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Journal of Chromatography B, 822 (2005) 339–346
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

A fast, sensitive method for the simultaneous determination of α-tocopherol and α-tocopheryl acetate in mixed micelles

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Received 26 March 2005; accepted 9 June 2005
Available online 7 July 2005

Abstract

This report improves analytical procedures to investigate the behaviour of the two Vitamin E forms, α-tocopherol (Tol) and α-tocopheryl acetate (Tac), in model systems mimicking the intestinal medium. We describe how to prepare mixed micelles as vehicle for Tac and Tol and the HPLC method for their quantification in the micelles. Tac and Tol were extracted using ethanol-hexane-drying procedure, whereas the separation and detection were performed in methanol and by UV method, respectively. Both compounds were eluted in less than 4 min. In the range between 1.7 μM and 54 μM of Tac or Tol in the micelles, their recovery were 89% and 81%, respectively, with correlation coefficient over 0.99 and R.S.D. of less than 7.2% in all cases. Limits of detection and quantification for Tac and Tol in mixed micelles ranged between 1 μM and 2 μM and between 3 μM and 5 μM, respectively. The behaviours of Tac and Tol were quite different during the extraction procedure and both were influenced by the vitamin concentration and the relative volume of organic solvents.

Keywords: α-Tocopherol; α-Tocopheryl acetate; Mixed micelles; Reverse phase HPLC

1. Introduction

High performance liquid chromatography methods have proven to be useful means of determining Vitamin E in various matrices, alone or mixed with vitamers such as γ-tocopherol or other vitamins such as Vitamin A and/or D, or with lipophilic compounds such as carotenoids.

According to the most common protocols to extract Vitamin E from biological or nutritional samples one has first to precipitate proteins then use apolar solvents. In some protocols, an aliquot of the organic phase is then directly injected into the HPLC system [1–3], whereas in other more frequent procedures the organic phase is dried before the vitamin is redissolved in a less apolar organic solvent, which is then injected into the chromatograph [4–9]. For separation, the most common mobile phase is methanol or methanol-based solvents, generally under isocratic conditions [1–3,5–9]. Detection is performed using either fluorimetric [10,11], electrochemical [12,13] or spectrophotometric [1–4,14] means. Coupled UV and fluorescence detection is also used if more than one compound has to be determined [6–8,15,16]. It is interesting to note that all these detection methods allow for determination of Vitamin E in almost all in vitro and in vivo situations, though the fluorescence detection is the most sensitive.

Only few papers have so far described simultanous analysis of α-tocopherol and α-tocopheryl acetate, althought the latter is, in terms of quantity, the most important form of Vitamin E used in animal nutrition and cosmetics. The published HPLC methods describe the simultaneous determination of Tac and Tol, for example, in human stools [3], diets for animals [8] and fish feed [15]. These methods have different extraction procedures and detection means, but commonly used methanol as mobile phase. For those methods, the time of elution of both Tol and Tac ranged from 8 min to 20 min.

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doi:10.1016/j.jchromb.2005.06.028

The limit of quantification of Tac ranged from 0.2 μg/ml of extract [15] to 102 μg/g of stool [3], whereas that of Tol, using fluorescence detection, was more than ten times lower [15].

In other respects, mixed micellar solutions containing bile salts and lipids have been used as model systems for solubilisation of fatty acids [17], carotenoids [18–20] and Vitamin E [21] in order to study the absorption of these lipophilic compounds. In the latter report [21], the absorption of Vitamin E was investigated in loops of rat intestine in line with the hydrolysis of Tac by local or added esterases. The composition of the micelle used as vehicle of Vitamin E was based on that of the micelles previously used for similar purposes [22–24].

Further information about the digestive and absorption mechanisms of Tac is now required, with special respect to its convertion into tocopherol, the biologically active form. Very few data are available in this area, although it is generally recognized that the acetylated form of Vitamin E is hydrolyzed into the alcohol form by means the cholesterol esterase (EC 3.1.1.13), secreted by the pancreas, and the released alcohol form is then absorbed in the small intestine [25].

The aim of the present study was to develop and validate a well-controlled analytical tools for the determination of both α-tocopherol and α-tocopheryl acetate in a medium mimicking the physiological conditions of the intestinal milieu. Based on literature, mixed micelles were prepared and used to solubilize the two forms of Vitamin E. Therefore, an HPLC method using an ethanol–hexane-drying extraction procedure, separation in methanol and UV detection means, was adapted for the simultaneous determination of Tac and Tol. This method should allow for minimizing retention time and maximizing precision and accuracy whilst retaining a sufficiently large range of concentration of Vitamin E. To our knowledge, no such analytical tools have previously been provided.

2. Experimental

2.1. Equipment

Analysis of tocopherol and tocopheryl acetate with UV detection was performed in an HPLC system consisting of a GP40 Dionex gradient pump (Dionex Corp., Sunnyval, CA, USA), an AS 3500 Spectra System autosampler from Termoelectron (Fremont, CA, USA) and a model 486 variable wavelength detector (Waters Assoc., Milford, MA, USA). The area under the peaks was integrated with an Olivetti P75i integrator (Olivetti, Paris, France) using the Borwin chromatography software program (JMBS, Grenoble, France). The UV spectra of the two forms of Vitamin E were obtained using a UV 1605 UV–vis spectrophotometer (Shimadzu Scientific Instruments, Inc. Columbia, USA). Centrifugation of the eppendorf tubes containing the aqueous–organic mixtures was performed using a Beckman-microcentrifuge. Organic phases containing Vitamin E were vacuum-dried using a Speed Vacuum Concentrator (Savant Instruments, Inc., NY, USA) connected to a Heto Drywinner freeze dryer (Birkerod, Denmark). Micelles were sonicated in a 50 W Sonicator bath (Ficher Bioblock Scientific), whereas vortexing was performed either manually using Reax 2000 (Heidolph, Germany) at maximum rate or automatically using Vortex-2 genie (Scientific Industries, USA) equipped with a 40-eppendorf tubes support.

2.2. Reagents

DL-α-Tocopherol (>98%), DL-α-tocopheryl acetate (98%) and sodium taurocholate (>97%) were from Fluka. Analytical grade butyl hydroxytoluene (BHT), cholesterol (99%), oleic acid (99%), egg yolk L-α-lysophosphatidylcholine (99%), 1-monopalmitoyl glycerol and retinyl acetate were from Sigma. HPLC grade solvents (methanol, chloroform, hexane and ethanol) were from SDS (Peypin, France). Ultra pure water was obtained in the laboratory using Elga Prima and Maxima Elga systems (Elga, UK). All the other reagents were of analytical grade.

Tac and Tol in the Fluka conditioning were stored at 4 °C under nitrogen during less than 3 months and their degree of purity was regularly controlled by HPLC. The two forms of Vitamin E were freshly prepared in ethanol at the desired concentration for use.

2.3. Standard

Retinyl acetate (Rac) was used as the internal standard as it is eluted before Tol under reverse phase conditions. The sample from Sigma was purified and its concentration in ethanol determined according to $\varepsilon_{1\%,\,325\,nm} = 1850$ [26]. Rac was stored at −80 °C.

2.4. Mixed micelles

The micellar solution constituents and their respective concentrations were based on the literature [18–24] and modifications were done according to the objective of the study. A stock micelle solution consisting of 40 mM sodium taurocholate and 4 mM oleic acid in 35 mM phosphate buffer (pH 6.5 containing 150 mM NaCl), was prepared under gentle magnetic agitation. This micelle solution was stored at −80 °C or used in order to prepare a further micelle solution to which monopalmitin, lysophophatidylcholin and cholesterol were added. Each of these three lipids was solubilized in chloroform–methanol (2:1) by vigourous manual vortexing (30 s) and dried in a vacuum. The lipids were then solubilized separately in the stock micellar solutions by vigourous manual vortexing (30 s) followed by a 30 min sonication step to obtain a final concentration of 3 mM for each. The three micelle solutions were finally mixed together by magnetic stirring gently for 30 min and then performing a 10 min sonication step. The final concentrations of monopalmitine, lysophophatidylcholine and cholesterol were 1 mM. The

micellar solution thus obtained, called micelle M40 with reference to its sodium taurocholate concentration (40 mM), was aliquoted and stored at −80 °C until use.

Working micelle (M4) was prepared in 2 ml colored microcentrifuge tubes by diluting 10 times M40 in phosphate buffer (35 mM, pH 6.5, NaCl 0.15 M) and vortexing manually the mixture for 15 s. After adding Tac or Tol (in ethanol), the micelle solution was vortexed for 30 s and then sonicated for 10 min. The final ethanol concentration in micelles was set at less than 0.5% (v:v) in all cases.

2.5. Extraction and analysis of Vitamin E

The extraction procedures of Vitamin E from plasma by mean of ethanol and hexane, including a drying step, have been described in several studies [3,4,8,10]. The general lines of our extraction procedure are similar to those decribed by Hoehler et al. [8] for serum, however specific modifications needed to be applied as micelles were concerned. The importance of some steps of the extraction procedure are discussed in the results and discussion section. In a 2 ml colored microcentrifuge tubes, 200 μL of the micellar solution was mixed with 200 μL of ethanol, containing retinyl acetate and in some cases BHT, by manual vigourous vortexing (15 s). Four hundred microlitres of hexane was then added under a hood and the mixture was automatically stirred during 2 min before being centrifuged for 5 min at 5000 rpm. Two hundred microlitres of the upper phase were subsequently collected, dried and then redissolved in 100 μL of ethanol. Fifty microlitres of the latter solution was finally injected into the HPLC system.

The effect of relative volumes of ethanol and hexane on Tac and Tol recovery has been determined (see Section 3).

HPLC separation was performed by mean of a LiChroCart column (125 mm × 4 mm) packed with 5 μm RP-18 Purospher gel (Merck, Darmstadt, Germany) and equipped with a LiChroCart precolumn (4 mm × 4 mm) packed with the same material (Merck). The precolumn and column were placed in a thermostated bath maintained at 38 °C. The elution procedure was carried out by a methanol–water solvent, using a 98–100% methanol gradient for 4.5 min. Tac and Tol were both detected at 284 nm.

3. Results and discussion

3.1. Spectra of α-tocopherol and α-tocopheryl acetate

The UV-absorbance spectra of α-tocopherol and α-tocopheryl acetate were first examined. With most HPLC methods, α-tocopherol is detected in a wavelength between 292 nm and 297 nm. It can be seen from Fig. 1 that the absorbance of Tol almost reaches a maximum in this wavelength range, although the absorbance value at 297 nm, generally used for its detection, is 12% lower than at 292 nm. Tac has mainly been detected below 290 nm, at 280 nm [1–3,7],

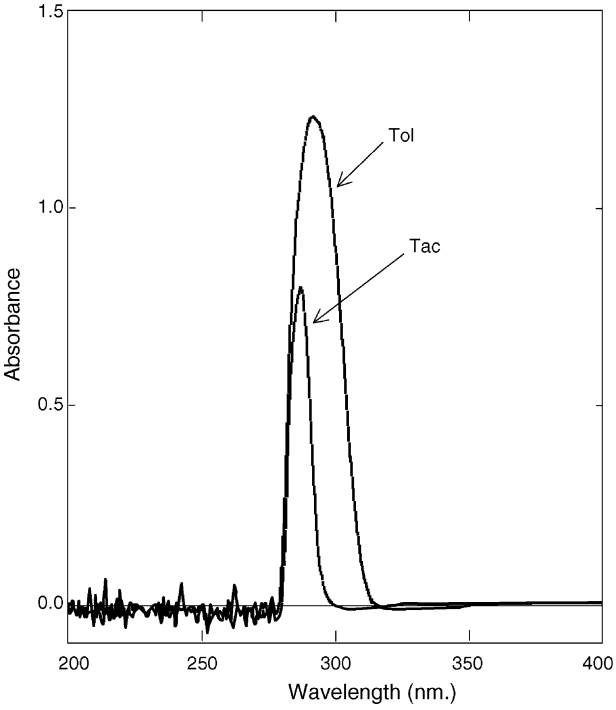

Fig. 1. UV spectra of α-tocopherol and α-tocopheryl acetate.

284 nm [8], and 285 nm [26], for example. As shown in Fig. 1, the most suitable wavelength values for Tac detection are in the 282–288 nm range. The extinction coefficient of Tac ($\varepsilon_{1\%}$ in ethanol) determined at 284 nm was 41.3, which is quite similar to the value (43.6) previously obtained at 285 nm [26].

On the other hand, the Tol:Tac absorbance ratio is about 2.0 at 280 nm and 1.2 at 284 nm, and then increases up to about 100 at 297 nm. These data indicate that in order to determine both Tac and Tol using UV detection methods, the wavelength should be set at a value around 284 nm.

3.2. HPLC determination of Tol and Tac

As mentioned in the introduction, methanol has been largely used for elution of Vitamins E and A [1–3,7–9]. Here, the best separation profile was obtained by using a linear methanol gradient ranging in 4.5 min from 98% (2% of water) to 100% in 4.5 min. A typical chromatogram is shown in Fig. 2.

The internal standard retinyl acetate elutes first at 1.44 min, just after the ethanol peak and two minor non-identified peaks. α-Tocopherol elutes at 2.65 min, followed by α-tocopheryl acetate at 3.57 min. The peaks corresponding to the two Vitamin E forms are clearly separated and the Tol/Tac ratio between the areas is about 1.4. Both Tol and Tac can be determined within less than 4 min. This value is almost two times shorter than the previously reported values [1–8,10–16]. Still, Gimeno et al. [9] reported similar running time value for α-tocopherol by using methanol–butanol–water mixture as mobile phase at 2 ml min^{-1} and a 15 cm column at 45 °C. Also, α-tocopherol

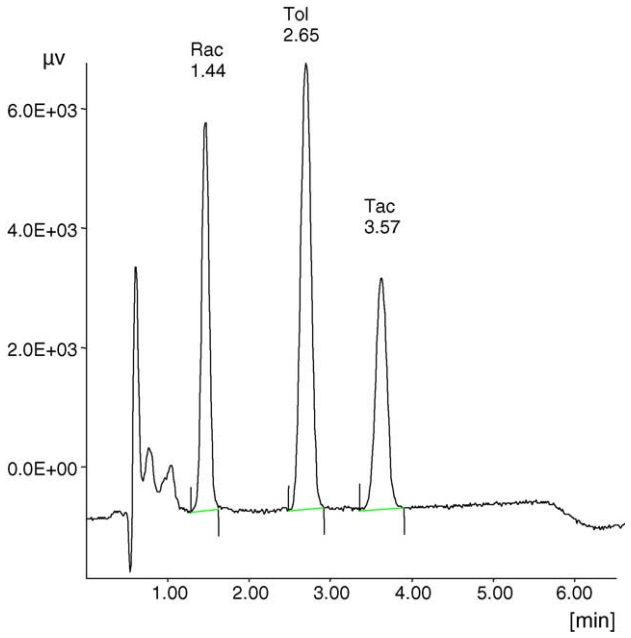

Fig. 2. A typical chromatogram of α-tocopherol and α-tocopheryl acetate with retinyl acetate as internal standard. Eight nanomoles of Tac and Tol and 100 nmol of Rac were solubilized in ethanol and injected into the HPLC system. Separation was performed as described in Section 2 at 2 ml min^{-1}. Column temperature: 38 °C.

was eluted in 100% methanol at 1.3 ml min^{-1} from a 7.5 cm column at about 4 min [27]. The temperature of the column was not indicated in the latter study. In all cases, this make emphasis on the importance of the lengh and temperature of the column for to get rapid separation, especially when a high number of determinations are to be carried out.

Fig. 2 also shows that retinyl acetate is an appropriate internal standard under the experimental conditions used in the present study. Whether or not it is suitable for use under other experimental conditions should be closely investigated. Indeed, retinyl acetate from Sigma, which was analyzed without purification under the experimental conditions shown in Fig. 2, the peak at 1.44 accounted for only about 50% of the total area of the peaks. There were three other main peaks eluting at 3.86 min, 4.56 min and 5.07 min. Only the peak at 1.44 min corresponded to retinyl acetate, according to the data published by Nierenberg et al. [3]. Also, Rac is likely to be present in a number of experimental samples such as nutritional supplements, pharmaceutical preparations, feces or specimens from the digestive tract.

3.3. Stability of the reactants

Special attention needs to be paid to the stability of Vitamins A and E when used in laboratory conditions. In the present study, retinyl acetate was degraded to some extent after 3 months of storage in ethanol at −20 °C (data not shown). This specific point was not examined in detail and the internal standard was rather stored at −80 °C where it was stable at least for 6 months. Tac and Tol in Fluka conditioning stored under nitrogen at 4 °C were stable for more than 3 months. The concerned items were regularly purchassed and the Vitamin E forms dissolved in ethanol just before use. Several studies adressed the stability of Vitamin E, Vitamin A and carotenoids in plasma or whole blood under handling conditions [4,7,28–30]. However, equivocal results have been reported when comparing different studies as well as ambivalent results within studies done by the same team [28,31]. In summary, these substances seem to be stable several months at −20 °C and more than a year at −80 °C. However, some caution should be paid to the number of freeze/thaw cycles [30]. This may explain the losses of retinyl acetate observed in the present study when the Vitamin A ester was stored at −20 °C. More severe losses of Vitamin E are likely to occur in feedstuffs stored under usual conditions [16,32]. According to Anderson et al. [32], 86% of the α-tocopherol were lost in pig diet after 3 weeks of storage at room temperature. Moreover, lower plasma Vitamin E concentrations were detected in pigs fed with tocopherol than in those fed with tocopheryl acetate, probably because tocopherol was less stable than tocopheryl acetate during the diet storage.

3.4. Calibration curves

Fig. 3 shows the calibration curves of Tol and Tac in ethanol from 10 pmol injected (0.2 μM) to 62.5 nmol (1250 μM) and from 10 pmol to 200 pmol (insert). The curves are linear and the regression coefficients (R^2) are above 0.999 in all cases. As low as 5 pmol of Tac and Tol (about 2 ng) may be detected by this method however the points below 20 pmol tended to have some down inflexion in the plot (data not shown). According to ISO recommendations, limits of detection and quantification were found to be 21 pmol and 37 pmol for Tol and 22 pmol and 38 pmol for Tac, respectively. The relative standard deviation values either for within-day assay or between-day assay were lower than 6%, except for the dose values between 20 pmol and 5 pmol where the values of R.S.D. ranged from 5% to 12%. These LOD and LOQ values are lower than those previously reported, especially for Tac [3,9,15].

Examination of the calibration data set from 0.8 μM (40 pmol) up to 1250 μM shows, nevertheless, that the slopes of the calibration curves slow down progressively as the range of Vitamin E concentration is enlarged (Table 1). From the range 0.8–5 μM to the range 0.8–1250 μM, the slope values decrease by 16% for Tol and 19% for Tac. The regression equations of the calibration curves in the five concentration ranges shown in Table 1 were used to calculate the concentrations of Vitamin E extracted from mixed micelles in the range of Vitamin E concentration from 1.7 μM to 215 μM.

3.5. Mixed micelles

Information is lacking about the behaviour of α-tocopheryl acetate in the intestinal tract, although it has been established that this ester and other esters of Vitamin

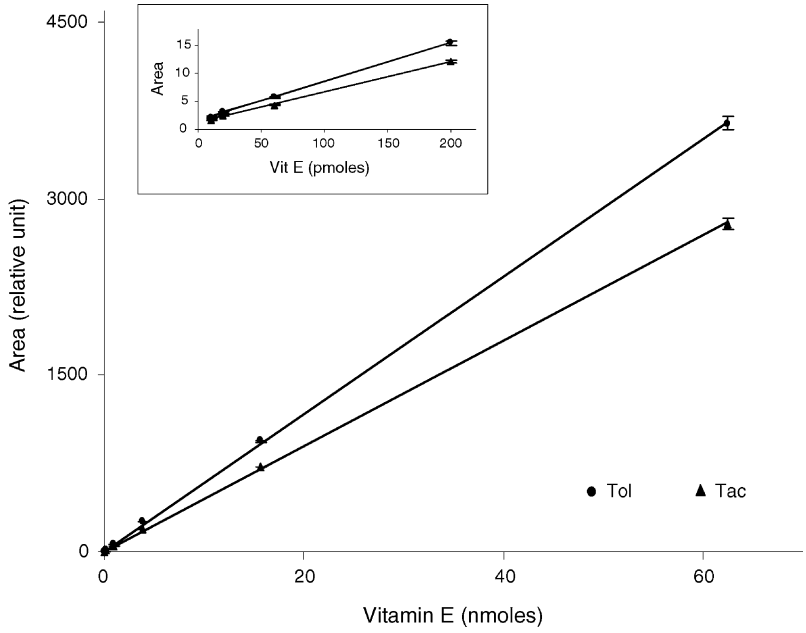

Fig. 3. Calibration curves of α-tocopherol and α-tocopheryl acetate. Each point is a mean value of five determinations.

E are hydrolyzed by pancreatic cholesterol esterase [21–24]. According to Mathias et al. [24], the maximum activity of cholesterol esterase on Tac was obtained using a mixture of sodium taurocholate (15–30 mM) and oleic acid (2–4 mM) in phosphate buffer (35 mM, pH 8.5). This fatty acid–bile salt mixture under buffered conditions, except for the pH (which was set at 6.5) was used in the present study. Cholesterol, lysophosphatidylcholin and monopalmitin were added to the micelle at concentrations similar to those used for solubilisation of lutein in absorption experiments [20]. The micellar solution thus obtained is clear to eye in reflected light, and has an absorbance at 750 nm (with water as the blank) of 0.02 and was thus considered as isotropic [24].

3.6. Extraction of Tac and Tol from micelles

The main factor influencing the extraction of Vitamin E from serum using the ethanol–hexane-drying procedure seems to be the relative volume of ethanol added to the sample [7]. Also, the same authors have reported that the recovery of Vitamin E decreased if the relative volume of hexane is increased, as much as 50% in some ethanol:hexane volume ratio sets. Here, in micelles, the relative volume of ethanol have dramatic infuence on the recovery of Tac and Tol, however this was not the case concerning the relative volume of hexane. As can be seen in Fig. 4a, using 54 μM of Vitamin E and an hexane:micelle volume ratio of 2:1, at least an equal volume of ethanol to micelle is necessary to obtain the maximum recovery of Tac and Tol from the micelles. Using the same concentration of Vitamin E and an ethanol:micelle volume ratio of 1:1, the relative volume of hexane to micelle from 0.5 to 4 has almost no infuence on the recovery values of Tac and Tol (Fig. 4b). Interestingly, the infuence of ethanol below ratio 1:1 and that of hexan below 0.5:1 depend on the Vitamin E form. Our results are well in line with those of Hoehler et al. [7] concerning the importance of the ethanol but not of hexane in the extraction procedure, as far as serum and micelles may be compared. The extended vortexing time (180 s) of the serum–solvents mixture in some ethanol:hexane volume sets was also found by Hoehler et al. to cause decrease of the Vitamin E recovery (up to 80% in some cases). The authors suggested that after prolonged vortexing, a substantial amount of emulsion is formed and a part of Vitamin E remain in the aqeuous fraction. Here, using a vortex equipped with a 40-eppendorf tube support performing medium strengh agitation, the optimal vortexing time was found to be 2 min (data not shown). In conclusion, these data suggest that an extraction procedure which is optimal for serum is not necessary optimal for a lipid-containing medium.

Table 1
Regression equations[a] of the calibration curves of α-tocopherol and α-tocopheryl acetate

Concentration range of Vitamin E (μM)	Tol	Tac
0.8–5	$y = 69.1x + 1.68$	$y = 55.2x + 1.11$
0.8–20	$y = 65.6x + 2.01$	$y = 52.7x + 1.35$
0.8–80	$y = 63.9x + 2.47$	$y = 49.3x + 2.29$
0.8–312	$y = 59.8x + 6.11$	$y = 46.0x + 5.22$
0.8–1250	$y = 58.2x + 10.89$	$y = 44.6x + 9.32$

[a] y = area of peak corrected with the area of the internal standard, x = pmol of Tac or Tol injected. The correlation coefficient (R^2) was above 0.999 in all cases. The regression equations correspond to the data of Fig. 3.

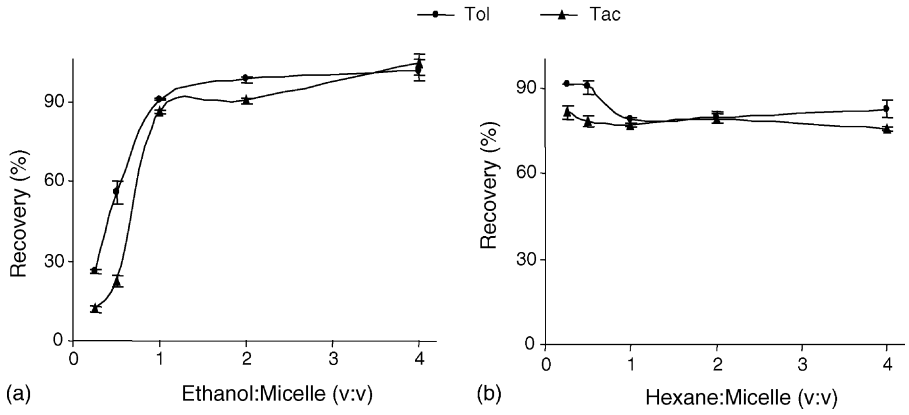

Fig. 4. Effects of relative volume of ethanol (a) and hexane (b) on the extraction recovery of Tac and Tol from mixed micelles. Tac or Tol: 54 μM. (a) 200 μL of micelles was mixed with different volumes of ethanol and then Tac or Tol extracted with 400 μL of hexane. (b) 200 μL of micelles was mixed with 200 μL of ethanol then Tac or Tol extracted with different volumes of hexane.

3.7. Validation

Fig. 5 shows the accuracy of the method in the concentrations of Tac and Tol in micelles ranging from 1.7 μM to 214 μM and from 1.7 μM to 54 μM (insert). The dose–response relationship is quite linear in the range 1.7–54 μM (insert, Table 2) giving mean recovery values of about 81% for Tol and 89% for Tac with correlation coefficient over 0.99 and R.S.D. of less than 7.2% in all cases. The recovery of Tac was almost constant, above 90%, except for 54 μM (88%), whereas that of Tol approach 100% below 13 μM but decreases progessively thereabove from 92% at 20 μM to 81% at 54 μM. At higher concentration values, the recovery curves continue to slow down, more rapidely for Tol (up to 52%) as compared to Tac, (46%) for a concentration of 214 μM. Note also that higher standard deviations occur at 107 μM and 214 μM as compared to the lower concentrations values of Vitamin E. It seems, therefore, that the present mixed micelles are not optimal for solubilization of more than 50 μM of Vitamin E.

Experiments were performed in order to highlight the behaviour of the non recovered Vitamin E at 107 μM and 214 μM. The remainder of the upper phase (hexane phase) of the eppendorf tubes was withdrawn and then excess volume of hexane with respect to micelle was added and the extraction procedure applied again for to reextract Tac or Tol still retained in the lower phase. Additionally recovered Tac and Tol was about 8% at 107 μM and 214 μM, and 3–7% at lower concentration values. Moreover, in order to check if part of Vitamin E is retained on the wall of the eppendorf tubes after the sonication, the vortexing and the redissolution steps, the tubes were rinced with ethanol (without agitation) and then hexane was added and the remaining steps of the extraction procedure performed. In this case, no more than additional 2% was recovered. The reextraction procedure allow, therefore, to approach 100% of recorery below 54 μM, but not above this concentration value. Suspecting oxidation of tocopherol during the extraction procedure, BHT (in 5 molar excess with respect to Tol) was added as an antioxidant to micelles containing 107 μM or 214 μM of Tol. In this case, lower recovery values of Tol were obtained (data not shown). Nierenberg and Lester [2] examined the effects of some compounds such as citrate, EDTA, oxalate and BHT on the stability of Vitamin E during the extraction process. According to these authors, BHT has no effect, whereas the other three compounds cause a loss of α-tocopherol. The question therefore arises how useful the addition of these substances may be and whether their use as additives should be recommended, especially in the case of BHT, as this substance has been reported to have toxic effects [33].

Table 2
Validation parameters in the concentration range of 1.7–54 μM of Tac or Tol in mixed micelles

	α-Tocopherol	α-Tocopheryl acetate
Precision[a]: R.S.D. (%)		
Within-day assay	1.5–5.3	1.3–2.7
Between-day assay	2.6–5.7	2.8–7.2
Linearity[b]	$y = 0.806x + 0.815$; $R^2 = 0.995$	$y = 0.885x + 0.218$; $R^2 = 0.997$
LOD (μM)	2.13	1.2
LOQ (μM)	4.75	3.4

[a] Mean values of 10 determinations.
[b] Regression equations of the plots of Fig. 4 (insert), y = recovery (μM), x = concentration of Tac or Tol in micelles (μM).

Based on the data described above, it is clear that the recovery of Tac and Tol from aqueous solution containing fixed concentrations of a bile salt and lipids is strongly dependant on their concentration. It seems that rapid interactions, which fluctuate in strength, occur between the constituents of the micelles depending on their respective concentrations. It is possible that at 107 μM and 214 μM of Tac or Tol lipidic stuctures are formed which trap Vitamin E and render it unavailable for extraction.

All in all, the method described in this paper permit accurate analysis of Tac and Tol in the concentration range below 50 μM in the micelles. The latter concentration correspond to about 25 mg/L, which is quite similar to the dietary

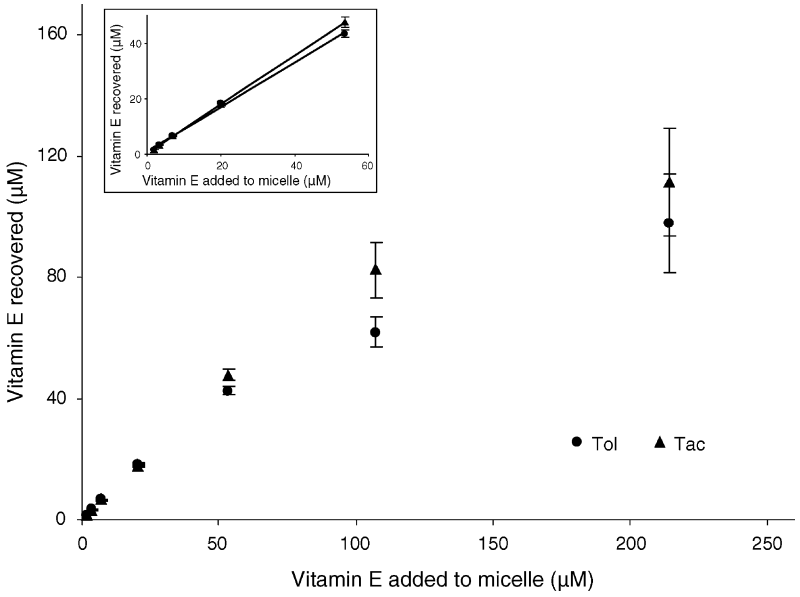

Fig. 5. Recovery of Tac and Tol from mixed micelles depending on the concentration of the two forms of Vitamin E. Each point is a mean value of five determinations.

references intakes of Vitamin E for humans [34]. Limits of detection are 1.2 μM for Tac and 2.1 μM for Tol, whereas limits of quantification are 3.4 μM and 4.8 μM, respectively (Table 2). The latter values which correspond to less than 2 mg/L of Vitamin E are too much lower than the nutritional Vitamin E intakes, even if we take into account the digestive dilutions.

4. Conclusion

It is in our opinion time to consider α-tocopheryl acetate as incoming form of Vitamin E and if necessary, as internal standard. Our aim is that our report may serve to design experiments for to get useful informations on the behaviour of tocopheryl acetate during digestion process. Indeed, the latest report of Food and Nutrition Board concerning Vitamin E [34] underlined the lack of data on the mechanism of absorption of this vitamin. The present analytical means may be used, for example, to design absorption experiments with organs or cultured cells, particularly in connection with the catalytic action of cholesterol esterase on tocopheryl acetate, or other Vitamin E esters. This may allow more precisely targeted nutritional supplementations by the ester forms of Vitamin E.

Acknowledgements

We would like to thank Dr. Pierre-André Geraert for his helpful advices and discussions. We are very grateful to Claude Arzouyan for advices concerning validation process and to Drs. Daniel Lafitte and Vincent Ollendorff for revising the english manuscript.

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