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Journal of Chromatography A, 771 (1997) 301–309

JOURNAL OF CHROMATOGRAPHY A

Determination of long-chained fatty acids using non-aqueous capillary electrophoresis and indirect UV detection

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Received 31 October 1996; revised 23 January 1997; accepted 12 February 1997

Abstract

A method for separation and determination of free saturated long-chained fatty acids (n-C_{14}–n-C_{26}) has been developed using non-aqueous capillary electrophoresis with indirect UV detection at 264 nm. The separation medium consisted of 2.5 m*M* anthraquinone-2-carboxylic acid and 40 m*M* Tris in N-methylformamide–dioxane (3:1, v/v). The electroosmotic mobility was about $3 \cdot 10^{-4}$ $cm^2/V\,s$ resulting in a separation time of about 15 min. Injection was done at the anode. Sorbic acid and the low mobility anthraquinone-2-sulphonic acid, 2,6-dihydroxy anthraquinone and all-*trans*-retinoic acid were less suitable as background absorbent. Maximum efficiency (up to $3.2 \cdot 10^5$ plates) were obtained in a 54 cm effective length×50 μm I.D. capillary at 300 V/cm. However, due to better sensitivity, 75 μm I.D. capillaries were preferred. Plate numbers of $1.4 \cdot 10^5$ were achieved in 75 μm I.D. capillaries of 46 cm effective length at 300 V/cm. Linear calibration curves were established for the fatty acids n-C_{16}–n-C_{20} (0.025–1.0 m*M*), n-C_{22} (0.025–0.6 m*M*) and n-C_{24}–n-C_{26} (0.025–0.3 m*M*) with correlation coefficients better than 0.985, using corrected peak area ratio and n-C_{14} fatty acid as internal standard. The concentration limits of detection were about 0.025 m*M*. The method has been applied for separation of the components in a hydrogenated fish oil, and for separation of dimeric and trimeric acids as well.

Keywords: Buffer composition; Fatty acids

1. Introduction

Long-chained fatty acids are usually determined by gas chromatography (GC) [1] or liquid chromatography (LC) [2]. Supercritical fluid chromatography (SFC) has also been suggested as a separation method [3,4]. The GC methods as well as most of the LC methods incorporate derivatization to obtain volatility and detectability, respectively. Even though these methods are widely used, alternative methods giving faster analysis, preferably without a derivatization step, are sought.

Capillary electrophoresis (CE) generally provides high efficiencies and fast analyses, and is therefore considered a potent alternative method.

Most CE methods use an aqueous electrolyte separation medium. However, the solubility of long-chained fatty acids in aqueous buffers is low, also when the water content is low. CE separations of fatty acids up to C_{18} have been achieved using 60% acetonitrile [5] or 60% methanol added cyclodextrin to increase solubility and selectivity [6]. A mixture of fatty acids up to C_{20} was separated in a water–

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PII S0021-9673(97)00183-0

acetonitrile medium added sodium dodecyl benzenesulfonate and Brij [7]. Fatty acids with longer chain lengths could not be satisfactory analyzed due to poor solubility in these media.

It therefore appears that a non-aqueous medium is required for electrophoretic separation of long-chained fatty acids. A few organic solvents have been investigated as electrophoresis media. Formamide, having a higher dielectric constant than water, has been shown to provide higher efficiencies and shorter analysis times than aqueous media [8]. N-Methylformamide (NMF), has an even higher dielectric constant and was recently reported to be a suitable separation medium in CE [9].

This work focuses upon the possibility of using a non-aqueous medium based upon NMF for the electrophoretic separation of long-chained saturated fatty acids. Since these compounds do not contain any usable chromophore, indirect UV detection was performed. The choice of an appropriate background absorbent as well as the limitations of the system upon quantitative determinations are discussed.

2. Experimental

2.1. Capillary electrophoresis system

The CE instrument was laboratory-built using mainly commercial available components and was run by an IBM compatible personal computer using a MC-MIO-16H-25 I/O-card from National Instruments (Austin, TX, USA). The high voltage source was a HCN 35–35 000 from FUG Electronik (Rosenheim, Germany) and the UV detector a Spectra 100 equipped with a capillary flow cell for on-column detection from Spectra-Physics. Hydrodynamic injection (10 cm) was performed using a pneumatically driven eight compartment autosampler. Mobilities were confirmed on a Beckman P/ACE 5500 system with temperature control.

Indirect detection was performed at 264 nm using anthraquinone-2-carboxylic acid as background absorbent (BGA). About 60% of the capillary was cooled by air in a sleeve, and the temperature stabilized at 6–7°C above the room temperature. The apparent pH (denoted by pH*) of the non-aqueous media were measured by a Flushtrode-P electrode from Hamilton (Bonaduz, Switzerland) using a Metrohm 654 pH meter (Herisau, Switzerland). Kinematic viscosities were measured using a calibrated Ubbelohde viscometer (±0.02°C).

All capillaries (50, 75 and 100 μm I.D.), obtained from Composite Metal Services (Hallow, Worcester, UK), were PTFE coated and had an outer diameter of 375 μm. The length from the detection point to the outlet was 21 cm. The total capillary length varied from 67 cm to 75 cm. Sample vials made of polypropylene were supplied by Elkay Products (Shrewsbury, MA, USA) and 0.45 μm Acrodisc or PFTE filters from Gelman Sciences (Northampton, UK) were used.

2.2. Chemicals

NMF (purum), sorbic acid (purum) and dioxane (puriss, absolute) were purchased from Fluka. Anthraquinone-2-carboxylic acid (98%), anthraquinone-2-sulphonic acid (97%) and 2,6-dihydroxy anthraquinone (97%) were supplied by Aldrich and Tris (enzyme grade) from Gibco BRL, Life Techn. (Gaithersburg, MD, USA). Saturated fatty acids (*n*-C_{14}–*n*-C_{20} and *n*-C_{24}) and retinoic acid (all trans, 97%) were all from Sigma. The fatty acids *n*-C_{26}, 2-hydroxy-C_{16} and 2-hydroxy-C_{22} were obtained from Larodan Fine Chemicals and *n*-C_{22} from Alltech. Trimeric and dimeric acids, as well as hydrogenated fish oil, were purchased from Chem. Service (West Chester, UK).

2.3. Separation media and standard solutions

The final separation medium consisted of 40 m*M* Tris and 2.5 m*M* anthraquinone-2-carboxylic acid in NMF–dioxane (3:1, v/v), pH*=10.4.

All standards and samples were dissolved in a 4.0 m*M* Tris, 0.25 m*M* anthraquinone-2-carboxylic acid in NMF–dioxane (3:1, v/v) solution.

Standard solutions for calibration (0.025, 0.05, 0.10, 0.30, 0.60 and 1.0 m*M*) of *n*-C_{16}–*n*-C_{26} and *n*-C_{14} as internal standard, were prepared from stock solutions of the acids. The following stock solutions were used: 4 m*M* of *n*-C_{14} dissolved in separation medium–NMF (2:1, v/v), 4 m*M* of *n*-C_{16}, *n*-C_{18} and *n*-C_{20} and 3 m*M* of *n*-C_{22} all dissolved in neat NMF,

1.5 m*M* of *n*-C_{24} and *n*-C_{26} both dissolved in neat dioxane.

2.4. Electrophoretic procedures

New capillaries were flushed with the separation medium for 1–2 h until a stable current was attained. Capillaries were conditioned daily with fresh separation medium for 10–30 min before an electric field was applied. The first injection was performed after the baseline had stabilized, usually after about 2 h. After the hydrodynamic injection, the capillary tip and the electrode were rinsed for 2 s in a wash vial before they were transferred to the separation medium. The outlet reservoir separation medium (10 ml) was changed daily while the inlet separation medium (1.5 ml) was changed several times during the day. Capillaries were stored in the separation medium over night and during weekends. All solutions were stored at room temperature and filtered at least 2 h before use. This procedure made it unnecessary to degas the non-aqueous solutions. All samples and separation media were temperature equilibrated in the injection compartment housing before use.

2.5. Calculations

Efficiency was calculated using Eq. (1).

$$N = 5.54(t_R/t_{W1/2})^2 \quad (1)$$

where t_R and $t_{W1/2}$ are the migration time and the width at half peak height, respectively.

Resolution was calculated using Eq. (2).

$$R_S = 2(t_2 - t_1)/(t_{W1} + t_{W2}) \quad (2)$$

where t_2 and t_1 are the migration time and t_{W1} and t_{W2} the width of the peak at baseline for component 1 and 2, respectively.

Internal standard calibration curves were based on time corrected peak areas.

The limit of detection (LOD) was calculated using a signal-to-noise (*S/N*) ratio of 3.

The electroosmotic flow (EOF) was determined using the migration time of the solvent peak.

3. Results and discussion

As the fatty acids have similar and high mass to charge ratio, their absolute mobilities (towards the anode) were less than the EOF in the investigated separation systems. Hence they could be transported to the detector by the EOF in the normal CE mode with injection at the anode.

3.1. Choice of solvent

Due to the amphiphilic character of the analytes, the solvent used in the separation medium should be able to both stabilize the ionized form and dissolve the hydrophobic chain. In order to achieve separation in an acceptable time, the EOF should be sufficiently large without being detrimental to the resolution. Since high electrolyte concentrations cannot be utilized without affecting the sensitivity in indirect detection, a solvent with high dielectric constant to viscosity ratio (ϵ_r/η) was required to obtain sufficient mobility. Amides have beneficially high dielectric constants, and proton acceptor properties. NMF, which has an ϵ_r of 182 [8] and a pK_a^* of 8 [9], was considered a potential candidate. The electroosmotic flow in NMF has been shown to be substantial even without added salts, due to the favorable ϵ_r/η ratio [9]. Separation of *n*-C_{14}–*n*-C_{22} fatty acids was achieved in less than 15 min in a NMF medium. However, the solubility of the long-chained fatty acids (C_n, $n>22$) was poor, making it necessary to add a modifier. Dioxane was chosen due to its proton acceptor properties [10]. Dioxane alone provides very high migration time of acids [11], but the remarkably high ϵ_r of NMF allows modifiers with very low ϵ_r. The high boiling points of dioxane and NMF are also favorable during stacking, preventing boiling due to thermal heating in the sample compartment. These solvents were compatible with the polypropylene vials used, since no interference in the electropherograms was observed as compared to glass vials.

3.2. Choice of background electrolyte (BGE) and absorbent (BGA)

The electrolyte composition in un-buffered solvents is very sensitive to electrolysis products, and a

pH change from 6 to 9 was reported for dimethylformamide (DMF) during 3 h of operation [12]. However, by adding a primary amine the reproducibility of EOF was improved. In this work Tris was chosen due to its low conductivity and favorable proton acceptor property ($pK_a = 8.5$). To prevent electrodispersion during the initial stage of separation, the concentration of background electrolyte (BGE) in the sample is usually kept less than 1/10 the concentration of BGE in the separation medium. Likewise, the ion strength of the sample should be less than 1/10 of the ion strength of the separation medium to obtain stacking. If this ratio is too low, however, diffusion at the zone boundaries will result in zone broadening [13]. Therefore an ion strength concentration ratio of 1/10 was used in this work. The Tris concentration chosen, 40 m*M*, was high enough to ensure an excess of Tris in the sample compartment even for highly concentrated samples.

To minimize peak distortion and poor transfer ratio due to differences in mobility between the BGA and the analytes, a BGA with similar mobility should be used [14,15]. Since the NMF–dioxane has a high UV cut-off, the BGA should have a UV absorbance maximum above 260 nm. None of the BGAs previously described in the literature combine these two properties, as most of them are used in aqueous media and for analytes of high mobilities [16–18]. Among others, sorbic acid has been used for detection of analytes with intermediate mobility [16,19]. However, the mobility of sorbic acid is also quite different from that of the long-chained fatty acids. The UV absorbance and the mobilities of other possible candidates were determined in the NMF–dioxane separation medium using sorbic acid as the BGA. When the sample compartment contained less BGA than the separation medium a large negative system peak appeared corresponding to the eigen mobility [20] of sorbic acid. This adds a third requirement to the choice of BGA, the BGA should not co-migrate with any of the analytes. The BGA candidates investigated in this work were all-*trans*-retinoic acid ($pK_a = 6–8$ [21]), anthraquinone-2-carboxylic acid ($pK_a = 3.37$ [22]), 2,6-dihydroxy-anthraquinone and anthraquinone-2-sulphonic acid ($pK_a = 0.38$ [23]). Both 2,6-dihydroxy-anthraquinone and retinoic acid co-migrated with the fatty acids and were hence abandoned. Anthraquinone-2-sulphonic acid has a lower molar absorbtivity and absolute mobility as compared to anthraquinone-2-carboxylic acid which was found to be a suitable BGA for the C_{14}–C_{26} fatty acids. A small shift in relative mobility of C_{14} and anthraquinone-2-carboxylic acid occurred when replacing the sorbic acid with anthraquinone-2- carboxylic acid as BGA. This change was confirmed on a Beckman P/ACE 5500 under temperature control (±0.1°C). The shift in relative mobility was probably due to differences in pH* of the medium caused by differences in pK_a values. The acids, both the investigated BGAs and the fatty acids, were expected to be completely ionized since the pH* of the separation media was in the range of 10–11, more than 5 pH units above their pK_a values. The pK_a of the *n*-C fatty acids and the 2-hydroxy fatty acids are about 4.8 and 3.8 [24], respectively. Hence the migration order was expected to be directly related to the molecular size. However, it is probable that the mobilities of the acids are related to their pK_a value in addition to their molecular size since the migration time of the 2-hydroxy C_{16} fatty acid was larger than that of *n*-C_{16}. This indicates that the pK_a^* values, and the difference in pK_a^* values, in NMF are larger than in aqueous solutions. Changes in pK_a of up to 4 units have been reported for benzene substituted carboxylic acids in non-aqueous media [25].

Separation of fatty acids and 2-hydroxy fatty acids in a 75 μm I.D. capillary is demonstrated in Fig. 1. The fatty acids were identified by standard addition.

The resolution between adjacent *n*-C fatty acids was larger than 2 and larger than 1 using the 50 and 75 μm I.D. capillaries, respectively (Fig. 2).

3.3. Detection

The high background signal in indirect detection generates increased noise compared to direct UV detection, and the *S/N* ratio was very sensitive to impurities, temperature variations and changes in solvent composition. Thus the sample should closely match the composition of the separation medium. An exception to this is the requirement of reduced ion strength to enhance stacking at low sample concentrations and reduce electrodispersion at high concentrations. The detection wavelength, the concentration of the BGA and the capillary I.D. were

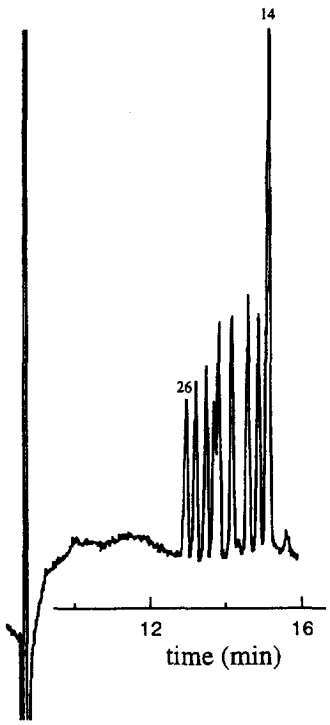

Fig. 1. Separation of fatty acids in a 67 cm×75 μm I.D. capillary using 20 kV. The migration order is: n-C_{26}, n-C_{24}, n-C_{22}, $C_{22\text{-}OH}$, n-C_{20}, n-C_{18}, n-C_{16}, $C_{16\text{-}OH}$ and n-C_{14}. Except for n-C_{14} (0.6 m*M*), the sample concentration of each acid was about 0.2 m*M* in a solution of 4.0 m*M* Tris and 0.25 m*M* anthraquinone-2-carboxylic acid in NMF–dioxane (3:1, v/v). The injection time was 6 s. The separation medium consisted of 40 m*M* Tris and 2.5 m*M* anthraquinone-2-carboxylic acid in NMF–dioxane (3:1, v/v). Detection was performed at 264 nm at L_{eff}=46 cm.

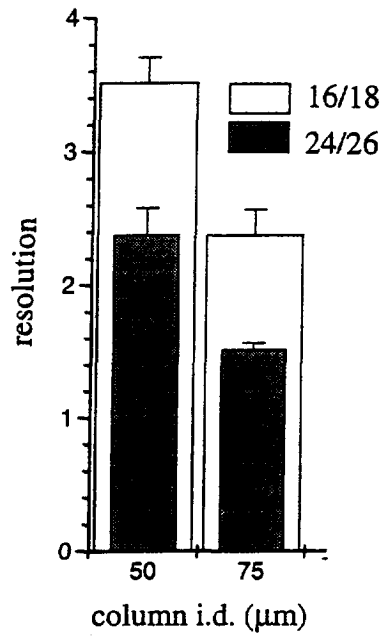

Fig. 2. Resolution between n-C_{26} and n-C_{24} (grey) and between n-C_{18} and n-C_{16} (white) using 50 and 75 μm I.D. capillaries. Separation medium were as in Fig. 1, while field strengths of 312 V/cm and 333 V/cm were used for the 50 μm (L_{eff}=60 cm) and 75 μm I.D. (L_{eff}=55 cm) capillaries, respectively. The mean and standard deviation of six replicates are presented.

optimized with respect to sensitivity and concentration LOD by monitoring corrected area, *S/N* ratio, efficiency, resolution and analysis time.

3.3.1. Detection wavelength and BGA concentration

The UV-maximum of anthraquinone-2-carboxylic acid was found to be 263 nm ($\epsilon_{263\ nm}$=25 480), and the solvent UV cut-off was about 260 nm. The optimum detection wavelength with respect to dynamic range was investigated by monitoring corrected areas at 264 nm and 275 nm, in the latter case the solvent contribution to noise was at a minimum. The reduction in noise, however, did not compensate for the reduction in dynamic range due to reduced molar absorptivity of anthraquinone-2-carboxylic acid at 275 nm. Hence 264 nm was considered to be the best choice of wavelength at the BGA concentration used, 2.5 m*M*. By lowering the BGA concentration to 1.25 m*M* a large reduction in signal response was found due to the loss in dynamic range. The reduced noise could not counterbalance this effect on the *S/N* ratio. No improvement in neither *S/N* ratio nor efficiency was observed by increasing the BGA concentration to 5 m*M*. However, the separation time increased considerably due to a reduction in EOF. This effect can be explained by an increased ionic strength and a pH* reduction which will both reduce the zeta potential at the wall. The mobilities of the fatty acids were little influenced by the ionic strength in the separation medium. Since the LOD in indirect UV detection is directly proportional to the concentration [26,27] an increase in BGA concentration does not expand the linear range. Hence increasing the BGA concentration would only move the range towards higher sample concentrations. Because the fatty acids have relatively low

solubility in this separation media, the low concentration range was preferred, and all further separations were carried out using 2.5 m*M* BGA.

3.3.2. Capillary

At low sample concentrations (less than 0.7 m*M*) efficiencies up to $3 \cdot 10^5$ plates were obtained using a 54 cm effective length 50 μm I.D. capillary at 300 V/cm. About 50% of the efficiency was lost by changing from a 50 to a 75 μm I.D. capillary. The loss in efficiency was less pronounced at high sample concentrations (1 m*M*) for which the efficiency was already low ($0.5–1 \cdot 10^5$ plates). However, due to better sensitivity and LOD using the 75 μm I.D. capillary (Table 1), this I.D. was preferred.

In this work the capillaries were conditioned using the separation medium. This method is frequently used in non-aqueous systems [12,28,29] to avoid introduction of new ions. No reduction in conditioning time was obtained using 0.01 *M* NaOH in NMF.

The efficiency is independent of capillary length as long as thermal and electrodispersive effects are negligible. The shortest possible capillary length was chosen (L_{eff} = 46 cm) to reduce contributions to plate height from diffusion. For the concentration range used in this work, the maximum applied field to achieve baseline resolution of the *n*-C_{26} and *n*-C_{24} using a capillary with L_{eff} = 46 cm was 298 V/cm. Using this field strength the resulting current was in the range of 3–5 μA. Linearity between current and field strength was obtained up to 470 V/cm, measured on the Beckman P/ACE 5500 system.

3.4. Validation

Injection volume as well as electrophoretic and electroosmotic mobility depends on the kinematic viscosity (η/ρ), which is strongly temperature dependent for organic solvents. The kinematic viscosity of NMF–dioxane (3:1, v/v) varied from 1.60 cSt at 25°C to 1.47 cSt at 30.5°C. The liquid expansion was found to be less than 3% in the temperature range from 18–29°C.

3.4.1. Reproducibility of migration times

Although the mobility was difficult to control under varying temperature conditions using the laboratory-built instrument, the migration time of analyte to migration time of an internal standard ratio remained relatively constant.

The C_{14} fatty acid was chosen as internal standard in this work and the R.S.D. of the migration time ratios were found to be less than 1.0% during a day and usually less than 0.3%.

3.4.2. Linearity

The peak area depends on the time spent in the detector cell and must be corrected for differences in migration rate when using internal standard quantification. Peak height calibration is difficult to achieve. At high sample concentrations the peak height depends on the degree of band broadening due to electrodispersion. This effect was most pronounced for components with migration rates very different from those of the BGA. In indirect detection mode

Table 1
Sensitivity, *S*, (slope × 10^3 of corrected area vs. concentration) of *n*-C_{16}, *n*-C_{18} and *n*-C_{20} fatty acids in the concentration range 0.1–1 m*M* using different background absorbents and capillary I.D.s

Fatty acid	Background absorbent					
	2.5 m*M* Sorbic acid		2.5 m*M* Anthraquinone-2-carboxylic acid			
	50 μm I.D.		50 μm I.D.		75 μm I.D.	
	S	R.S.D. (%)	*S*	R.S.D. (%)	*S*	R.S.D. (%)
n-C_{16}	52	5.7	90	3.2	218	4.7
n-C_{18}	47	7.3	88	2.7	204	3.7
n-C_{20}	42	13	n.d.	-	180	3.7

Field strengths of 312 V/cm and 335 V/cm were used for the 50 μm I.D. (80 cm) and 75 μm I.D. (67 cm) capillaries, respectively. Other parameters were the same in the three systems. Relative standard deviation, R.S.D. (%), was calculated from the standard deviation of the calibration curve.
n.d.: Not determined.

combined with sample stacking, a nonlinear relationship between peak height and sample concentration exists for low sample concentrations as well. This is due to the fact that the degree of stacking depends on the total ion strength in the sample compartment [30], which is sample dependent. The effective mobilities of analyte and BGA do also affect the stacking as a high transfer ratio will reduce the ion strength in the analyte zone [31]. Hence the influence of stacking and electrodispersion on peak height of the analyte and the internal standard will differ. When the fatty acid chain length increased, transfer ratio was reduced due to an increased difference in mobility between analyte and BGA. As a consequence the sensitivity of the larger fatty acids decreased, and this effect was most pronounced when peak height calibration was used. Calibration curves should therefore be established based on corrected peak areas, and not peak heights, although peak height precision can be better at low concentrations [32]. To reduce the uncertainty in quantitative determinations, the fatty acid with the mobility closest to that of the BGA, n-C_{14}, was chosen as internal standard. Replicates of seven different concentrations ($0.025_{n=6}$, $0.05_{n=1}$, $0.1_{n=6}$, $0.2_{n=1}$, $0.3_{n=6}$, $0.6_{n=1}$ and $1.0_{n=1}$ mM) of n-C_{16}–n-C_{20}, six different concentrations of n-C_{22} ($0.025_{n=6}$, $0.05_{n=1}$, $0.1_{n=6}$, $0.2_{n=1}$, $0.3_{n=6}$ and $0.6_{n=1}$ mM) and five different concentrations of n-C_{24} and n-C_{26} ($0.025_{n=6}$, $0.05_{n=1}$, $0.1_{n=6}$, $0.2_{n=1}$, $0.3_{n=6}$ mM) were injected (as a mixture) and their corrected area ratios plotted against concentration. The correlations were calculated by least square regression and found to be linear in the region 0.025–1.0 mM, with correlation coefficients (r) larger than 0.994 for n-C_{16} to n-C_{20}, but less (0.985) for the larger fatty acids. These values are expected to improve using a thermostatted system. In addition improvement in correlation factors is possible by using another background absorbent for the larger fatty acids. The calibration curves for n-C_{16} and n-C_{26} are shown in Fig. 3. The reduced sensitivity due to reduced transfer ratio of n-C_{26} as compared to n-C_{16} is obvious. The same trend has been observed by others [15]. The upper limit of the calibration curves was restricted by electrodispersion, due to higher ion strength in the sample compartment when the fatty acids were injected as a mixture. Thus the calibration

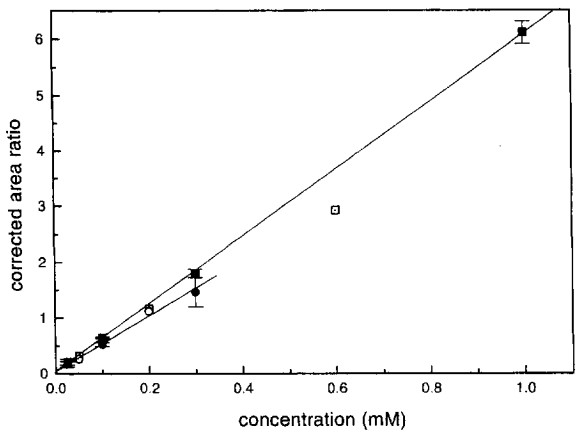

Fig. 3. Calibration curves of n-C_{16} (■) and n-C_{26} (●) using 0.15 mM n-C_{14} as internal standard. Open symbols are used for concentrations with one replicate. Separation conditions were as in Fig. 1. The injection times were 20 s for 0.025–0.05 mM solutions, 10 s for 0.1 mM solution and 5 s for 0.2–1 mM solutions.

curve of n-C_{22} alone was found to be linear up to 6.5 mM. The upper limit of the calibration curve was also influenced by the low solubility of the long fatty acids.

3.4.3. Limit of detection

The concentration limit of detection (cLOD) and the mass limit of detection (mLOD) were found to be 0.025 mM and about 50 fmol, respectively, using a 75 μm I.D. capillary.

The cLODs were determined using 10 s injection time. If the sample contains few ionic species, stacking can be achieved, the injection time increased and the cLOD reduced. On the other hand, trace determinations of an analyte in the presence of high concentrations of ionic species, may be difficult to achieve. Hence no further attempts were made to improve the cLODs. The sensitivity obtained using anthraquinone-2-carboxylic acid as BGA was better than that obtained using sorbic acid (Table 1). Previously reported cLODs for fatty acids in CE were $1–2 \cdot 10^{-6}$ M for n-C_8–n-C_{18} by indirect UV [6] and 700–800 ppb by indirect fluorescence [33].

3.4.4. Repeatability

Six replicate injections at four (three) concentration levels were performed. The results are pre-

sented in Table 2. The repeatability was satisfactory except for the longer fatty acids where electrodispersion result in poor resolution.

The main problem associated with this non-aqueous system was baseline instability and the long time (2 h) required for stabilizing the system. However, some of these problems are related to the indirect detection mode. This type of non-aqueous system may become very useful for separation of amphiphilic/hydrophobic components containing a chromophore, both in CE as well as electrochromatography.

3.5. Applications

A hydrogenated fish oil was analyzed (Fig. 4) using the same conditions as in Fig. 1. The five fatty acids n-C_{14}–n-C_{22} were identified by standard addition. In this case another internal standard than n-C_{14} should be applied for quantitation. Attempts to separate the components in a tall oil, containing isomers of both saturated and unsaturated fatty acids, were unsuccessful. The electropherogram showed only one broad peak.

One interesting feature of this method is that group separation of monomeric, dimeric and trimeric acid is possible as shown in Fig. 5. The migration behavior of the dimeric and trimeric acids are in accordance with their lower mass-to-charge ratio compared to the monomeric acids. The trimeric sample from Greyhound was found to contain dimeric acids and vice versa by this method.

Table 2
Repeatability

	Concentration (mM)	Corrected area ratio (mean)	R.S.D. (%)	n
C_{16}	0.025	0.21_1	24	6
	0.1	0.64_5	2.1	5
	0.3	1.80_0	4.2	6
	1.0	6.11_2	3.3	6
C_{18}	0.025	0.23_5	30	6
	0.1	0.62_2	3.7	5
	0.3	1.73_3	4.2	6
	1.0	5.77_4	5.2	6
C_{20}	0.025	0.20_7	24	6
	0.1	0.61_8	3.1	5
	0.3	1.70_5	8.1	6
	1.0	5.29_0	7.4	6
C_{22}	0.025	0.21_5	39	6
	0.1	0.57_4	3.6	5
	0.3	1.68_5	11	6
C_{24}	0.025	0.18_1	32	6
	0.1	0.57_3	7.3	5
	0.3	1.61_8	11	6
C_{26}	0.025	0.17_2	30	6
	0.1	0.52_6	6.3	5
	0.3	1.46_0	18	6

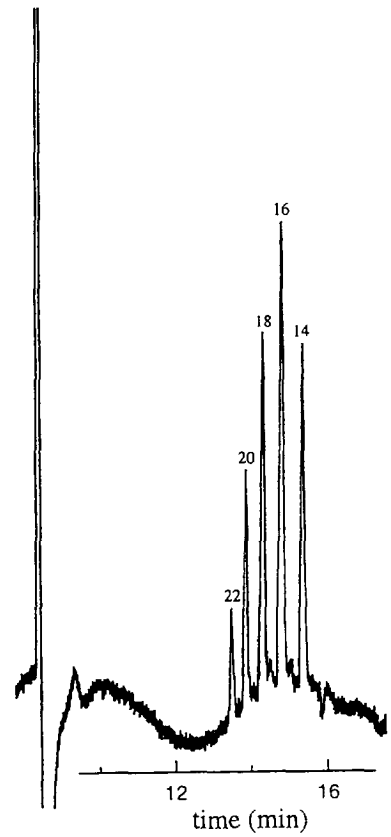

Fig. 4. Separation of a hydrogenated fish oil (0.454 mg/ml) added to 0.15 mM n-C_{14}. The separation conditions were as in Fig. 1. The injection time was 5 s.

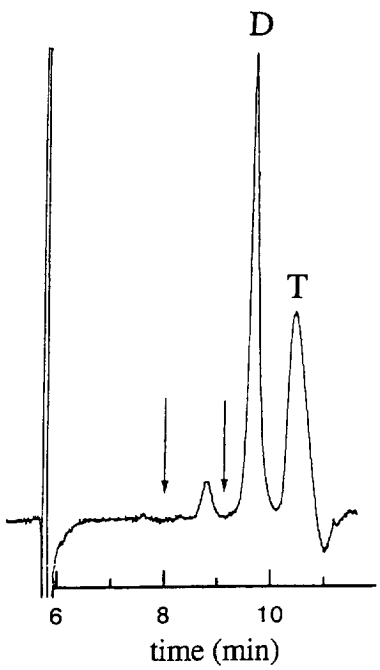

Fig. 5. Separation of dimeric (D) and trimeric (T) acids. Separation conditions were as in Fig. 1 except for the field strength which was 373 V/cm. The migration time window of the monomeric fatty acids is indicated with arrows.

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