



Simplified HPLC-UV method for the determination of α -tocopherol in plasma

Marco Renzi¹, Federico Righi¹, Carla Quarantelli²,
Afro Quarantelli¹, Alberto Bonomi¹

¹Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti. Università di Parma, Italy

²Facoltà di Scienze Matematiche, Fisiche e Naturali. Università di Parma, Italy

Corresponding author: Dr. Marco Renzi. Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti. Facoltà di Medicina Veterinaria, Università di Parma. Via Del Taglio 8, 43100 Parma, Italy - Tel. +39 0521 032612 – Fax: +39 0521 032611 - Email: renzi@unipr.it

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ABSTRACT

Vitamin E, known for its great nutritional importance, is normally included in animal diets as DL- α -tocopherol acetate. The authors propose a method that makes it possible to determine the concentration of vitamin E in plasma without saponification. This method enable to avoid aggressive treatments on the analyte and complex procedures; it detects vitamin E only in form of DL- α -tocopherol.

Lipoproteins of analysed plasma were denaturated by methanol. Vitamin E was extracted by petroleum ether in presence of NaCl. The extract was dried by rotavapor at 45 °C, solubilized by methanol and injected in HPLC (C18 column, reversed phase). The quantitative determination was carried out by UV detector settled on 294 nm.

Tests of repeatability inter-analysis and intra-analysis gave coefficient of variability (CV%) respectively of 1.64 and 2.41%. The mean recovery was 100%.

Key Words: Vitamin E, Simplified method, Quantitative analysis, Plasma, HPLC-UV.

RIASSUNTO

METODO SEMPLIFICATO PER LA DETERMINAZIONE DELL' α -TOCOFEROLO NEL PLASMA VIA HPLC

La Vitamina E, nota per la sua grande importanza nutrizionale, viene integrata alla razione degli animali normalmente sottoforma di DL- α -tocoferoil acetato. Gli Autori propongono un metodo che permette di determinare la Vitamina E nel plasma, senza saponificazione, evitando così trattamenti aggressivi all'analita e procedure laboriose. Non vengono presi in considerazione congeneri della Vitamina diversi dall' α -tocoferolo. Il plasma da analizzare è sottoposto a denaturazione delle lipoproteine mediante metanolo. La Vitamina E liberata, è successivamente estratta con etere di petrolio in presenza di sodio cloruro. L'estratto è portato a secco con Rotavapor a 45 °C, ripreso con metanolo ed iniettato in colonna C18 per il dosaggio in HPLC in fase inversa, con rivelatore UV regolato a 294 nm. Le prove di riproducibilità inter-analisi ed intra-analisi hanno portato all'ottenimento di Coefficienti di Variabilità (CV %) rispettivamente di 1,64 % e di 2,41 %. Si è ottenuto un Recupero medio del 100 %.

Parole chiave: Vitamina E, Metodo semplificato, Analisi quantitativa, Plasma, HPLC-UV.

Introduction

It has been demonstrated that the addition of vitamin E in animal diet reduces the incidence of many reproductive diseases and increases the general state of health of animals (Quarantelli *et al.*, 2004). In order to verify animal deficiency, a quantitative determination of vitamin E in animal plasma may be necessary. Previous methods described in literature (Kayden *et al.* 1973; Bieri *et al.*, 1979; Indyk, 1988) are often laborious, expensive and sometimes characterised by aggressive procedures on the analyte (saponification, high temperatures), involve the use of complex instruments (specific HPLC column and detectors) and uncommon, relatively toxic reagents. The objective of this paper is to evaluate an alternative method for the determination of vitamin E in the plasma of domestic animals (cows, horses and poultry).

Because of the clinical relevance of vitamin E in animal health, it may be necessary to simultaneously evaluate its concentration in several samples. For this reason, the analytical method has to be simple, fast and inexpensive, but also reliable.

In addition, the instruments have to be common and the reagents have to be safe for both the operator and the environment.

Material and methods

Attention was focused only on the DL- α -tocopherol because this form is the only one utilised in animal feeding.

Reagents

Methanol for HPLC (Lichrosolv MERCK), Petroleum ether 40-60° for analysis (BDH), NaCl for analysis (CARLO ERBA).

Standard solutions were prepared from a stock solution (1000 mg/l) obtained by dissolving 100 mg of DL- α -tocopherol (SUPELCO 4-7783) in 100 ml of methanol.

Appropriate aliquots of stock solution are diluted with methanol to make working standard solutions.

Instruments

LC-system: HPLC system equipped with spectra series P100 pump (Thermoquest), 20 μ l loop.

Analytical column: Supelcosil C18, 25 cm X 4.6 mm, 5 μ m (Supelco).

Detector: Micro UV-Vis 20RS (Carlo Erba) with flow cell microhole (3 mm; 1.2 μ l).

Vacuum rotary evaporator: Buchi.

Centrifuge: ALC4226, 6000 rpm.

Vortex: VWR international.

Extraction

Plasma was defrosted at 37° for 1h; blended for ~30 seconds with vortex and centrifuged at 4000 rpm for 5 minutes. A portion of 1 ml was transferred into a Teflon centrifuge test tube and 1 ml of methanol was added; the mixture was shaken manually for ~30 seconds and then with vortex for another 30 seconds. Petroleum ether (8 ml) and NaCl (~1 g) were added. The mixture was shaken with a vortex for ~30 seconds, with ultrasounds for 5 minutes and centrifuged for 5 minutes at 4000 rpm.

The apolar supernatant was quantitatively transferred to a 50 ml round-bottom flask by plastic Pasteur pipet. The liquid-liquid extraction with petroleum ether was repeated without NaCl, adding new supernatant by the same pipet, in the round-bottom flask. The extract was evaporated by Rotavapor at 45 °C, taken up with methanol (1 ml), centrifuged for 5 minutes at 4000 rpm and injected in column.

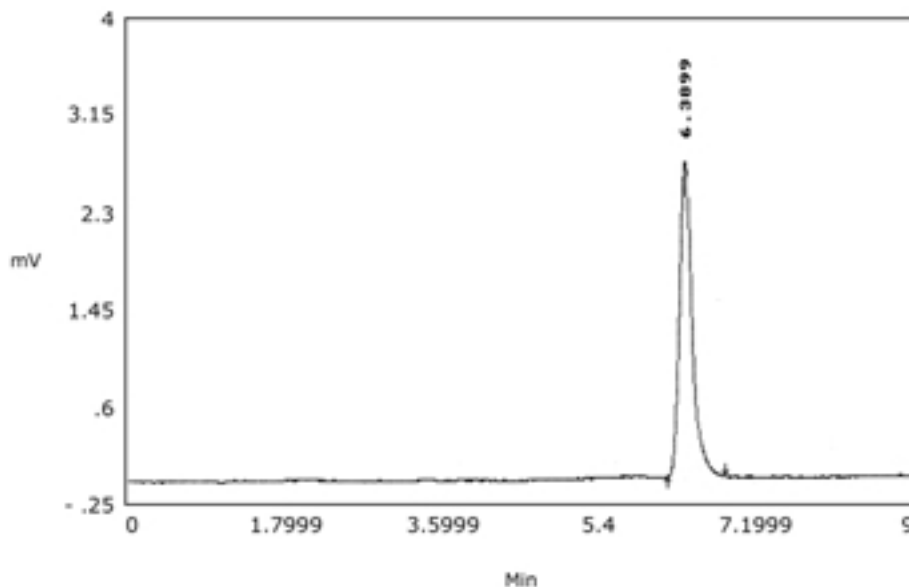
Lc Analysis

As isocratic elution methanol (100%) was used with a flow rate of 1.5 ml/min.

The λ of the detector was adjusted on 294 nm. The column was conditioned for at least 1 hour before analysis. The obtained retention time of α -tocopherol was 6-7 minutes and the total run time was 9 minutes for each sample (Fig. 1).

Calculation

The linearity of the method was evaluated by a straight line of calibration ($y = mx + q$). The equation was obtained by injecting 7 standard solutions at concentration of 0.2; 0.6; 1.0; 1.2; 1.6; 2.0; 10.0 mg/l, respectively, and calculating the linear

Figure 1. Standard solution (α -tocopherol = 10 mg/l).

regression per obtained area. The coefficient of correlation and coefficient of regression obtained were 0.9998 and 1.0103, respectively.

The calculated limit of quantification was 0.10 mg/l, while the calculated limit of revelation was 0.03 mg/l.

Results and discussion

The elution of α -tocopherol in the chromatographic conditions described above, provided a good peak-to-peak resolution for each plasma sample analysed (Fig. 2).

Repeatability of the inter-analysis method was determined by analysing 6 samples of plasma, fortified with 1 ml of standard solution at a concentration of 2 mg/l of DL- α -tocopherol, without the addition of methanol in phase of extraction. The obtained CV(%) value was 1.64 (Table 1).

Intra-analysis repeatability was determined by analysing the same extract four consecutive times. The obtained CV(%) value was 2.41 (Table 2).

Recovery was obtained analysing 6 samples of plasma fortified with 1 ml of standard solution at the concentration of 2 mg/l of DL- α -tocopherol, without the addition of methanol in phase of

extraction. The mean recovery was 100% (Table 3).

The described method results easier than others described in the bibliography (Kayden *et al.*, 1973; Bieri *et al.*, 1979; Indyk, 1988; Keller, 1988) because it involves the use of common instruments and there are no laborious treatments or strong chemical-physical stresses on the analyte, factors that can reduce recoveries.

Lipoproteins are denatured and precipitate using methanol and vitamin E release is obtained by manual and vortex shaking, without saponification, in concordance with other authors (Vuilleumier *et al.*, 1983; Cooper *et al.*, 1997; Gawlik *et al.*, 2003).

Use of NaCl to reduce solubility of analyte in methanol and favour extraction with petroleum ether appear crucial in reducing CV (%) value and in increasing the percentages of recovery, as described above.

Total time of analysis was ~20 minutes for each sample; this time includes conditioning of column, injection of standard solutions, extraction and chromatography runs. In each analytical session ten samples could be executed.

Used reagents are inexpensive, easy to find, with relatively low toxicity for operators and without excessive problems of waste disposal.

Figure 2. Bovine plasma (α -tocopherol = 5.87 mg/l).

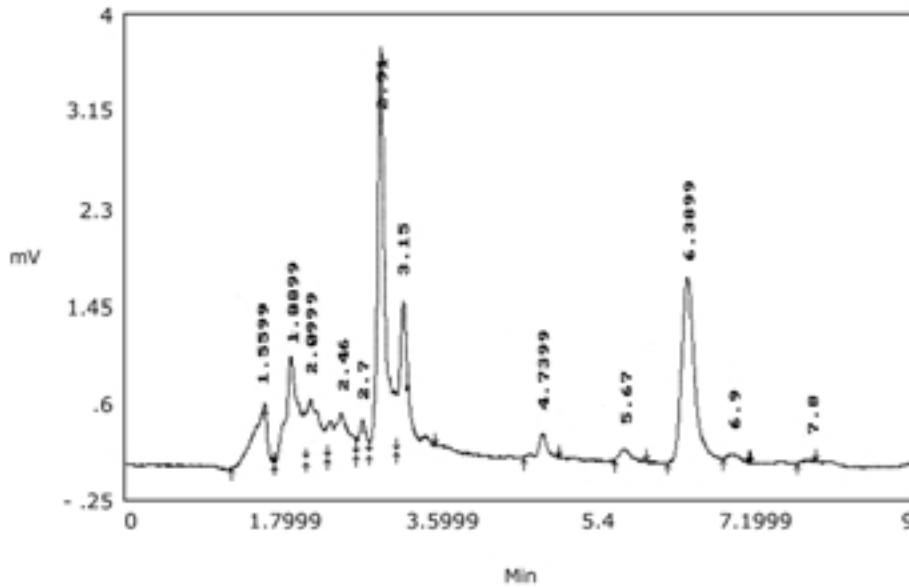


Table 1. Repeatability of inter-analysis method.

Parameter

Mean	7.61	α -tocopherol (mg/l)
SD	0.12	
CV %	1.64	
n. (number of injections of different extractions)	6	

Table 2. Repeatability of intra-analysis method.

Parameter

Mean	7.43	α -tocopherol (mg/l)
SD	0.18	
CV %	2.41	
n. (number of injections of the same extraction)	4	

Table 3. Mean Recovery.

Descriptions

Mean of n. 3 control plasma samples	5.60	α - tocopherol (mg/l)
Mean of n. 6 control plasma samples fortified with 2 mg/l of DL- α -tocopherol	7.61	α - tocopherol (mg/l)
Mean recovery	100	%

Conclusions

The described method is reproducible, reliable, simple, fast, inexpensive, relatively safe, with low environmental impact and it appears to be an important contribution for the determination of vitamin E in plasma.

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REFERENCES

- BIERI, J.G., TOLLIVER, T.J., CATIGNANI, G.L., 1979. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin. Nutr.* 32:2143-2149.
- COOPER, J.D.H., THADWAL, R., COOPER, M.J., 1997. Determination of vitamin E in human plasma by high-performance liquid chromatography. *J. Chromat. B.* 690:355-358.
- GAWLIK, M.T., GAWLIK, M.B., GORKA, A., BRANDYS, J., 2003. Optimization and validation of a high-performance liquid chromatographic method for the determination of α - and γ -tocopherol in rat plasma and erythrocytes. *Acta Chromat.* 13:185-195.
- INDYK, H.E., 1988. Simplified saponification procedure for the routine determination of total vitamin E in dairy products, foods and tissue by high-performance liquid chromatography. *Analyst.* 113:1217-1221.
- KAYDEN, H.J., CHOW, C.K., BJORNSON, L.K., 1973. Spectrophotometric method for determination of tocopherol in red blood cells. *J. Lipid Res.* 14:533-540.
- KELLER, H.E., 1988. *Analytical Methods for Vitamins and Carotenoids in Feed.* Hoffmann-La Roche Inc., Basel, Switzerland.
- QUARANTELLI, A., RIGHI, F., RENZI, M., BONOMI, A., NANI, E., TADDEI, S., CAVIRANI, S., 2004. La Supplementazione di vitamina E nella fase di asciutta: effetti sulla distribuzione di α -tocoferolo nel plasma delle vacche, dei vitelli e sulla sintesi delle immunoglobuline. *Proc. 36th Nat. Congr. SIB, Salsomaggiore (PR), Italy,* 36:81-93.
- VUILLEUMIER, J.P., KELLER, H.E., GYSEL, D., HUNZIKER, F., 1983. Clinical Chemical methods for the routine assessment of the vitamin status in human populations. *Int. J. Vitam. Nutr. Res.* 53:265-272.