Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 855 (2007) 152–158
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantitative gas chromatographic method for the analysis of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of the conjugated linoleic acid in liver

A. Zabala [a], M.P. Portillo [a], V. Navarro [a], M.T. Macarulla [a], L.J.R. Barron [b], A. Fernández-Quintela [a,*]

[a] *Department of Nutrition and Food Science, University of the Basque Country, Paseo de la Universidad 7, 01006 Vitoria, Spain*
[b] *Department of Food Technology, University of the Basque Country, Paseo de la Universidad 7, 01006 Vitoria, Spain*

Received 1 February 2007; accepted 25 April 2007
Available online 10 May 2007

Abstract

A quantitative GC method for conjugated linoleic acid (CLA) isomers of physiological significance (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) as non-esterified fatty acids (NEFA) or triacylglycerols (TAG) was developed. Furthermore, the effect of the internal standard addition point (sample or fat extract) was studied. Response linearity, recovery and precision assays, detection and quantification limits were determined. Linearity was demonstrated over a range from 0.1 to 10 μg/mL. When CLA isomers were present as NEFA, the recovery significantly decreased ($P \leq 0.05$) from 76% to 27.1% (*cis*-9, *trans*-11 CLA) and 28.5% (*trans*-10, *cis*-12 CLA) when the standards were added to the fat extract or to the initial tissue, respectively. As an application, liver samples from hamsters fed a diet supplemented with both CLA isomers were analyzed. The CLA isomers in liver samples were detected with reasonable reproducibility.

Keywords: Quantitative method; GC; CLA analysis; Liver

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of octadecadienoic acid first described by Dr Pariza's group in the 1980s [1]. These isomers possess conjugated double bonds (from 2, 4 – 18:2 to 15, 17 – 18:2) instead of the typical methylene interrupted configuration. The position of double bonds and the isomeric configuration (*cis* or *trans*) make numerous CLA isomers possible [2]. However, only a few are naturally found in foods such as ruminant meats, dairy products and processed cheeses, the *cis*-9, *trans*-11 CLA being the predominant isomer [3], and even fewer are commercially available.

In order to establish the complete isomeric distribution it is essential to use a combination of analytical methods (Ag^+-HPLC, GC/MS) [4–6]. However, in physiological and mechanistic studies only *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers are important at present, since beneficial effects including anticarcinogenic, antiatherogenic, antidiabetogenic, antiobesity and immunomodulatory activities have been attributed to them [7,8]. Therefore, Kramer et al. [9] suggested that a good GC analysis might be sufficient for their quantitative analysis when the study is applied to monogastric animals or humans.

Thus, considerable efforts have been made to supply data related to CLA isomer content in biological samples, most of them, however, being related as percentage of total fat [10–12] usually making it difficult to reach metabolic conclusions. This is because these isomers are always in limited amounts, which are linked to changes in the profile of the major fatty acids when they are expressed as normalized area. Nevertheless, when quantitative data are provided another doubt is raised, since a review of the literature shows that there is a lack of consistency concerning the addition point of the internal standard, it being applied either to the fat extract [13,14] or to the initial tissue [15,16].

* Corresponding author at: Nutrición y Bromatología, Facultad de Farmacia, Universidad del País Vasco, Paseo de la Universidad 7, 01006 Vitoria, Spain. Tel.: +34 945 013066; fax: +34 945 013014.
E-mail address: alfredo.fernandez@ehu.es (A. Fernández-Quintela).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.04.041

As a consequence, due to the potential health benefits of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers reported in the literature, accurate quantification of these fatty acids in biological samples is crucial in order to establish the biochemical mechanism(s) of the physiologically relevant CLA isomers, including their incorporation to the fat depots, and to definitively verify CLA-related response [17–19].

Therefore, the aim of this work was to describe a method that would provide accurate data of the CLA isomers of physiological significance (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) in liver as non-esterified fatty acids (NEFA), or in the main storage form, esterified as triacylglycerols (TAG), focusing on the incidence of the internal standard addition point.

2. Material and methods

2.1. Animals, diets and experimental design

Sixteen male Syrian Golden hamsters (105 + 1 g) purchased from Harlan Ibérica (Barcelona, Spain) were individually housed in polycarbonate metabolic cages (Techniplast Gazzada, Guguggiate, Italy) following procedures in accordance with the institution's guide for the care and use of laboratory animals. Animals were randomly assigned to two groups ($n = 8$) and fed semi-purified atherogenic diets supplemented with 0.5 g/100 g diet of *cis*-9, *trans*-11 (Diet A) or *trans*-10, *cis*-12 CLA (Diet B) in free fatty acid form, respectively. At the end of the experimental period, animals were killed under anaesthesia (diethyl ether) with cardiac puncture. Liver was dissected, weighed and immediately dropped in liquid nitrogen. Samples were stored at −80 °C until analysis.

2.2. Chemicals and gases

All reagents were of analytical grade (Merck, Sigma–Aldrich, Panreac). 99% purity standards of triacylglycerol and free fatty acid were purchased from Sigma–Aldrich except for CLA isomers which were acquired from Matreya (Matreya Inc., Pleasant GAP, PA, USA). Gases used were of 99.999% purity (Alphagaz, Air-Liquide, Spain).

2.3. Sampling and lipid extraction

Samples of 0.5–1 g of liver were accurately weighed in screw-capped glass tubes and, at this point, 1 mg of *n*-nonadecanoic acid (19:0) and 1 mg of trinonadecanoylglycerol were added as internal standards in dry form. After flushing the tubes with nitrogen, lipid extraction was carried out with trichloromethane:methanol (2:1, v/v) and the sealed tubes were incubated for 30 min at room temperature (20 °C) following the method described by Folch et al. [20]. Samples were flushed with nitrogen until dry and were dissolved in 1 mL of trichloromethane.

2.4. Separation

TAG and NEFA were separated by TLC on pre-coated silica plates (DC-Platten Kiesegel 60 F254, Merck, Frankfurt, Germany) with *n*-hexane:diethyl ether:glacial acetic acid 70:30:1 (v/v/v) for NEFA. The solvent mixture used for TAG was trichloromethane:*n*-hexane:glacial acetic acid 65:35:1 (v/v/v) to avoid migration problems. The lipids were visualised with Rhodamine 0.05% in absolute ethanol and bands were identified by using standards for TAG, trioleylglycerol, and NEFA, capric (10:0), palmitic (16:0) and arachidonic (20:4) acids to determine the R_F, scraped off, dissolved in trichloromethane and filtered, first through a glass wool and then through a syringe filter (Millipore, PVDF 0.22 μm 13 mm diameter). The sample obtained was dried under nitrogen and redissolved in 1 mL trichloromethane.

2.5. Methylation

Methylation of TAG: 500 μL of sodium methoxide in methanol (0.5 M) (Sigma–Aldrich, Steinheim, Germany) was added to lipid extracts in a screw-capped test tube to prepare the fatty acid methyl esters (FAMEs). The mixture was heated at 50 °C for 10 min and the reaction was stopped with 100 μL of glacial acetic acid. Water (5 mL) was added, vortexed and the required esters were extracted twice with *n*-hexane (5 mL). The *n*-hexane layer was dried over anhydrous sodium sulphate (Merck, Frankfurt, Germany) and filtered with a Whatman filter paper (#1). The solvent was removed under reduced pressure on a rotary evaporator (20 °C) and dissolved in 500 μL of *n*-heptane containing 50 ppm of butylated hydroxytoluene (BHT) as stabilizer [21]. Completion of methylation was checked by TLC with *n*-hexane:diethyl ether:glacial acetic acid 70:30:1 (v/v/v). Finally, samples were transferred into vials and stored at −80 °C until analysis.

Methylation of NEFA: 400 μL of (Trimethylsilyl)diazomethane (TMS-DM) in *n*-hexane (2 M) (Sigma–Aldrich) was added to lipid extracts. After 30 min at room temperature, the reaction was stopped with glacial acetic acid added dropwise (5–10 drops) until the yellow colour had gone. The solvent was evaporated under a gentle nitrogen stream, and samples reconstituted in 500 μL of *n*-heptane containing 50 ppm of BHT, and stored in capped vials at −80 °C [22]. Despite TMS-DM is a safer alternative source of diazomethane, the whole process was carried out in a fume-hood. Completion of methylation was also checked by TLC at the aforementioned conditions.

2.6. Analytical conditions

Samples were analyzed by GC using a Hewlett Packard HP6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), split/splitless injection port, HP7673 autosampler (Hewlett Packard). The analytical column was a fused silica capillary SP-2380 column (100 m, 0.32 mm i.d., 0.20 μm film thickness) from Supelco (Bellafonte, PA, USA). The oven temperature was initially programmed at 150 °C (hold 4 min) and raised to 220 °C at a 4 °C/min rate (hold 25 min). Injection (1 μL) was run in split (40:1) mode. Helium was the carrier gas at constant flow (1 mL/min) and make-up gas for the FID. The injector was maintained at 200 °C and detector at 250 °C. Chromatographic

data were evaluated with the HP Chemstation software data system.

FAMEs in liver samples were identified by comparison of similar peak retention times using pure fatty acid standards.

2.7. Quantification

Quantification procedure was based on peak areas using 19:0 and trinonadecanoylglycerol as internal standards for NEFA and TAG, respectively. Each TAG and NEFA standard was derivatized with methanolic sodium methoxide or TMS-DM as described above, and injected (1 μL) six times in order to obtain the response factors. The relative response factor (RRF) of each FAME was determined by the formula:

$$\mathrm{RRF_c} = \frac{A_{\mathrm{is}}}{A_{\mathrm{c}}} \times \frac{n_{\mathrm{c}}}{n_{\mathrm{is}}}$$

where A_c and A_{is} were the peak areas of studied compound and of the internal standard, respectively; n_c and n_{is} were the moles of studied compound and of the internal standard, respectively.

Method validation procedure included detector response linearity, recovery and precision assays, detection and quantification limits [23]. Linearity was determined by injecting three times quantities between 0.1 and 10 μg of selected fatty acid standards. Recovery trials were carried out for some fatty acids by adding different amounts of selected pure NEFA and TAG to the liver. The standards added to the samples were: palmitic (16:0), stearic (18:0) and docosahexahexaenoic (22:6 (*cis*-4,7,10,13,16,19)) acids, trilaurylglycerol, tripalmitoylglycerol, tristearoylglycerol, trioleylglycerol, trilinoleylglycerol and trilinolenoylglycerol. Each sample was analyzed four times. Further, a CLA recovery assay was performed. Two different mixtures of CLA isomers (NEFA) containing 90% of *cis*-9, *trans*-11, and 95% of *trans*-10, *cis*-12 CLA, as the main CLA isomers, respectively, as well as, two more mixtures of CLA isomers (TAG) containing 84% of *cis*-9, *trans*-11 and 84% of *trans*-10, *cis*-12 CLA were added both to the initial tissue (liver) and to the fat extract from the same sample obtained after the Folch method.

Chromatographic and analytical method intra-assay precision (assay repeatability) was evaluated. For chromatographic method, six injections were done in a blank vial. Analytical method repeatability was checked and five complete analyses for each sample were done.

The limit of detection (LOD) and limit of quantification (LOQ) were determined by measuring noise based on area values. The average noise value was determined by analysing six blanks. Therefore, LOD for the chromatographic peaks was established by multiplying the average noise area by 2 and LOQ by 5.

2.8. Statistical analysis

The SPSS statistical package, version 14.0 (SPSS Inc. Chicago, IL, USA) was used for the statistical analysis. Linear regression analysis was applied to fit the increase of absolute peak area with standard concentration. Student's *t*-test was used to evaluate the significance of the differences ($P \leq 0.05$) in fatty acid composition of hepatic non-esterified fatty acids between hamsters fed experimental diets.

3. Results and discussion

This paper focuses on validation aspects of the quantification method for fatty acid composition in liver, mainly in the internal

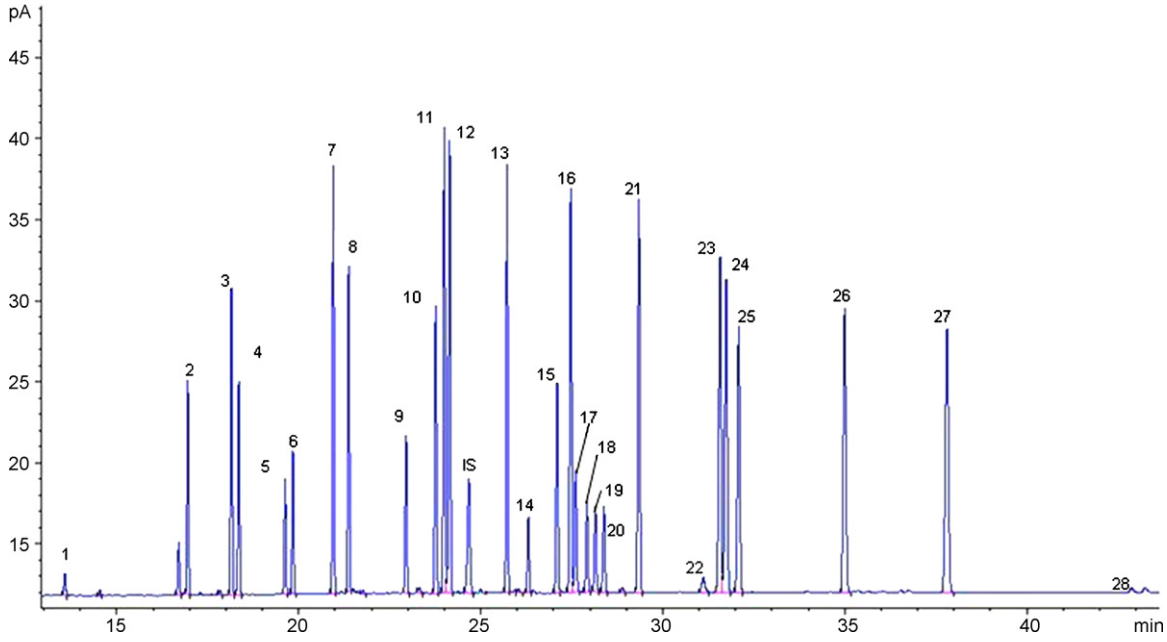

Fig. 1. GC chromatogram of a mix of FAME standards. Chromatographic conditions: SP-2380 column (100 m, 0.32 mm i.d., 0.20 μm film thickness). The oven temperature was initially programmed at 150 °C (hold 4 min) and raised to 220 °C at a rate of 4 °C/min (hold 25 min). The carrier gas and make-up gas for the FID was helium at constant flow (1 mL/min). The injector and detector temperatures were maintained at 200 and 250 °C, respectively. For peak identification see Table 1. IS: internal standard.

standard addition point and the recovery of the method. This issue is especially relevant when very small amounts of fatty acids are measured, as occurs with CLA isomers in biological tissues. Quantitative data are achievable mainly by means of an internal standard. The internal standard choice in our study was 19:0 and trinonadecanoylglycerol instead of that more used in the literature: *n*-heptadecanoic (17:0) acid. Kramer et al. [24] suggest 17:0 as internal standard for tissue lipids analysis due to the lack of resolution between 19:0 with oleic (18:1 (*cis*-9)) and γ-linoleic (18:2 (*cis*-9,12)) acids. However, we found small amounts of 17:0 in biological samples (i.e. liver and muscle) [25,26]. This fact makes 17:0 inappropriate for studies related to lipid metabolism reporting quantitative data. Moreover, in our experimental conditions relative retention (α) values were 0.98 between *trans*-vaccenic acid (18:1 (*trans*-11)) and 19:0 FAMEs, and 0.96 for 19:0 and 18:2 (cis-9,12) FAMEs (Fig. 1).

Internal standard quantitative procedure is based on the relationship between the responses factors of each fatty acid and that of the internal standard (RRF). Therefore, RRF for each fatty acid was determined both for NEFA and TAG (Tables 1 and 2). Mean of RRF values were close to 1.0 except for docosatetraenoic acid (22:4 (*cis*-7,10,13,16)) (1.60) and *trans*-9, *trans*-11 CLA (2.29). The relative standard deviation was, in general, lower than 9%, with a value of 12.2% for eicosapentaenoic acid (20:5 (*cis*-5,8,11,14,17)).

Table 1
Mean values of relative response factor (RRF) for non-esterified fatty acid (NEFA) pure standards (internal standard: 19:0)

Peak #	Fatty acid standard	RRF	RSD%
1	C12:0	1.04	6.26
2	C14:0	0.67	6.98
3	C14:1 (*cis*-9)	1.03	3.17
4	C15:0	0.97	8.51
5	C15:1 (*cis*-10)	1.73	4.70
6	C16:0	0.90	2.74
7	C16:1 (*cis*-9)	0.73	2.48
8	C17:0	1.04	3.85
9	C18:0	0.95	3.39
10	C18:1 (*trans*-11)	0.94	5.10
11	C18:1 (*cis*-9)	0.88	0.40
12	C18:1 (*cis*-11)	0.78	1.24
13	C18:2 (*cis*-9,12)	0.86	4.40
14	C18:3 (*cis*-9,12,15)	0.86	2.06
15	*cis*-9, *trans*-11 CLA	1.14	1.03
16	C21:0	1.00	4.27
17	*trans*-10, *cis*-12 CLA	1.30	8.65
18	*trans*-9, *trans*-11 CLA	2.29	3.22
19	*cis*-9, *cis*-11 CLA	1.97	0.82
20	C20:2 (*cis*-13,16)	1.33	2.50
21	C22:0	0.87	6.32
22	C20:3 (*cis*-11,14,17)	1.18	4.76
23	C20:4 (*cis*-5,8,11,14)	0.81	3.83
24	C22:2 (*cis*-13,16)	1.37	6.34
25	C20:5 (*cis*-5,8,11,14,17)	0.75	12.16
26	C24:1 (*cis*-15)	1.01	2.38
27	C22:4 (*cis*-7,10,13,16)	1.60	5.38
28	C22:6 (*cis*-4,7,10,13,16,19)	1.13	7.82

RSD: relative standard deviation.

Table 2
Mean values of relative response factor (RRF) for fatty acids esterified in triacylglycerols (FA-TAG) pure standards (internal standard: trinonadecanoylglycerol)

FA-TAG	RRF	RSD%
C12:0	0.75	7.57
C14:0	1.27	8.94
C16:0	1.01	6.47
C16:1 (*cis*-9)	1.18	2.58
C18:0	1.01	0.75
C18:1 (*cis*-9)	0.79	7.32
C18:1 (*trans*-9)	0.79	4.43
C18:2 (*cis*-9,12)	1.31	9.91
C18:3 (*cis*-9,12,15)	0.91	0.21
C20:0	0.98	9.08
C20:4 (*cis*-5,8,11,14)	1.11	2.10
C22:0	1.21	9.71
C22:1 (*cis*-11)	1.48	5.47
trans-10, *cis*-12 CLA	0.95	4.08
cis-9, *trans*-11 CLA	0.99	7.46

RSD: relative standard deviation.

Linearity values are presented in Table 3. The coefficient of determination (R^2) values was higher than 0.994 for the fatty acids studied. The slopes of the regression lines were similar for all the fatty acids, however, that of the fatty acids in TAG form showed less response, and consequently lower slopes than those of NEFA, probably due to differences in the molecular weight of TAG as compared to NEFA. The response–concentration ratio of the two CLA isomers was also determined, showing relative standard deviation (RSD) values of ∼4.40% and ∼3.47% for *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomer, when considered as NEFA, and ∼4.67% and ∼1.98% for *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in TAG form.

Results of LOD and LOQ were calculated from the mean noise (0.0275 pA s). Detectable peaks were considered from 0.055 pA s, and quantifiable peaks from 0.1375 pA s. These values were obtained in the case of CLA isomers either in NEFA or in TAG form. Thus, LOD values were 0.06 and 0.07 μg for the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA NEFA, respectively, and when TAG were considered, both isomers showed the same LOD (0.05 μg). LOQ values were as follows: 0.16 and 0.18 μg for the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, respectively, and 0.14 and 0.13 μg for *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers in TAG form.

Table 3
Estimated regression parameters for some non-esterified fatty acids (NEFA) and fatty acids esterified in triacylglycerols (FA-TAG) standards in the linearity study

	Equation	R^2
NEFA		
19:0	$A = 444.6c + 6.02$	0.994
trans-10, *cis*-12 CLA	$A = 532.46c - 33.87$	0.999
cis-9, *trans*-11 CLA	$A = 396.65c - 9.83$	0.999
FA-TAG		
18:1 *cis*-9	$A = 283.28c + 79.86$	0.997
trans-10, *cis*-12 CLA	$A = 260.58c - 28.25$	0.999
cis-9, *trans*-11 CLA	$A = 237.46c - 2.47$	0.998

R^2, coefficient of determination; A, area; c, amount (μg).

Table 4
Mean percentages of non-esterified fatty acids (NEFA) recovery in liver samples from repeated analysis of the same sample ($n = 4$)

	Mean	RSD%
C12:0	69.97	7.40
C16:0	95.61	4.22
C18:0	99.53	8.38
C19:0 (IS)	87.54	19.71
C18:1 (*cis*-9)	77.80	20.37
C18:2 (*cis*-9,12)	58.93	0.70
C18:3 (*cis*-9,12,15)	79.39	7.82
C22:6 (*cis*-4,7,10,13,16,19)	73.67	9.20

RSD: relative standard deviation; IS: internal standard.

Table 5
Mean percentages of fatty acids esterified in triacylglycerols (FA-TAG) recovery in liver samples from repeated analysis of the same sample ($n = 4$)

	Recovery (%)	RSD%
Trilaurylglycerol	70.37	7.47
Tripalmitoylglycerol	94.26	6.83
Tristearoylglycerol	96.29	5.32
Trinonadecanoylglycerol (IS)	88.93	9.39
Trioleylglycerol	71.88	6.14
Trilinoleylglycerol	89.24	4.97
Trilinolenoylglycerol	87.68	6.36

RSD: relative standard deviation; IS: internal standard.

Recovery trials were performed by adding different amounts of pure NEFA and TAG to the liver (Tables 4 and 5). The recovery data of the internal standard was close to 90% either in NEFA form or in TAG form. In general, NEFA produced poorer recoveries than TAG. The lower value was approximately 59% for 18:2 (*cis*-9,12) as NEFA, the mean recovery value being 83% for the rest of NEFA. For TAG, recovery values ranged from 88% to 96% except for trilaurylglycerol (70.4%) and trioleylglycerol (71.9%).

On the other hand, recovery of the analytes is of pivotal importance for all the fatty acids, but those with conjugated double bounds or other labile fatty acids can be more susceptible to some losses in the quantitative procedure. A review of the literature brings up the fact that the internal standard is added to the fat extract [13,14] instead of to the initial tissue [15,16,23]. This could lead to some mistakes, especially if the extraction yield is not assessed. Thus, in order to assay CLA recovery two different experiments were performed, and CLA isomers, as NEFA or TAG form, were added both to the initial tissue (liver) and to the fat extract.

The recovery observed in both experiments (with the standard added either to the tissue or to the fat extract) was about 90–95% for the CLA isomers in TAG form showing no statistical differences ($P > 0.05$). Nevertheless, when CLA isomers were present as NEFA, the recovery was about 76% when the standard was added to the fat extract, and the recovery significantly decreased to 27.1% and 28.5% ($P \leq 0.05$) for *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, when the standard was added to the initial tissue (Fig. 2). This remarkable decrease in CLA recovery must be taken into account in order to make an accurate quantification of CLAs as NEFA in biological tissues.

In order to measure the precision of the chromatographic method, liver from hamsters fed atherogenic diets supplemented with 0.5 g/100 g diet of 18:2 (*cis*-9,12), *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA, were analyzed (Figs. 3 and 4). The intra-assay precision (repeatability) of the chromatographic method showed a mean RSD ~1.34%, and that for the analytical method was ≤10% for each fatty acid. Considering the long experimental procedure employed and the lack of uniformity attached to biological matrices, these values can be considered acceptable.

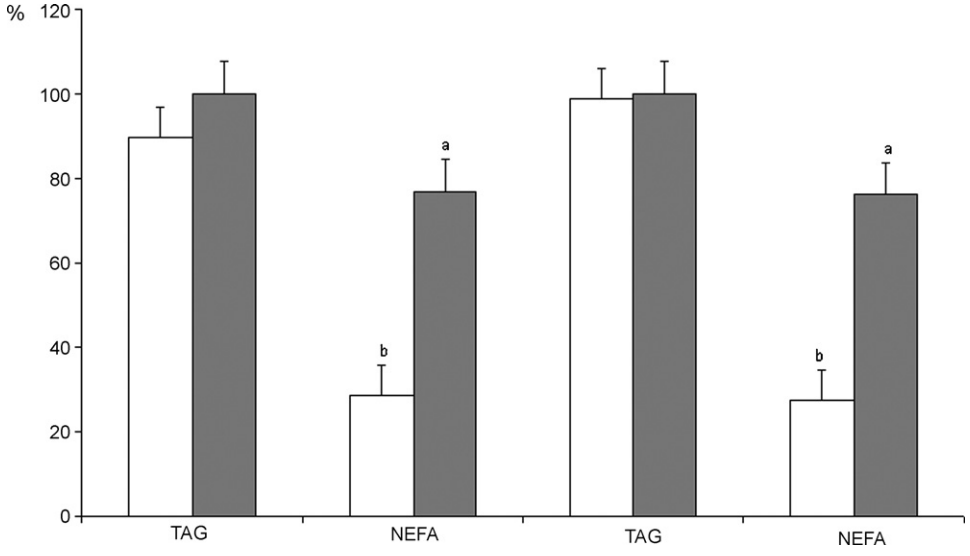

Fig. 2. Mean recovery data (%) and standard deviation of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers from liver when they are added to the fat extract (white bars) or to the initial tissue (grey bars) as non-esterified fatty acid (NEFA) or as triacylglycerol (TAG). Chromatographic conditions are as Fig. 1. ([a,b]) Bars with different letters are statistically different ($P \leq 0.05$).

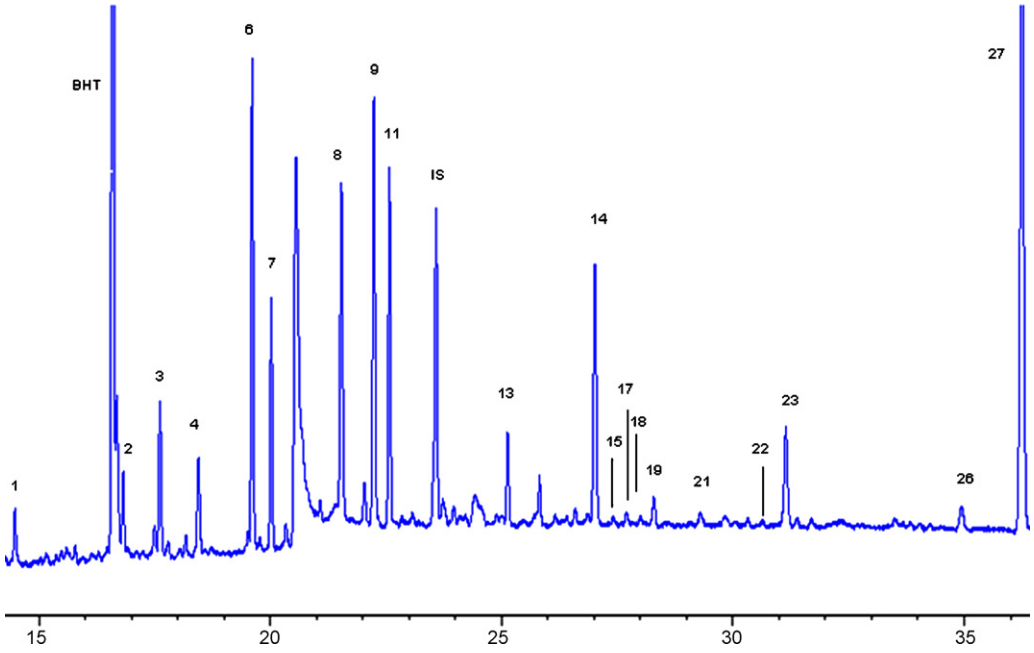

Fig. 3. GC chromatogram of FAMEs of non-esterified fatty acids in liver obtained from hamsters fed semi-purified atherogenic diets supplemented with 0.5 g/100 g diet of *trans*-10, *cis*-12 CLA. Chromatographic conditions are as Fig. 1. Solutes (mg/g tissue): 1: C12:0 (0.008); 2: C14:0 (0.015); 3: C14:1 (*cis*-9) (0.036); 4: C15:0 (0.022); 6: C16:0 (0.101); 7: C16:1 (*cis*-9) (0.048); 8: C17:0 (0.064); 9: C18:0 (0.079); 11: C18:1 (*cis*-9) (0.070); IS: internal standard; 13: C18:2 (*cis*-9,12) (0.029); 14: C18:3 (*cis*-9,12,15) (0.042); 15: *cis*-9, *trans*-11 CLA (0.001); 17: *trans*-10, *cis*-12 CLA (0.011); 18: *trans*-9, *trans*-11 CLA (traces); 19: *cis*-9, *trans*-11 CLA (0.019); 21: C22:0 (0.010); 22: C20:3 (*cis*-11,14,17) (0.018); 23: C20:4 (*cis*-5,8,11,14) (0.013); 26: C24:1 (*cis*-15) (0.021); 27: C22:4 (*cis*-7,10,13,16) (0.103).

NEFA profile in liver is shown in Table 6. The more abundant fatty acids in liver were 18:1 (*cis*-9), 16:0, 22:6 (*cis*-4,7,10,13,16,19), 18:2 (*cis*-9,12) and 18:0. With regard to CLAs, the present results show a different pattern of CLA isomer incorporation, consisting of a greater appearance of the *trans*-10, *cis*-12 isomer, with similar amounts independently of the proportion of this isomer in the diet.

In conclusion, this analytical method provides valuable data to quantify TAG and NEFA in liver for a broad variety of fatty acids, including *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers. Taking into account the low recovery of CLA isomers in the non-esterified fatty acid fraction, when quantitative data of these CLA isomers as NEFA are required it is of pivotal importance the addition of the internal standard before the lipid extraction procedure.

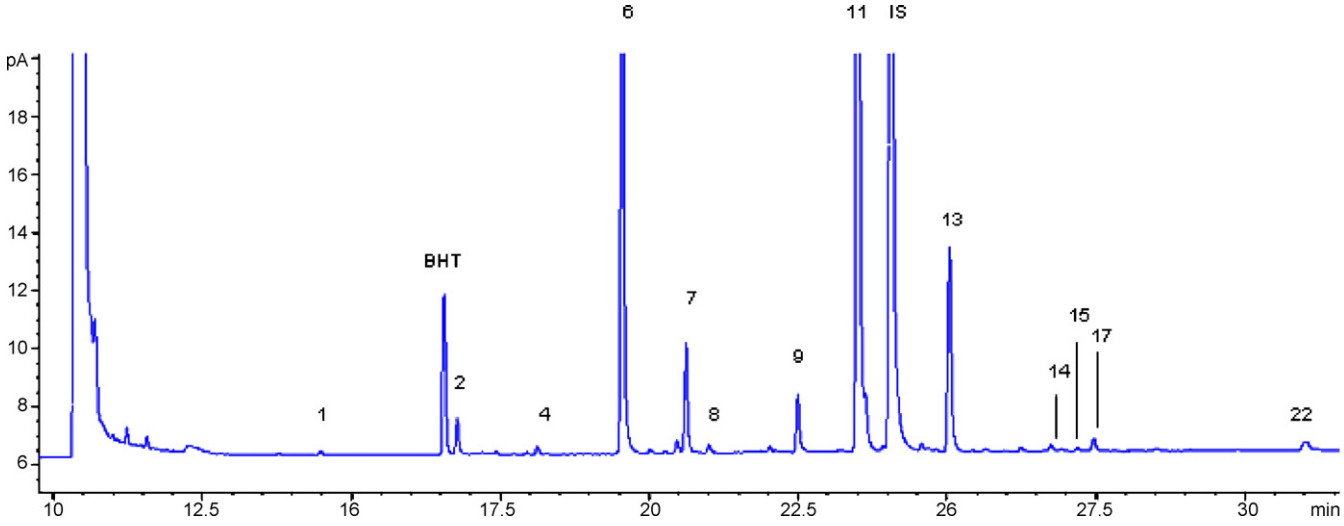

Fig. 4. GC chromatogram of FAMEs of fatty acids esterified in triacylglycerols in liver obtained from hamsters fed semi-purified atherogenic diets supplemented with 0.5 g/100 g diet of *trans*-10, *cis*-12 CLA. Chromatographic conditions are as Fig. 1. Solutes (mg/g tissue): 1: C12:0 (traces); 2: C14:0 (0.083); 4: C15:0 (0.007); 6: C16:0 (1.148); 7: C16:1 (*cis*-9) (0.310); 8: C17:0 (0.008); 9: C18:0 (0.164); 11: C18:1 (*cis*-9) (1.804); IS: internal standard; 13: C18:2 (*cis*-9,12) (0.750); 14: C18:3 (*cis*-9,12,15) (0.006); 15: *cis*-9, *trans*-11 CLA (0.010); 17: *trans*-10, *cis*-12 CLA (0.015); 22: C20:3 (*cis*-11,14,17) (0.033).

Table 6
Fatty acid composition (mg/g tissue) of hepatic non-esterified fatty acids (NEFA) of hamsters fed experimental diets supplemented with 0.5 g/100 g diet of *cis*-9, *trans*-11 (Diet A) or *trans*-10, *cis*-12 CLA (Diet B) for 6 weeks

	Diet A	Diet B
14:0	0.022 + 0.004	0.023 + 0.002
16:0	0.14 + 0.019	0.13 + 0.017
16:1 (*cis*-9)	0.018 + 0.003	0.023 + 0.005
17:0	0.017 + 0.002	0.010 + 0.001
18:0	0.07 + 0.007	0.06 + 0.005
18:1 (*cis*-9)	0.16 + 0.022	0.18 + 0.019
18:1 (*cis*-11)	0.005 + 0.001	0.005 + 0.000
18:2 (*cis*-9,12)	0.07 + 0.009	0.06 + 0.007
18:3 (*cis*-9,12,15)	0.009 + 0.001	0.009 + 0.001
20:0	tr	tr
20:3 (*cis*-5,8,11)	0.016 + 0.003	0.017 + 0.001
20:4 (*cis*-5,8,11,14)	0.004 + 0.001	0.004 + 0.001
22:0	0.008 + 0.002	0.008 + 0.001
22:4 (*cis*-7,10,13,16)	0.10 + 0.018	0.10 + 0.011
cis-9, *trans*-11 CLA	0.015 + 0.001[a]	0.004 + 0.001[b]
trans-10, *cis*-12 CLA	0.029 + 0.001[b]	0.032 + 0.001[a]

Mean values ± standard deviation. tr: Traces; ([a,b]) mean values within columns without a common superscript are significantly different ($P \leq 0.05$).

Acknowledgement

This study was supported by grants from the Ministerio de Ciencia y Tecnología (BFI2002-00273) and the Ministerio de Educación y Ciencia (AGL2005-02494).

References

[1] J. Ha, N.K. Grima, M.W. Pariza, Carcinogenesis 8 (1987) 1881.
[2] P. Delmonte, J.A.G. Roach, M.M. Mossoba, G. Losi, M.P. Yurawecz, Lipids 39 (2004) 185.
[3] J.M. Griinari, D.E. Bauman, in: M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, G.J. Nelson (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 1, AOCS Press, Champaign, IL, USA, 1999, p. 180.
[4] M.A. de la Fuente, P. Luna, M. Juárez, Trends Anal. Chem. 25 (2006) 917.
[5] C. Cruz-Hernandez, Z. Deng, J. Zhou, A.R. Hill, M.P. Yurawecz, P. Delmonte, M.M. Mossoba, M.E.R. Dugan, J.K.G. Kramer, J. AOAC Int. 87 (2004) 545.
[6] P. Delmonte, A. Kataoka, B.A. Corl, D.E. Bauman, M.P. Yurawecz, Lipids 40 (2005) 509.
[7] M.W. Pariza, Y. Park, M.E. Cook, Progr. Lipid Res. 40 (2001) 283.
[8] M.A. Belury, Annu. Nutr. Rev. 22 (2002) 505.
[9] J.K.G. Kramer, C. Cruz-Hernandez, Z. Deng, J. Zhou, G. Jahreis, M.E.R. Dugan, Am. J. Clin. Nutr. 79 (Suppl.) (2004) 1137S.
[10] P. Benito, G.J. Nelson, D.S. Kelley, G. Bartolini, P.C. Schmidt, V. Simon, Lipids 36 (2001) 229.
[11] E.J. Noone, H.M. Roche, A.P. Nugent, M.J. Gibney, Br. J. Nutr. 88 (2002) 243.
[12] B.A. Corl, D.M. Barbano, D.E. Bauman, C. Ip, J. Nutr. 133 (2003) 2893.
[13] N.C. Santha, E.A. Decker, B. Hennig, J. AOAC Int. 76 (1993) 644.
[14] J. Loor, X. Lin, J. Herbein, Br. J. Nutr. 90 (2003) 1039.
[15] G. Hynes, J. Heska, K. Chadee, P. Jones, J. Lipid Res. 44 (2003) 893.
[16] N. Shirai, H. Suzuki, S. Wada, Anal. Biochem. 343 (2005) 48.
[17] J. Fritsche, S. Fritsche, M.B. Solomon, M.M. Mossoba, M.P. Yurawecz, K. Morehouse, Y. Ku, Eur. J. Lipid Sci. Technol. 102 (2000) 667.
[18] A. Andersson, C. Nälsén, S. Tengblad, B. Vessby, Am. J. Clin. Nutr. 76 (2002) 1222.
[19] M.H. Gaíva, R.C. Couto, L.M. Oyama, G.E.C. Couto, V.L.F. Silveira, E.B. Ribeiro, C.M.O. Nascimento, Nutrition 19 (2003) 144.
[20] J. Folch, M. Lees, G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[21] W.W. Christie, Inform 12 (2001) 147.
[22] N. Sehat, M.P. Yurawecz, J.A. Roach, M.M. Mossoba, J.K.G. Kramer, Y. Ku, Lipids 33 (1998) 217.
[23] N. Aldai, K. Osoro, L.J.R. Barrón, A.I. Nájera, J. Chromatogr. A 1110 (2006) 133.
[24] J.K.G. Kramer, C. Cruz-Hernández, J. Zhou, Eur. J. Lipid Sci. Technol. 103 (2001) 594.
[25] M.T. Macarulla, A. Fernández-Quintela, A. Zabala, V. Navarro, E. Echevarria, I. Churruca, V.M. Rodríguez, M.P. Portillo, Nutrition 21 (2005) 512.
[26] A. Zabala, A. Fernández-Quintela, M.T. Macarulla, E. Simón, V.M. Rodríguez, V. Navarro, M.P. Portillo, Nutrition 22 (2006) 528.