VALIDATION OF A METHOD TO DETERMINE VITAMIN E (ALPHA TOCOPHEROL) FROM FEED INGREDIENTS BY HPLC USING REVERSED PHASE CHROMATOGRAPHY

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Abstract

Keeping farm animals healthy is supported by feed quality. To prevent the high unsaturated fatty acids ingredients from getting rancid it is necessary to add antioxidants such as vitamin E (alpha tocopherol). EU and Romania regulations imposed special requirements on the performance of related laboratories on the validation of vitamin E determination in feeds according to the requirements of SR EN ISO/CEI 17025:2005 standard for the following performance categories: exactness, accuracy, repeatability, reproducibility, sensitivity, detection limit and quantification limit. The following results were obtained during the determinations on standard alpha tocopherol solution: exactness = 100.64 %, accuracy with RSD = 1.06% and 1.29%, repeatability with RSD = 1.06%, 0.62% and 0.74 %, reproducibility cu RSD 2.5% and 1.59%, sensitivity with R^2 = 0.998, the detection limit was lower than the quantification limit and within the admitted performance range. The model was tested on feed samples containing corn, rice, wheat, soybean meal, gluten used for layer feeding. The dietary vitamin E was calculated using the equation from the standardizing curve of the equipment obtaining 15 mg/100 g feed sample. Our work delivers to the specialists in feed quality control a tested model for the validation of the method to assay vitamin E from feed ingredients.

Key words: vitamin E, chromatography, validation, feed.

INTRODUCTION

The European regulations concerning feed quality are part of the legislation on food safety, based on risk factors analysis in terms of ensuring the public health. The economic development and modernization of our country impose a special attention to product quality; the products must be competitive and must meet the quality requirements demanded by this circuit. Method validation became compulsory for foods and feeds in order to get as close as possible to the requirements for human feeding [12, 13]. The amount and quality of dietary vitamin E is very important because it has a strong antioxidant character, protecting against oxidative degradation the biologically active substances such as vitamins A, D, F, the unsaturated fatty acids, hormones, erythrocytes, enzymes. Vitamin E influences the activity of tissues and organs, of endocrine glands, of the erythrocytes, of the muscle and nervous system, they take part in many metabolic processes, playing an active role in animal and vegetal organisms [1, 18, 4]. Because of the very high biological activity of α-tocopherol, the nutritionists prefer to introduce this form of vitamin E in animal feeding [11, 14, 6]. Knekt et al. and Kushi et al, have shown that the food with a high level of dietary tocopherol is associated to a low mortality caused by cardiovascular diseases [9,10].

HPLC methods are currently used to determine vitamin E in raw feed ingredients and in finished feeds, with methanol and/or acetonitrile mobile phases [18, 3, 15, 2, 8]. Svetlana et al. used in parallel the two mobile phases: methanol/water (95:5) and acetonitrile/water (78:22) to determine vitamin E from sunflower oil, observing that a better determination of the vitamin was achieved using the mobile phase based on methanol [18].

The validation of this analytical method is a process by which, using laboratory studies and mathematical-statistical analyses, the characteristic performances of a method are confirmed to meet the requirements for the intended practical application [19]. This is a procedure for the verification and confirmation of the performances of an analytical method, having several purposes: to increase the confidence in the results obtained with an analytical method; to show that the method is fit for the intended use; to meet customer demands and reach the performance parameters required for testing [5, 7, 16].

The purpose of this paper was to develop an experimental model, easy to apply practically in the laboratories for feed quality control, including the stages necessary to validate an analytical method for vitamin E assay.

MATERIAL AND METHOD

Materials HPLC PerkinElmer series 200 with the following components (pump, UV-VIS detector, chromatographic column Thermo 150x4.6mm, Hypersil GOLD , autosampler); analytical scales with accuracy to the fourth decimal; water bath with adjustable temperature GFL Gesellschaft

Reagents: potassium hydroxide (KOH); ethylic alcohol; methanol HPLC; petrol ether; Sigma chemicals α-tocoferol blank. All reagents were of the highest purity, being purchased from Merk.

Vitamin E determination from the feed sample:

The sample was processed according to STAS SR EN ISO 6867:2001-Feeds-Determination of vitamin E content – Method by high performance liquid chromatography.

We used 2 g feed, dried and ground. The sample was saponified with an ethanol solution of potassium hydroxide; extraction was done in petrol ether. The resulting samples were concentrated in a rotavapor; the residue was dissolved in 2 ml methanol.

Chromatographic separation: the mobile phase was methanol:water (97:3); flow 1.2 ml/min; wavelength 295 nm; column temperature 24ºC [17].

RESULTS AND DISCUSSION

In this work we conducted tests to validate an analytical method to determine vitamin E by HPLC from a poultry feed with 42.72% corn, 10% rice, 10% sorghum, 20% soybean meal, 5% gluten. The reference documents are mentioned in the literature. The analytical validation is the first step of quality in a laboratory. The analytical works on the poultry feed intended to validate the method for vitamin E determination aimed to determine:

EXACTNESS, which expresses the degree of agreement between the result of a test and the accepted (real) reference value of the measuring device. The exactness was determined on 15 determinations on the certified reference material (α-tocopherol) of 50 ppm concentration.

$$\text{Exactness } \% = \frac{\text{Xmediu}}{\mu} 100$$

where:
Xmediu = average of the 15 determinations
μ = real value of the reference material

$$\text{Bias } \% = \frac{\text{Xmediu} - \mu}{\mu} 100$$

The performance criterion demanded for exactness ranges between: 80-120%, for concentrations ≥ 10 μg/kg, i.e. 0.001% (g/g).

ACCURACY (fidelity) which is the measure of the degree of agreement between the independent results of a series of determinations done for the same analyt from a sample, using the same working conditions.

For the analysis of this parameter we conducted 5 determinations of the analyt of 50 ppm concentration during the same day and in different days.

$$\text{CV(RSD) } \% = \frac{s}{\text{Xmediu}} 100$$

where:
Xmediu = average of the 5 determinations
s = standard deviation

The maximal RSD value is set according to the analyt concentration for concentrations ≥1000 µg/kg, CV(RSD)max = 10%, according to ANSVSA Order no.51/2005.

REPEATABILITY is a measure of the measure variability when the same analyst works in the same conditions. We conducted three series of 5 repeated analyses of the reference material (α-tocopherol), by a single analyst, at short intervals, using the same measuring equipment and the same working method.

The maximal RSD value is determined function of the analyt concentration according to Horwitz equation(for concentrations of 1 ppm, RSD max = 10.72%), according to ANSVSA Order no.51/2005

REPRODUCIBILITY is the way in which the value of fidelity is found under different experimental conditions. We conducted two series of 10 repeated analyses of the reference material. We worked on an initial concentration of 50 ppm. The analyses were conducted by two analysts using different glassware, the same working method and the same equipment.

The maximal RSD value is determined function of the analyt concentration according to Horwitz equation(for concentrations of 1 ppm, RSD max = 10.72%), according to ANSVSA Order no.51/2005

SENSITIVITY – variation of instrument response to the variation of the analyt concentration. We plotted the standard curve in 10 points of the reference material. The slope must be constant on the working domain, R2 – correlation coefficient, must tend towards 1 (Figure 1).

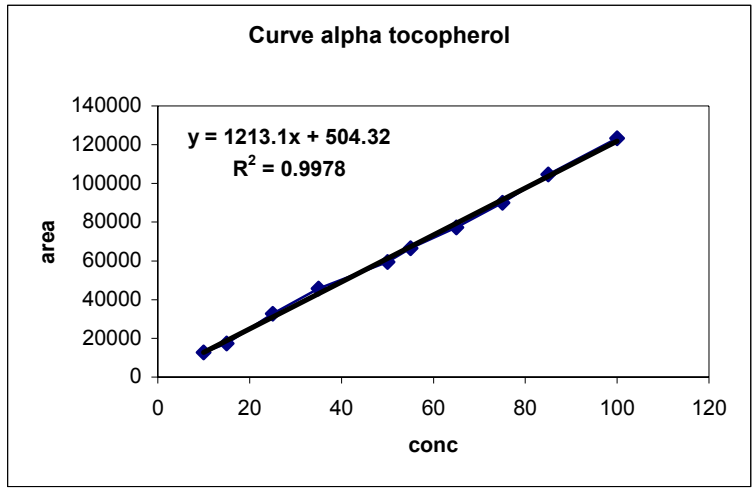

Fig.1 Standardization curve for vitamin E

LIMIT OF DETECTION (LOD)- the lowest analyt concentration that can be detected.
LIMIT OF QUANTIFICATION (LOQ) – the lowest analyt concentration that can be detected quantitatively, at an acceptable level of incertitude.
We conducted 5 analyses of the reference material with an initial concentration of 5 ppm. The demanded performance criterion is LoQ > LoD.

Table 1
Results obtained for the validation of HPLC method for vitamin E determination

Determined parameters	Vitamin E (α-tocoferol)
EXACTNESS	
BAlue (%)	100.64
Bias (%)	0.64
ACCURACY (FIDELITY)	
Same day – RSD (%)	1.06
Different days – RSD (%)	1.29
REPETABILITY	
series 1 (50ppm)-RSD (%)	1.06
series 2 (40ppm)-RSD (%) – (80% of the concentration from series 1)	0.62
series 3 (60ppm)- RSD (%) – (120% of the concentration of series 1)	0.74
REPRODUCIBILITY	
Analyst 1 – RSD (%)	2.57
Analyst 2 – RSD (%)	1.59
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION	
LoD (Mm)	6.79
LoQ (Mm)	9.34

In the case of the internal reproducibility, determined on 2 series of 10 analyses repeated by two analysts, we obtained the following values of the intralaboratory variation coefficient CV(RSD)2calc = 2.57% (analyst I), and CV(RSD)2calc = 1.59 (analyst II), both values being lower than the highest limit admitted by ANSVSA Order 51/2005.

Regarding the sensitivity, we obtained it from the linear regression equation Y = a + bx = 1213.1x + 504.32 and of the regression coefficient R2 = 0.997. It resulted that the function is linear and is close to the ideal case, the Burguer-Lambert-Beer law.

For the quantification limit LOQ, which usually is the lowest point on the standardization curve, we obtained a value of 9.34 µg/mL which is higher than the value obtained by for the limit of detection LOD of 6.79 µg/mL, being in agreement with ANSVSA Order 51/2005.

The incertitude may appear from several possible sources, including sample collection, environmental conditions, used glassware, used equipment. We also calculated the extended incertitude, with a value of 6.07ppm. Thus, we have found a vitamin E concentration of 149 ±6.07 ppm.

CONCLUSIONS

The results of the tests conducted to validate the analytical method for vitamin E determination in the feeds, shows that they are within the performance parameters admitted for this method and they meet the requirements of SR EN ISO 17025:2005 standard.

The characteristic performance of the method meet the requirements for its practical utilization. We consider that this validation method can be an experimental, practical model for those working in feed quality evaluation and want to become licensed according to SR EN ISO 17025:2005.

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