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Procedure for the determination of retinol and α-tocopherol in poultry tissues using capillary gas chromatography with solvent venting injection

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Abstract

A procedure designed for the determination of retinol (vitamin A) and α-tocopherol (vitamin E) in poultry tissues has been developed. The procedure involves lipid extraction, saponification, solid-phase clean-up and capillary gas chromatography (cGC). Retinol and α-tocopherol were determined separately by cGC–flame ionisation detection using a fused-silica open tubular capillary column, 30 m×0.25 mm I.D. coated with 5% phenylmethylsilicone and with a film thickness of 0.25 μm. Solvent extraction followed by saponification were sufficient to provide a purified extract which was directly analyzed for retinol by cGC in the solvent venting mode. However, in order to accurately determine α-tocopherol by cGC, further purification of the extract by solid-phase extraction was necessary. A silica SPE column was used to remove interfering cholesterol from the extract. α-Tocopherol was analyzed in its derivatized form. Absolute and relative recoveries for both vitamins from spiked samples were evaluated. Absolute and relative recoveries ranging from 80 to 95% were obtained for both compounds. 5α-Cholestane and α-tocopheryl acetate were used as internal standards. Poultry muscle meat and liver tissue were analyzed for their retinol and α-tocopherol content and the peaks detected by cGC were confirmed by cGC–mass spectrometry. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Vitamins; Retinol; α-Tocopherol

1. Introduction

The analytical determination of retinol (vitamin A) and α-tocopherol (vitamin E) in tissues provides useful information about the concentration of these vitamins in animal or human tissues after e.g. a feeding trial. Until so far, high-performance liquid chromatography (HPLC) either in normal-phase or in reversed-phase mode has been the analytical tool mainly used for tocopherol and retinol analysis. Most of the work reported in the literature deals with the determination of these vitamins in serum. The assessment of the vitamins concentration in tissues such as muscles and organs is a more complex problem [1]. For the detection of retinol and tocopherols in plasma samples, rapid extraction with hexane or hexane–ethanol is performed while in most of the cases, the analysis of tissues and food products requires a saponification prior to detection [1–10]. The simultaneous detection of retinol and α-tocopherol by HPLC involves the use of a programmable multiwavelength detector or a dual wavelength detector in order to shift wavelengths during the HPLC run [3–5]. Capillary gas chromatography

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(cGC) has not been extensively applied to determine retinol and tocopherols in tissues [11]. Nonetheless, retinol and tocopherols were successfully analyzed by cGC either as such, or in their derivatized form [9–13]. On-column cGC was recommended in order to minimize the thermal degradation and/or dehydration of the retinoid [10,13]. The procedures used for retinol or α-tocopherol analysis involve the saponification in order to hydrolyze the retinyl esters or tocopherol esters present in the organ, tissue or in animal feed and also help purifying the compounds of interest from triglycerides and phospholipids [6,7,9,11]. Retinoids are labile to oxygen and light and care must be taken during sample extraction and handling [11]. This paper proposes a procedure designed for the determination of retinol and α-tocopherol in poultry tissues. The procedure includes a saponification step and the use of solid-phase extraction (SPE). Retinol and α-tocopherol were detected by cGC. In contrast to the works of Furr and co-workers [12,13], the cGC analysis involved injection in solvent splitting mode (solvent venting). The efficiency of the procedure was evaluated by recovery experiments. Samples of chicken liver and muscle were analyzed for their vitamin A and E contents. cGC–mass spectrometry (MS) was also used to confirm the cGC detection of the vitamins in samples.

2. Experimental

2.1. Chemicals

α-Tocopherol, α-tocopheryl acetate, retinol, retinyl palmitate, 5α-cholestane, 2,6-di-*tert*.-butyl-4-methylphenol (BHT) and pyrogallol were purchased from Sigma (Madrid, Spain). ACS grade solvents were used. Chloroform, hexane, and diethylether for organic trace analysis were obtained from Merck (Darmstadt, Germany); ethanol, methanol and 2-propanol were from Panreac (Barcelona, Spain). Anhydrous Na_2SO_4 was obtained from Merck. SPE reservoirs were purchased from Varian Associates, (CA, USA). Muscle and liver tissues were purchased form a local supermarket.

2.2. Preparation of standards stock solutions

Stock solutions of α-tocopherol, α-tocopheryl acetate, retinol, retinyl acetate were prepared in 100% ethanol. Concentrations (μg/μl) of the standard solutions were determined by using the following extinction coefficients (E 1%/cm) [3]: retinol, 1780 at 325 nm; and α-tocopherol, 75.8 at 292 nm. Stock solution of retinyl palmitate was prepared in chloroform.

2.3. Sample pretreatment

Raw broiler muscle tissue (3 g) was transferred in a centrifugation tube and 5 g of anhydrous Na_2SO_4 were added. 5α-Cholestane (20 μg) was also added as analytical internal standard (I.S.). In an ice bath, the mixture was ground with 30 ml of hexane–isopropanol (3:2, v/v) and further homogenization was conducted by using a Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany) at speed 5 for 10 s. The extracting solvent contained 0.5% BHT and was flushed with nitrogen prior to use. The extract were centrifuged at 1500 *g* for 5 min. The supernatant was removed and stored at 4°C. The pellet was resuspended in 30 ml of hexane–isopropanol (3:2, v/v) and centrifuged at 3000 *g* for 5 min. The pooled extract were evaporated to dryness at a temperature not exceeding 40°C by means of a Büchi rotavapor (Switzerland). The residue was redissolved in 1 ml 100% EtOH in an amber screw-capped test tube. The final extracts were stored at −80°C under nitrogen.

2.4. Saponification of the extract (based on the procedure developed by Buttriss and Diplock 1)

A 2-ml volume of 1% pyrogallol in EtOH was added to the ethanolic extract. The mixture was equilibrated for 2 min at 70°C under nitrogen atmosphere. A 500-μl volume of 10 M $KOH_{(aq)}$ was then added and saponification was carried out at 70°C for 30 min under nitrogen atmosphere. After the completed reaction, the tube was ice-cooled. Distilled water (1 ml) and hexane (5 ml) were added and the mixture was blended. The hexane layer was recovered after centrifugation at 1500 *g* for 5 min. Extraction was repeated with 3 ml of hexane. The hexane extracts were pooled and evaporated under

vacuum. The residue was redissolved in 500 μl of hexane and 25 μg of α-tocopheryl acetate were added.

2.5. Determination of retinol by capillary gas chromatography

Retinol determination was performed by using a Dani gas chromatograph HR 3800-PTV equipped with flame ionization detection (FID). A fused-silica open tubular (FSOT) capillary column 30 m×0.25 mm I.D. coated with 5% phenylmethylsilicone (DB-5) and with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA, USA) was used. Helium was the carrier gas and delivered to the column at a head pressure of 2.1 bar. THe temperature programme used was: initial column temperature at 220°C programmed at a rate of 5°C/min to 310°C which was held for 16 min. Solvent venting injection was used [14,15]: the inlet was ballistically heated from 50 to 300°C which was held for 90 s. The split flow was 100 ml/min and the split valve was opened for 6 s and then closed for 60 s. A 1-μl volume of the saponified extract was injected. 5α-Cholestane was used as I.S.

2.6. Determination of α-tocopherol

2.6.1. Solid-phase extraction

SPE reservoirs were filled with 1 g of silica and conditioned with hexane. The saponified extract was applied to the column. A single fraction was eluted with 15 ml of hexane–diethyl ether (85:15, v/v). The eluted fraction was evaporated to dryness and redissolved in 200 μl of 33% hexadimethylsilazane (HMDS)+11% trimethylchlorosilane (TMCS) in pyridine (Sylon HTP, Supelco, Bellefonte, PA, USA) in an amber screw-capped test tube. Trimethylsilyl (TMS) ether derivatives were obtained by reaction for 1 h at 60°C. The silylating reagent was evaporated under nitrogen stream and the residue was redissolved in hexane.

2.6.2. Capillary gas chromatography

α-Tocopherol determination was performed by cGC as described in Section 2.5 except the helium head pressure was 1.2 bar. α-Tocopheryl acetate was the I.S.

2.7. Evaluation of the saponification step (saponification and recovery of the organic phase)

Each α-tocopherol and retinol (20 μg) was submitted to the saponification step described in Section 2.4. α-Tocopherol and retinol were recovered and monitored by cGC as described earlier. SPE was not applied in this case. Peak areas were compared with those obtained for external standards of α-tocopherol and retinol. In this case, α-tocopherol was not derivatized. A 20-μg amount of α-tocopheryl acetate and of 50 μg retinyl palmitate were also submitted to the saponification step.

2.8. Response factors

The response factors were calculated by varying standard α-tocopherol and retinol concentrations vs. a fixed concentration of the I.S.s (5α-cholestane for retinol and α-tocopheryl acetate for α-tocopherol).

2.9. Linearity and recoveries

A regression analysis was performed with five samples (2 g) spiked with 2, 10, 20, 40 μg of α-tocopherol and retinol. The recovered α-tocopherol and retinol were analyzed by cGC. 5α-Cholestane and α-tocopheryl acetate were used as I.S.s. Linear plot of concentration vs. peak areas were calculated. A blank sample was also run.

Another five samples (2 g) were spiked with 10 μg of retinol and α-tocopherol. Absolute and relative recoveries for each compound were evaluated. R.S.D. (relative standard deviation) values were determined. A nonspiked sample was analyzed for its retinol and α-tocopherol content in order to determine the amount of these compounds originally presents in the raw chicken meat.

2.10. Reproducibility of the solvent venting injection

A sample spiked with 10 μg of α-tocopherol and retinol was manually injected five times. R.S.D. values for retention times and peak areas were determined.

2.11. Application of the procedure to the detection of α-tocopherol and retinol in chicken liver by cGC and cGC–MS

Two samples of 1 g each from the same chicken liver were submitted to the entire procedure. The samples were analyzed for their α-tocopherol and retinol contents. Detection and confirmation of the peak identity were performed by cGC and cGC–MS, respectively. The mass spectra of α-tocopherol and retinol were obtained by cGC–MS in the total ion current (TIC) mode. cGC–MS analysis was performed under the following conditions:

(1) An HP 5890 gas chromatograph equipped with a capillary column coated with 5% phenylmethylsilicone (DB-5, 20 m×0.18 mm I.D., 0.18 μm film thickness) and coupled to an HP 5970 mass-selective detector (Hewlett-Packard, USA) by direct interface at 280°C was used. In this case, a two-step temperature program was applied: (a) initial column temperature at 80°C, held for 1 min and programmed to 250°C at a rate of 10°C/min and (b) final column temperature (280°C) reached by means of a temperature increase of 4°C/min and held for 20 min. The injector temperature was 270°C and the inlet pressure was 0.7 bar.

(2) A Fisons Instruments GC 8000 series equipped with a capillary column coated with 5% phenylmethylsilicone (Ultra II, 30 m×0.25 mm I.D., 0.25 μm film thickness; Hewlett-Packard) and coupled to an MS Fisons Instruments Trio 2000 by direct interface at 280°C was also used with a two-step temperature program: (a) initial column temperature at 80°C, held for 2 min and programmed to 250°C at a rate of 10°/min and (b) final temperature (280°C) reached by means of a temperature increase of 4°C/min and held for 19 min. The injector temperature was 290°C and the inlet pressure was 1.2 bar.

The samples were introduced onto the capillary columns by splitless injection (split valve closed for 1 min). The mass spectra of retinol and α-tocopherol were obtained in the electron-impact mode (70 eV).

3. Results and discussion

Careful extraction (low temperatures, amber tubes) followed by saponification provided an extract which was directly analyzed by cGC for its retinol content. A SPE clean-up of the extract was necessary to separate α-tocopherol from cholesterol. Both vitamins were analyzed by cGC. α-Tocopherol was analyzed in its TMS-derivatized form. The derivatization process decreased the thermal instability and improved the peak shape of α-tocopherol. It was preferable to analyse retinol in its native form since the derivatization may induce decomposition of the vitamin [16]. A high vaporization temperature used during injection caused the retinol to elute as a broad peak, possibly due to its conversion to anhydroretinol (Fig. 1A). Anhydroretinol is believed to consist of several isomers which could be responsible for the broadening of the peak. Nonetheless, the use of higher inlet pressures (>2 bar) gave a single sharper peak for retinol (Fig. 1B). Maybe a faster transfer of the solute into the capillary column reduced the residence time within the hot injector and minimized the conversion to anhydroretinol. Another explanation is that retinol was still analyzed as anhydroretinol but the high helium pressure reduced the resolution of its isomers causing the anhydroretinol to elute as a single peak. Retinol was analyzed by cGC–MS in the TIC mode and eluted as a single gaussian peak (Fig. 2). The TIC chromatogram did not show the presence of coeluting peaks as a result of the conversion of retinol to anhydroretinol (Fig. 2). Contrasting with the results of Furr et al. [12,13] and Clifford et al. [16], the ion at *m/z* 268 ($M-H_2O$), indicative of the dehydration of retinol in the ion source, was not the predominant ion. It seemed that the dehydration of retinol was minimized for both cGC and cGC-MS analyses. The molecular ion (*m/z* 286) and the fragmentation ion (*m/z* 255) were the major abundant ions characterizing retinol (Fig. 2). Moreover, the ion at *m/z* 268 was observed only when the liver samples were analyzed (Fig. 2). The latter fact suggests that retinol in the presence of other solutes is more subjected to dehydration either in the injection port or in the ion source. On-column injection for cGC and cGC–MS analyses was used by Furr et al. and Clifford et al. [12,13,16] whereas in the present study, solvent venting and splitless injection were used for cGC and cGC–MS analysis, respectively.

The recovery after the saponification step was evaluated and the two compounds were simultan-

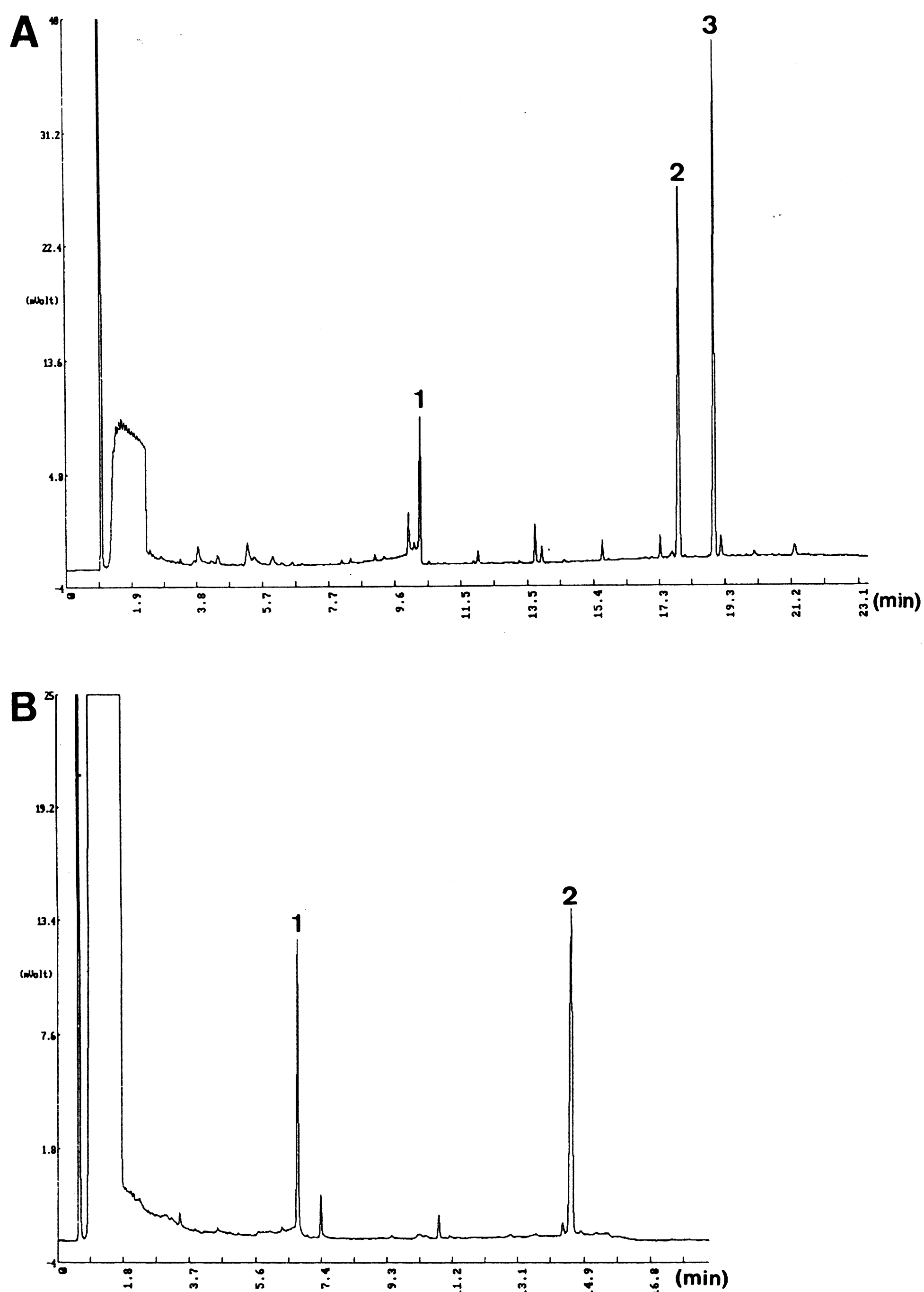

Fig. 1. cGC analysis of retinol and α-tocopherol. A FSOT capillary column of 30 m×0.25 mm I.D. coated with 5% phenylmethylsilicone and with a film thickness of 0.25 μm was used. Samples introduced by solvent venting injection. (A) Temperature program: 220→310°C (held for 8 min) at a rate of 5°C/min. Helium inlet pressure: 1.4 bar. 1=retinol; 2=α-tocopherol; 3=19-hydroxycholesterol. (B) Temperature program: 220→310°C (held for 2 min) at a rate of 5°C/min. Helium inlet pressure: 2.1 bar. 1=retinol; 2=α-tocopherol.

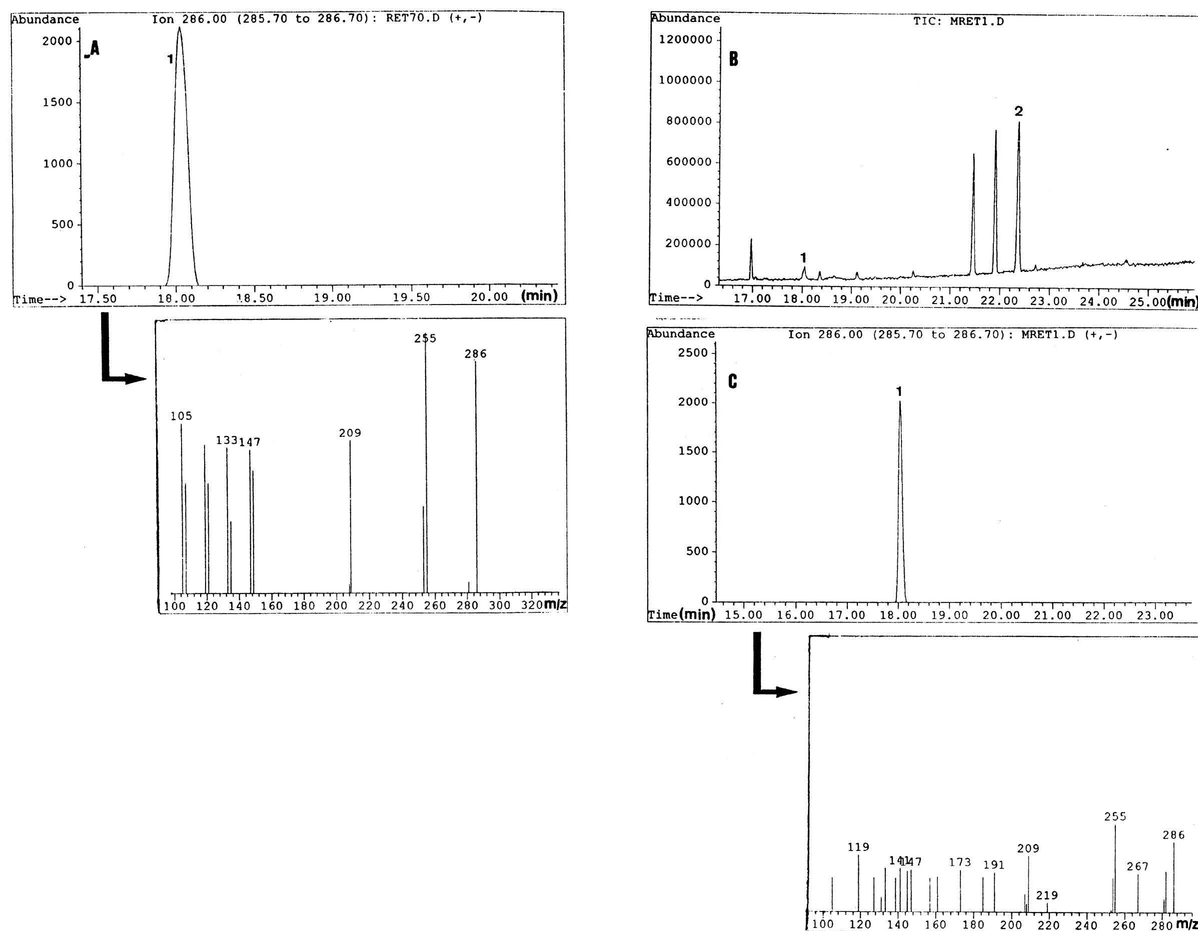

Fig. 2. cGC–MS analysis of retinol. (A) cGC–MS chromatogram of retinol standard reconstructed for m/z 286 and mass spectrum obtained by cGC–MS in the TIC mode; (B) liver sample (TIC mode); (C) identification of the retinol present in the liver sample. cGC–MS chromatogram reconstructed for m/z 286. 1=Retinol; 2=5α-cholestane. Analytical conditions as described in Section 2.

eously analyzed by cGC without previous derivatization. The retinol recovery was less (80%) than that of α-tocopherol (89%), maybe due to its greater thermal instability (Table 1). Therefore, large amounts of added pyrogallol with BHT (in the extracting solvent) prevented the destruction of α-tocopherol and retinol in the presence of alkali and high temperatures [17,18]. Buttriss and Diplock reported a 95% recovery of α-tocopherol after the saponification at 70°C for 30 min with recovery performed with hexane [1]. Results were quite reproducible as R.S.D. values were lower than 2% (Table 1). The conditions used for saponification were found to be adequate since the retinyl palmitate

Table 1
Revoveries for retinol and tocopherol after the saponification step (saponification+recovery of the organic phase)

	Recovery (%)	
	Retinol	α-Tocopherol
Sample 1	81.50	90.00
Sample 2	80.90	88.84
Sample 3	78.57	88.42
Mean	80.32	89.09
R.S.D. (%)	1.9	0.92

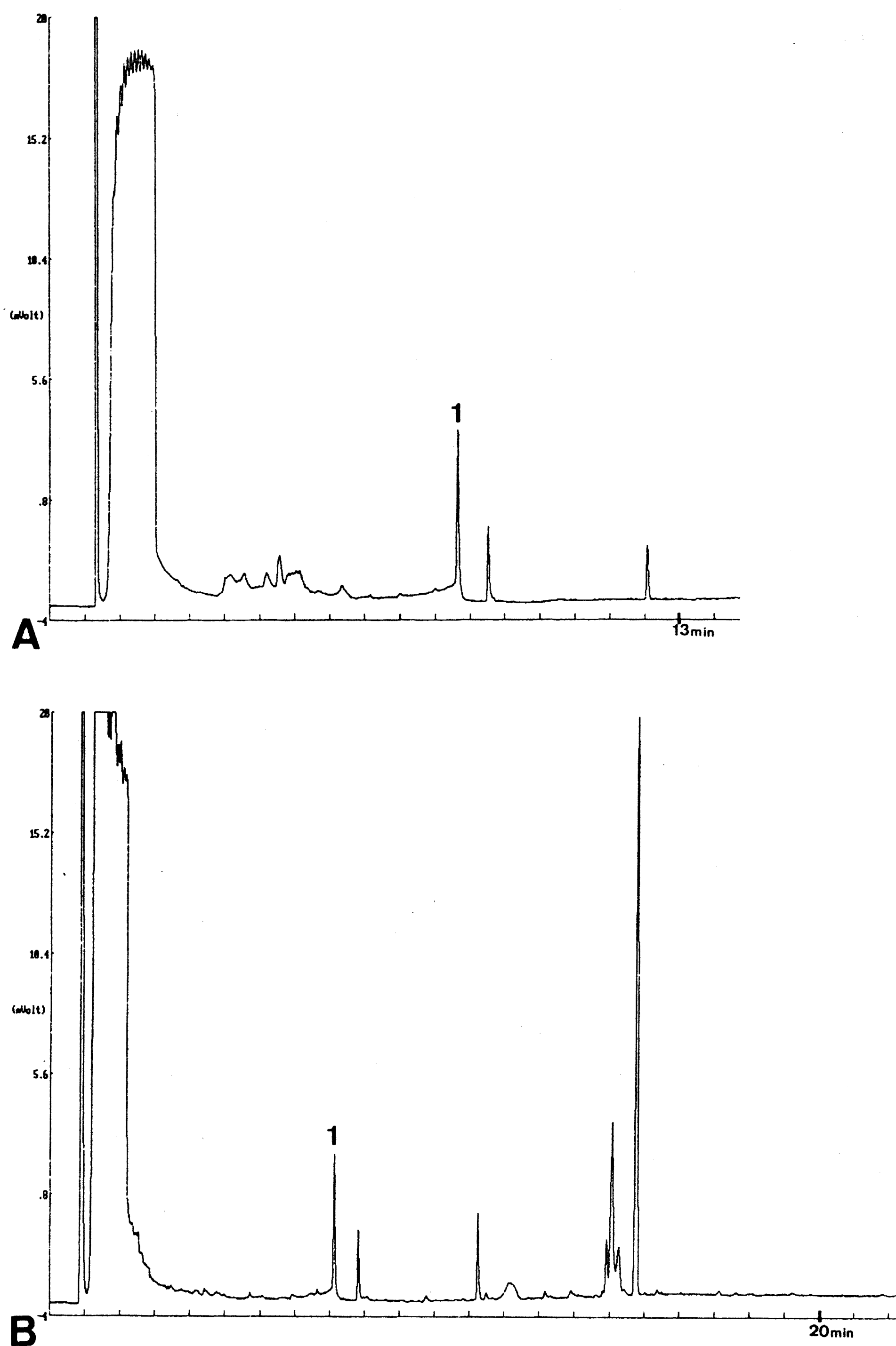

Fig. 3. Efficiency of saponification; (A) retinol standard; (B) hydrolysis of retinyl palmitate; 1=retinol. Analytical conditions as described in Section 2.

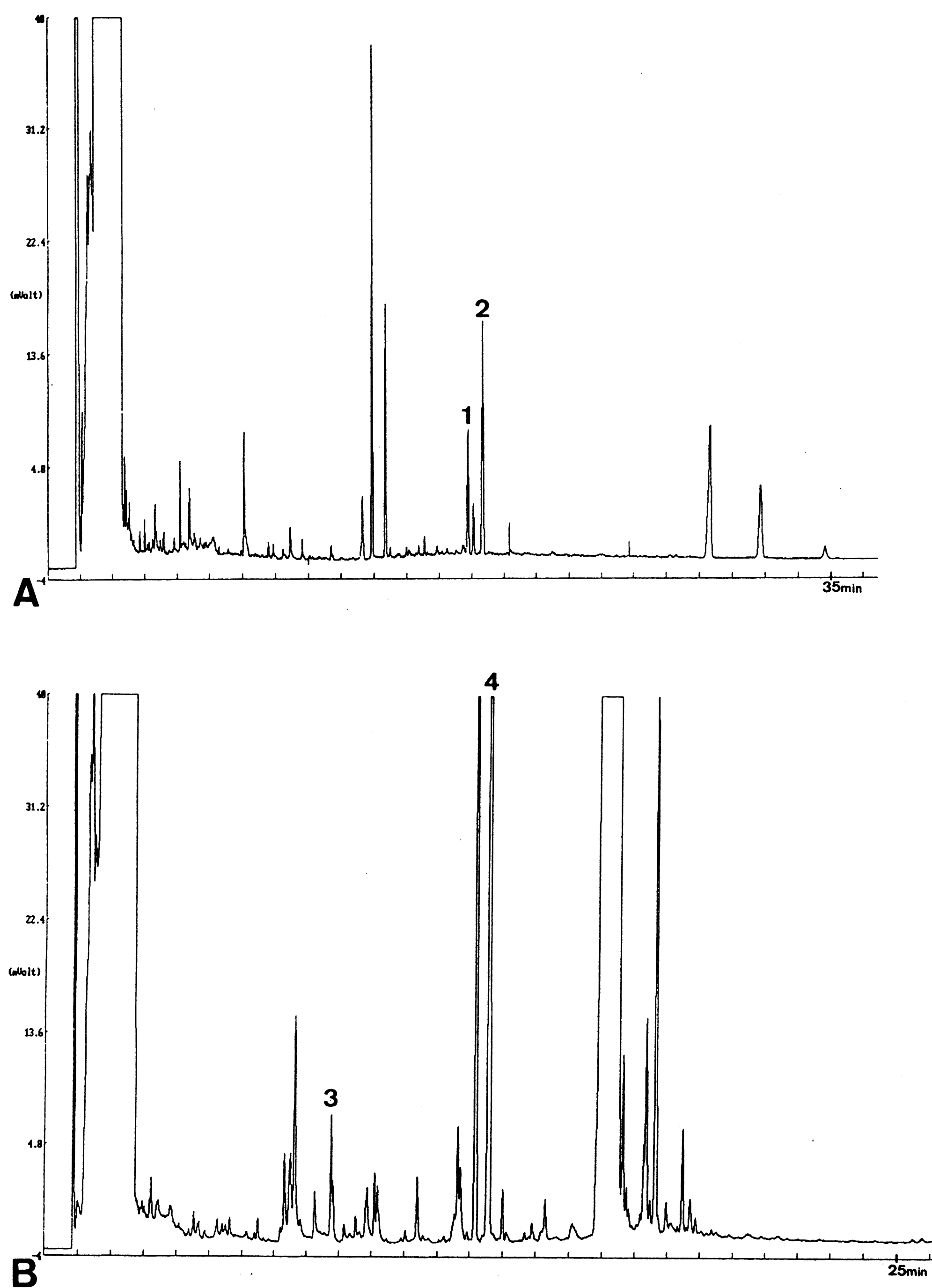

Fig. 4. cGC analysis of a meat sample spiked with 10 μg of retinol and α- tocopherol. (A) Detection of α-tocopherol; 1=α-tocopherol; 2=α-tocopheryl acetate. (B) Detection of retinol; 3=retinol; 4=5α-cholestane. Analytical conditions as described in Section 2.

Table 2
Evaluation of the absolute recoveries from spiked muscle samples[a]

Compound	Absolute recoveries (%)						
	1	2	3	4	5	Mean	R.S.D. (%)
5α-Cholestane	89.17	86.51	96.59	99.83	90.36	92.49	5.97
Retinol	76.78	89.19	88.82	85.14	74.89	82.96	8.11
α-Tocopheryl acetate	75.79	84.98	90.58	99.90	89.17	85.75	9.96
α-Tocopherol	70.37	78.00	79.27	83.35	91.03	79.79	9.42

[a] Samples were spiked with 10 μg of retinol and α-tocopherol; for details, see Section 2.

and the α-tocopheryl acetate were efficiently hydrolyzed to retinol and α-tocopherol, respectively (Fig. 3). Retinyl palmitate is the major retinyl ester found in biological tissues [11].

When tissues were analyzed (muscle or liver), SPE was necessary to separate cholesterol, which interfered with the cGC analysis, from α-tocopherol. Fig. 4 shows the detection of retinol and α-tocopherol in spiked samples. Tables 2 and 3 report the absolute and relative recoveries evaluated from spiked samples and for both compounds. High absolute recoveries (80%) were obtained for the two vitamins. The absolute recovery values for retinol was similar to that obtained when only the saponification step was evaluated (Tables 1 and 2). The lower recoveries observed for α-tocopherol suggest that some α-tocopherol was degraded during the derivatization step. The reproducibility of the procedure was quite satisfactory since the R.S.D. values (<10%), evaluated for retinol and α-tocopherol, reflect the total procedure from the extraction to the cGC analysis step. 5α-Cholestane and α-tocopheryl acetate (I.S.s) presented similar absolute recoveries. The use of retinyl acetate as I.S. for retinol analysis was not possible because it must be added after the saponification step and could not provide information about the efficiency of the hexane–isopropanol extraction. Hence, we opted for 5α-cholestane which is liposoluble and soluble in organic solvents such as hexane, as are retinol and α-tocopherol. The 5α-cholestane was then added prior to the extraction. Furthermore, the results obtained for absolute recoveries indicated that 5α-cholestane can be used as an appropriate I.S. for retinol quantification (Table 2). The use of I.S.s corrected the losses of retinol and α-tocopherol which could have occurred during the procedure including the derivatization step for α-tocopherol. Relative recoveries superior to 90% were obtained (Table 3). Furthermore, the use of I.S.s lowered the standard deviation of the results (Table 3). The reproducibility of the injection was tested and retinol presented higher R.S.D. values than 5α-cholestane, probably due to the thermal instability presented by retinol at the high temperatures used in the solvent venting injection (300°C) (Table 4). An alternative could be the use of cold on-column injection as reported by other authors [10–12,16]. The regression analyses performed on spiked samples gave satisfactory results with calibration curves characterized by a coefficient of determination (R^2)

Table 3
Evaluation of the linearity[a] and the relative recoveries from spiked muscle samples (2 g)

	F[b]	RRT[b]	Calibration curves	R^2	Relative recoveries (μg)[c]						
					1	2	3	4	5	Mean	R.S.D. (%)
Retinol[d]	3.37	0.609	$y=1.65+0.87x$	0.9751	10.04	10.42	8.92	8.94	9.66	9.60 (96%)	6.93
α-Tocopherol[e]	0.89	0.960	$y=0.34+0.63x$	0.9955	10.08	9.96	9.50	8.14	9.64	9.46 (95%)	8.20

[a] Samples were spiked with 40, 20, 10 and 2 μg of retinol and α-tocopherol.
[b] F=response factor; relative retention times (RRT)=t_R (solute)/t_R (I.S.).
[c] Samples were spiked with 10 μg of retinol and α-tocopherol. Results are expressed in μg/g.
[d] Relative recoveries were calculated using 5α-cholestane as the I.S..
[e] Relative recoveries were calculated using tocopherol acetate as the I.S..

Table 4
Reproducibility of the solvent venting injection[a]

Compound	R.S.D. (%)	
	Retention times	Absolute areas
Retinol	0.70	5.42
5α-Cholestane	0.38	2.46
α-Tocopherol	0.83	8.87
α-Tocopheryl acetate	0.94	9.03

[a] Sample spiked with 10 μg of both compounds was manually injected five times.

Table 5
Quantification of retinol and α-tocopherol in chicken liver

Compound	Sample 1 (μg/g)	Sample 2 (μg/g)	Mean (μg/g)	R.S.D. (%)
Retinol	51.39	58.70	55.05	9.39
α-Tocopherol	4.20	4.77	4.49	8.99

Two samples from the same liver were analyzed.

superior to 0.97 (Table 3). It can be inferred that retinol was minimally adsorbed to active sites in the injector or in the column [13]. The limit of detection of the procedure was 1 μg/g for retinol but an increased sensitivity can be obtained by the large volume injection (10 μl) allowed by cGC in the solvent venting mode [15]. Retinol was detected by cGC in chicken liver but not in chicken muscle and was confirmed by cGC–MS in the TIC mode (Table 5, Figs. 2 and 5). The use of single ion monitoring was not necessary. α-Tocopherol was detected in chicken liver and muscle, and was confirmed by cGC–MS in the TIC mode (Table 5, Figs. 6 and 7). No trace of other tocopherols (δ, γ), resulting from dietary vegetable oils, was evidenced by cGC–MS analysis. Nearly 60 μg of retinol per gram of liver tissue were quantified (Table 5), a value similar to that reported by the US Department of Agriculture [19]. The tissue contents in retinol and α-tocopherol

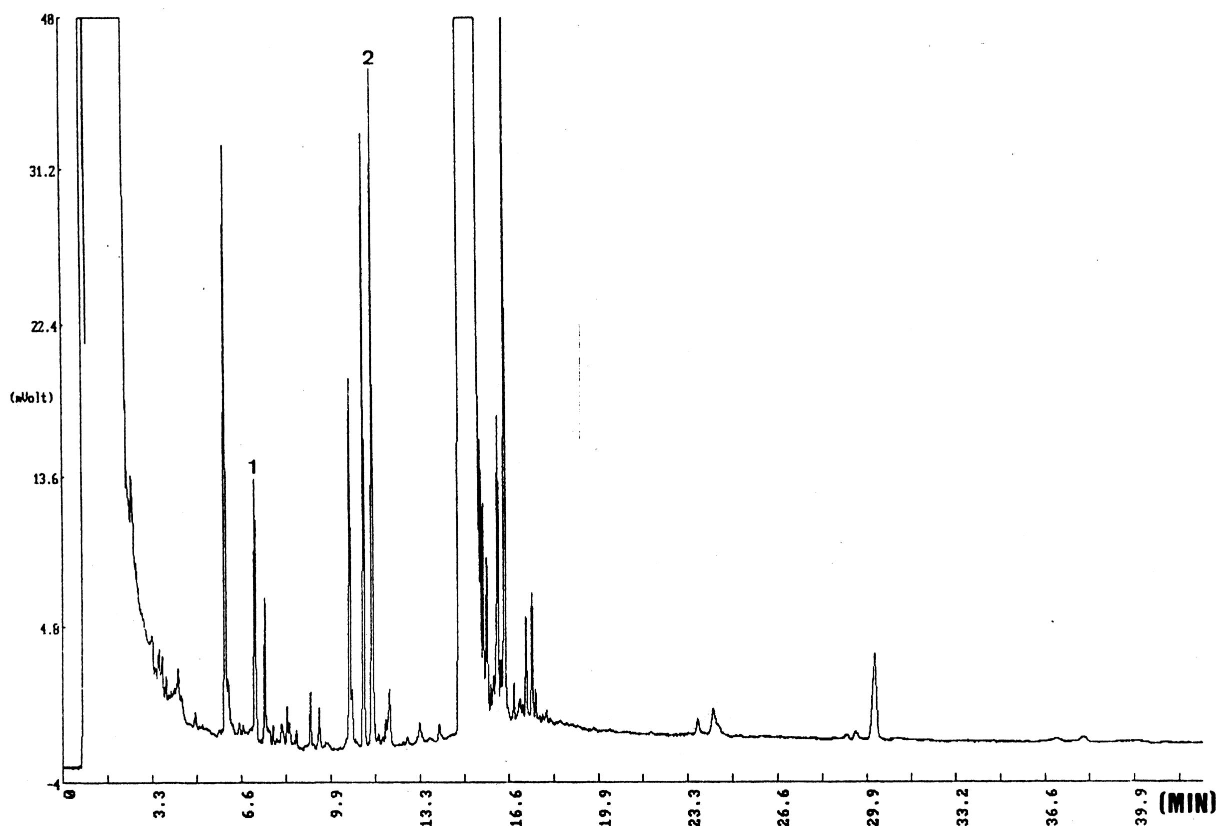

Fig. 5. Detection of retinol by cGC in a liver sample. 1=Retinol; 2=5α-cholestane. Analytical conditions as described in Section 2.

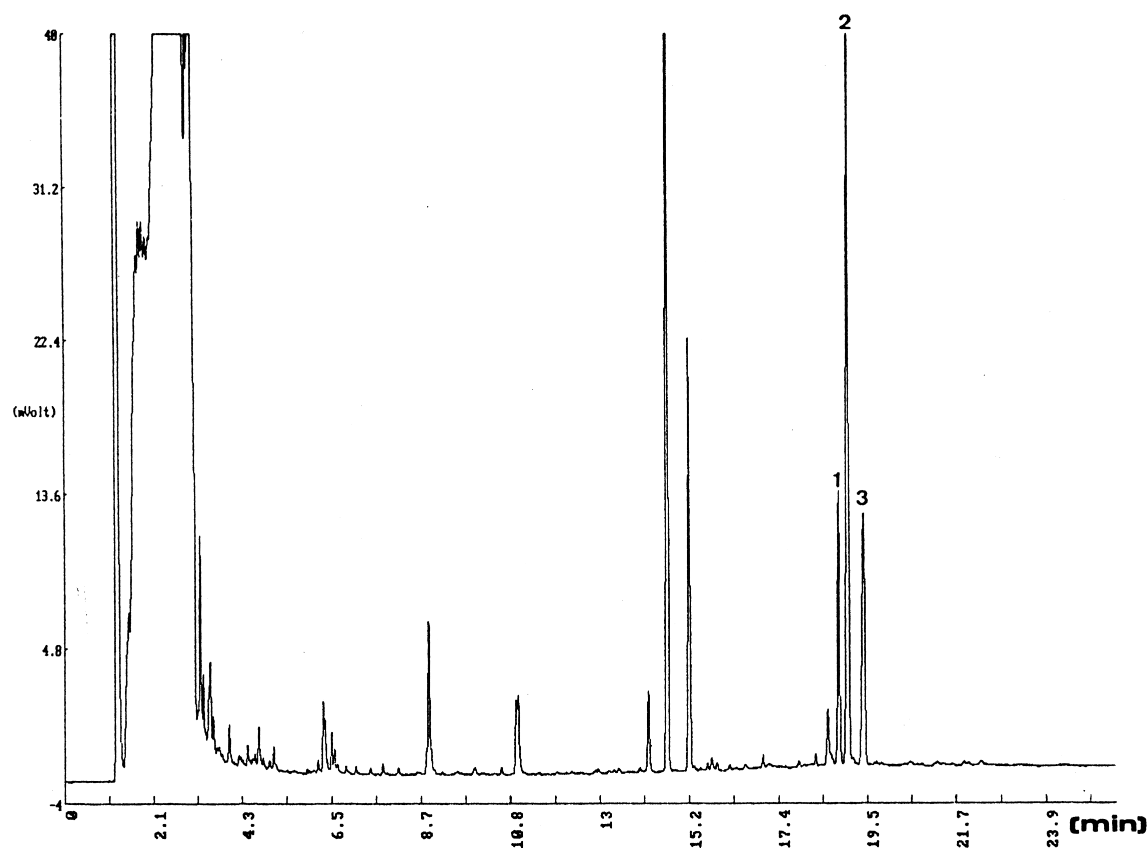

Fig. 6. Detection of α-tocopherol by cGC in a meat sample. 1=α-tocopherol; 2=cholesterol; 3=α-tocopheryl acetate. Analytical conditions as described in Section 2.

are influenced by the diet or by the genetic make-up of the animal. The retinol values evaluated for chicken liver were lower than the 120–140 μg/g reported by Engberg et al. [20], who found that the chicken thigh muscle contained trace levels of vitamin A (0.18 μg/g). King et al. [21] reported higher concentrations of α-tocopherol in liver. They also found an α-tocopherol concentration of 3.6 μg/g in the meat from chickens fed normal quantities of vitamin E [21].

4. Conclusions

The procedure developed in this work can be used as an alternative to the commonly-used HPLC procedures for retinol and α-tocopherol analysis in tissues. The problem of equilibration of the column between runs, in the described procedure, is nonexistent. α-Tocopheryl acetate does not fluoresce appreciably and is therefore unsuitable for use as the I.S. for α-tocopherol quantification by HPLC procedure [22]. Zaspel and Csallany used UV detection with their HPLC procedure in order to quantify plasma tocopherol causing a loss in sensitivity [22]. In this work, 5α-cholestane and α-tocopheryl acetate were found to be appropriate for the quantification of both vitamins in muscle and liver tissues by cGC. The described procedure involved rapid organic solvent extraction, saponification and detection of vitamins A and E in tissues by cGC–FID in the solvent venting mode. The procedure is reproducible and allows the detection of concentrations as low as 1 μg/g.

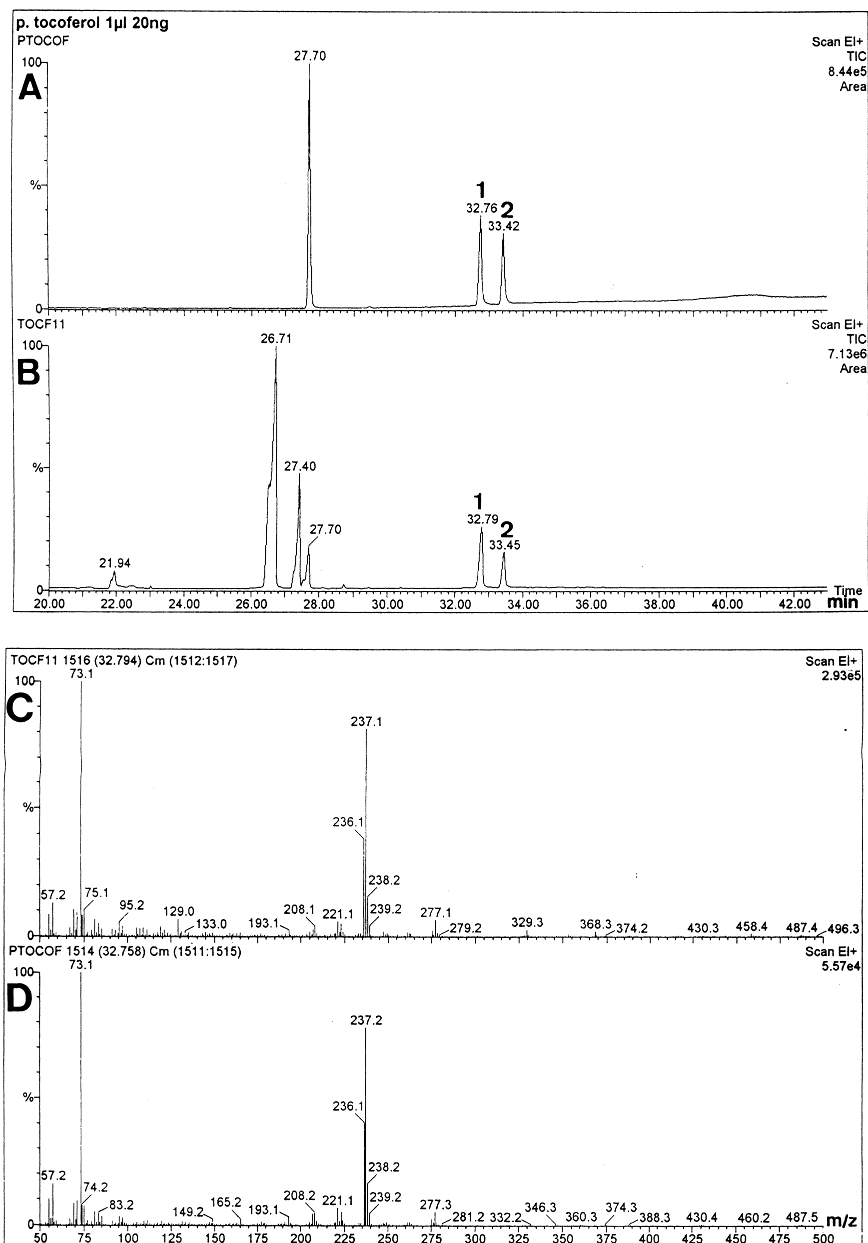

Fig. 7. cGC–MS analysis of the TMS-ether derivative of α-tocopherol. (A) Total ion chromatogram (TIC) of standards; (B) TIC of a meat sample; (C) mass spectrum of TMS α-tocopherol; (D) confirmation of the identity of α-tocopherol in its derivatized form in the meat sample; 1=α-tocopherol; 2=α-tocopheryl acetate. Analytical conditions as described in Section 2.

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References

[1] J.L. Buttriss, A.T. Diplock, Methods Enzymol. 105 (1984) 131.
[2] A.P. De Leenheer, V.O. De Bevere, M.G. De Ruyter, A.E. Claeys, J. Chromatogr. 162(3) (1979) 408.
[3] K.W. Miller, N.A. Lorr, C.S. Yang, Anal. Biochem. 138 (1984) 340.
[4] K.W. Miller, C.S. Yang, Anal. Biochem. 145 (1985) 21.
[5] W.A. McCrehan, E. Schönberger, Clin. Chem. 33 (1987) 1585.
[6] N. Bilic, R. Sieber, Z. Lebensm. Unters. Forsch. 186 (1988) 514.
[7] S. Ciapellano, F. Brighenti, M. Porrini, G. Testolin, Acta Vitaminol. Enzymol. 7 (1985) 223.
[8] N.D. Mostow, R. O'Neill, D.L. Noon, B.R. Bacon, J. Chromatogr. 344 (1985) 137.
[9] H.T. Slover, R.H. Thompson, G.V. Merola, J. Am. Oil Chem. Soc. 60 (1983) 1524.
[10] C.R. Smidt, A.D. Jones, A.J. Clifford, J. Chromatogr. 434 (1988) 21.
[11] H.C. Furr, A.B. Barua, J.A. Olson, in: A.P. De Leenheer, W.E. Lambert, H.J. Nelis (Editors), Modern Chromatographic Analysis of Vitamins, Marcel Dekker, New York, Basel, 1992, pp. 1–71.
[12] H.C. Furr, S.H. Zeng, A.J. Clifford, J.A. Olson, J. Chromatogr. 527 (1990) 406.
[13] H.C. Furr, A.J. Clifford, A.D. Jones, Methods Enzymol. 213 (1992) 281.
[14] C. Maraschiello, I. Díaz, J.A. García Regueiro, J. High Resolut. Chromatogr. 19 (1996) 165.
[15] J.A. García Regueiro, C. Maraschiello, J. Chromatogr. A 764 (1997) 279.
[16] A.J. Clifford, A.D. Jones, H.C. Furr, Methods Enzymol. 189 (1990) 94.
[17] I.D. Desai, Methods Enzymol. 102 (1984) 138.
[18] J.K. Lang, M. Schillaci, B. Irvin, in: A.P. De Leenheer, W.E. Lambert, H.J. Nelis (Editors), Modern Chromatographic Analysis of Vitamins, Marcel Dekker, New York, Basel, 1992, pp. 153–195.
[19] US Department of Agriculture, Composition of Foods (Consumer and Food Economics Institute), US Gov. Print. Office, Washington, DC, 1976–1984, Agricultural Handbook No. 8, revisions 8.1–8.12.
[20] R.M. Engberg, C. Lauridsen, S.K. Jensen, K. Jakobsen, Poultry Sci. 75 (1995) 1003.
[21] A.J. King, T.G. Uittenboogaart, A.W. de Vries, J. Food Sci. 60 (1995) 1009.
[22] B.J. Zaspel, A.S. Csallany, Anal. Biochem. 130 (1983) 146.