conditions (Figure 2-B), considering the signal-to-noise ratio approach [25]. LODs were 0.0011 and 0.0013 mmol L⁻¹ for DHA and EPA, respectively while LOQ were 0.0037 for DHA and 0.0043 mmol L⁻¹ for EPA. In the study of Porto *et al.*, LOD and LOQ for DHA accounted for 0.11 mg 100 g⁻¹ and 0.38 mg 100 g⁻¹, respectively. Soliman *et al.* reported LODs of 0.3 and 0.5 mg L⁻¹ for EPA and DHA (equivalent to 0.0009 mmol L⁻¹ for EPA and 0.0015 mmol L⁻¹ for

DHA), and LOQs under 10 mg L⁻¹ for both analytes (equivalent to 0.030 and 0.033 mmol L⁻¹ for DHA and EPA, respectively). These results indicate that our optimized method is able to quantify EPA and DHA amounts roughly 10 times smaller in comparison to the previous method.

Linear models obtained for DHA and EPA, to quantification in CE and GC are presented in table 1. Normality and homoscedasticity tests provided p-values higher than 0.05. Besides, no lack of fit was observed and a satisfactory regression significance was obtained for both FA ($F_{cal} > F_{tab}$). To quantify EPA and DHA in marine oil samples by CE, the calibration curve slope was included as R_f in Equation 3. In the equation, [IS] is the concentration of C18:1 *trans*-9, V is the volume in liters, MW_{FA} is the molecular weight of each FA, D_f is the dilution factor, A_{FA} is the peak area of each FA, A_{IS} is the peak area of C18:1 *trans*-9 and m is the sample mass in grams.

$$\%FA = \frac{[IS].V.MW_{FA}.D_f.A_{FA}}{A_{IS}.R_f.m} \cdot 100 \text{ (Equation 3)}$$

Method accuracy was verified by comparison of EPA and DHA percentages obtained by CZE-UV with the results from conventional GC-FID, by a paired t-test. Table 2 exhibits EPA and DHA percentages, expressed in g/100g of sample, obtained by CZE-UV and GC-FID of the 10 commercial omega-3 supplements. p-values > 0.05 were obtained, which would indicate that no significant differences exist between CZE-UV and GC-FID results within 95 % confidence interval. Parametric tests were performed since Ryan-Joiner normality test was verified for both FA and it was not violated (p-values > 0.100 for EPA and DHA).

Finally, in order to evaluate the precision of the CZE-UV methodology, an inter-day repeatability test was performed and a marine oil sample was analyzed in quintuplicate. Table 3 shows the RSD of concentration percentages and migration time of both FA. DHA showed a RSD of 4.11 % and 1.72 % for concentration and migration time, respectively, while EPA exhibited a RSD of 2.52 % in

241 concentration and 1.55 % in migration time. In this concern, the slight variance ($< 5\%$) of both
242 concentration percentage and migration time demonstrates the acceptable repeatability of the optimized
243 CZE-UV method.

Conclusions

A fast CZE-UV method to determine EPA and DHA content in marine oils omega-3 supplements was optimized and validated. The method is repeatable, accurate and selective, without requiring specific columns or costly solvents. Sample preparation comprised only a previous saponification step and CZE-UV analysis time was 8 min. In addition, no significant differences were observed between CZE-UV results and conventional GC-FID quantifications within 95 % confidence interval. Overall, the proposed CZE-UV method was successful in EPA and DHA determination and it could be used for fast FA monitoring during omega-3 supplement manufacture, final product quality assurance and labelling.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Linearity parameters for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) determined by capillary zone electrophoresis - UV detector (CZE-UV) and gas chromatography - flame ionization detector (GC-FID).

Method	Fatty Acid	R ²	Equation	Lack of fit ^a	Regression significance
CZE-UV	EPA	0.9988	$y = 5.9x + 0.018$	$F_{\text{calc}}=0.53 < F_{\text{tab}}=4.76$	$F_{\text{calc}}=6886.93 > F_{\text{tab}}=5.12$
	DHA	0.9996	$y = 6.4x + 0.332$	$F_{\text{calc}}=2.26 < F_{\text{tab}}=4.07$	$F_{\text{calc}}=26094.69 > F_{\text{tab}}=4.84$
GC-FID	EPA	0.9986	$y = 1013.5x - 30.415$	$F_{\text{calc}}=1.39 < F_{\text{tab}}=4.07$	$F_{\text{calc}}=8079.13 > F_{\text{tab}}=4.84$
	DHA	0.9963	$y = 1047.6x - 8.752$	$F_{\text{calc}}=1.96 < F_{\text{tab}}=3.71$	$F_{\text{calc}}=3517.26 > F_{\text{tab}}=4.67$

^a Degrees of freedom for lack of fit consideration in CE: Regression=1 for both FA; Residual=11 1 for DHA and 9 for EPA; Lack of fit= 3 for both fatty acids; Pure Error=8 for DHA and 6 for EPA | Degrees of freedom for lack of fit consideration in GC: Regression=1 for both FA; Residual=13 1 for DHA and 11 for EPA; Lack of fit= 3 for both fatty acids; Pure Error=10 for DHA and 8 for EPA

Table 2. Contents of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in marine oil supplements analyzed by capillary zone electrophoresis - UV detector (CZE-UV) and gas chromatography - flame ionization detector (GC-FID).

Sample	EPA (g/100g of sample)		DHA (g/100g of sample)	
	GC-FID	CZE-UV	GC-FID	CZE-UV
1	18.3	15.7	10.1	10.4
2	16.4	14.7	10.2	11.1
3	20.2	15.7	12.2	12.2
4	16.3	17.2	9.7	11.2
5	17.4	17.1	11.6	12.4
6	19.7	19.4	12.4	12.5
7	16.2	16.6	9.4	9.9
8	17.9	18.1	10.0	9.5
9	18.0	17.7	10.6	9.9
10	11.5	11.1	5.1	5.8
Paired t test p-value	0.065		0.060	

Table 3. Repeatability of concentration and migration time data for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using a marine oil sample analyzed five times by capillary zone electrophoresis - UV detector. RSD: relative standard deviation.

Number of injection	Concentration (g/100 g of sample)		Migration time (Min)	
	EPA	DHA	EPA	DHA
1	15.93	9.88	9.40	8.08
2	15.97	10.92	9.45	8.11
3	15.12	10.56	9.36	8.02
4	15.53	10.91	9.22	7.90
5	15.22	10.34	9.10	7.78
RSD (%)	2.52	4.11	1.55	1.72

Figure Captions

Fig. 1. Electropherograms of a standard mixture at 0.1 mmol L^{-1} using 12 (I), 24 (II), 30 (III) and 36 (IV) mmol L^{-1} of $\text{Na}_2\text{B}_4\text{O}_7$ in the composition of the background electrolyte, also containing 12.0 mmol L^{-1} Brij 35, 33% v/v methanol, 17% v/v acetonitrile.

Instrumental Conditions: Cartridge temperature: 27°C ; voltage: $+27 \text{ kV}$; hydrodynamic injection of 25 mbar for 7 s, detection at 200 nm (2.5 Hz); capillary length: 48.5 cm (40 cm effective length), $50 \mu\text{m}$ i.d. and $375 \mu\text{m}$ o.d. Trans = t and cis = c.

Fig. 2. Electropherograms of a standard mixture at 0.1 mmol L^{-1} injected for (A) 3, (B) 5 and (C) 7 s

Electrophoretic Conditions: Cartridge temperature: 27°C ; voltage: $+27 \text{ kV}$, detection at 200 nm (2.5 Hz); capillary length: 33.5 cm (25 cm effective length), $50 \mu\text{m}$ i.d. and $375 \mu\text{m}$ o.d. Background electrolyte composition: 30 mmol L^{-1} $\text{Na}_2\text{B}_4\text{O}_7$, 12.0 mmol L^{-1} Brij 35, 33% v/v methanol, 17% v/v acetonitrile and hydrodynamic injection of 25 mbar. Trans = t and cis = c.

Fig. 3. Background electrolyte (BGE) signal and electropherograms of a fish oil sample injected alone (solid line) and spiked with a mixture of C18:1 trans-9 (Internal Standard, IS), eicosapentaenoic acid (C20:5 n3) and docosahexaenoic acid (C22:6 n3) (dotted line).

Electrophoretic Conditions: Cartridge temperature: 27°C ; voltage: $+27 \text{ kV}$, detection at 200 nm (2.5 Hz); capillary length: 33.5 cm (25 cm effective length), $50 \mu\text{m}$ i.d. and $375 \mu\text{m}$ o.d. BGE composition: 30 mmol L^{-1} $\text{Na}_2\text{B}_4\text{O}_7$, 12.0 mmol L^{-1} Brij 35, 33% v/v methanol, 17% v/v acetonitrile and hydrodynamic injection of 25 mbar for 5s. EOF: electroosmotic flow.

FIG 1

FIG 2

FIG 3

SUPPLEMENTARY MATERIAL S1

SUPPLEMENTARY MATERIAL S2