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Determination of tocopherol in oil pharmaceuticals by using the spectrofluorimetry and method validation

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Abstract The paper presents research results regarding the establishing of the optimal conditions for the determination of fat-soluble vitamin E in multivitamin pharmaceutical products, by using the native fluorescence of the compound in n-hexane. Two spectrofluorimetric methods were tested, with solubilisation directly in n-hexane and after n-hexane extraction from non-polar matrices using ethanol as a carrier. The excitation and emission wavelengths were 290 nm and 306 nm, respectively, and a comparative study was made. The first method is linear in the range 1 – 100 μg/mL, having a detection limit of 1 μg/mL and a quantification limit of 2 μg/mL. The second method was developed for vitamin E concentrations in the range 2 - 60 μ/mL. The method is linear in the range 2 – 50 μg/mL, with a 0.68 μg/mL detection limit and a 2.27 μg/mL quantification limit.

Keywords: tocopherol, pharmaceutical, spectrofluorimetric assay, validation

1. Introduction

A large number of methods have been developed for quantifying vitamin E in pharmaceuticals because this vitamin is basic to human health and its determination gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food applications.

There have been isolated from natural sources three substances with vitamin E activity: α-, β- and γ-tocopherol, all being methyl derivatives of the mother substance – tocol.

Tocopherol can be condensed with o-phenyldiamine after oxidation the product being a fluorescent derivative [1,2]. One product is obtained with α-tocopherol, excitation at 270 nm and 370 nm and green colour emission. In the case of β- and γ-tocopherol, a mixture of different compounds compared to the adduct of α-tocopherol can be obtained. The literature reports fluorescent analytical methods for the determination of tocopherol by oxidation; e.g., a strong fluorescence was observed at 365 nm after oxidation [3].

Other specialists validated a method for simultaneous identification of water- and fat-soluble vitamins (A, E, B_1, B_2 and B_6) in aqueous micellar medium of hexadeciltrimethylammonium using fluorescence techniques [4].

In the last years, the coupling of one chromatographic separation method with fluorescence detection methods gained a great popularity. Most test were made using the native fluorescence (λ_{ex} = 295 nm, λ_{em} = 330 nm) and hexane as eluent on silice column [5]. It was tried a separation method using capillary electrophoresis coupled with fluorescence detector used to assay vitamin E analysis from oils, with methanol/acetonitril 50/50 v/v with ammonium acetate 0.01% as mobile phase and C18 column as a stationnary phase [6].

The papers aims to present two spectrofluorimetric methods for establishing of the optimal conditions for the determination of fat-soluble vitamin E in multivitamin pharmaceutical products, by using spectrofluorimetric methods. A comparative study was made and certain validation parameters for the methods were calculated.

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2. Experimental

Two methods have been used. **The first method** uses n-hexane as a solvent for α-tocopherol spectrofluorimetric assay, as n-hexane is the only non-polar solvent for α-tocopherol that results in a fluorescent species [2,7]; the method can be used to assay tocopherol in pharmaceutical formulations as soft jellylike capsules. **The second method** is an extractive one that uses ethanol as a carrier between the aqueous solution and the n-hexane fluorescent solution of α-tocopherol; the method can be adapted and further used to assay α-tocopherol from biological matrices (e.g. serum, tissue homogenate, etc.).

For the first method, DL-α-tocopherol acetate purchased from Merck; vitamin E soft jellylike capsules (Biofarm S.A. Bucharest) and n-hexane (Merck) have been used. The oily content of 5 soft jellylike capsules was extracted by means of a syringe. A series of DL-α-tocopherol acetate standard solutions in the range 1 to 230 μg/mL, as well as appropriate dilutions of vitamin E soft jellylike capsules content in n-hexane have been made. The fluorescence emission spectrum of the solutions was scanned using the instrumental parameters listed in Table 1. The calibration graph of vitamin E in n-hexane was made by linear regression [8] using the corrected for solvent background values of emission peak intensities at 306 nm.

For the second method, DL-α-tocopherol acetate purchased from Merck; vitamin E soft jellylike capsules (Biofarm S.A. Bucharest); physiological serum, ethanol (Chimopar) and n-hexane (Merck) have been used. Vitamin E and the content of 5 soft jellylike capsules were solubilised in ethanol to obtain the standards, than isotonic saline solution was added, followed by a two-step liquid-liquid extraction with hexane [7]. Blank samples lacking tocopherol and vitamin E samples were prepared similar to standards. The calibration curve was plotted by linear regression using the corrected fluorescence intensity values

The tests were performed using a Perkin Elmer LS 50B spectrofluorimeter and the values of the performance characteristics of the methods were estimated through statistical calculation using the *Microcal Origin 6.0* software.

3. Results and discussions

Fluorescence emission spectra were plotted using instrumental parameters given in Table 1. Earlier observations indicate the apparition of a Raman band of the solvent in the 315 nm zone, so a background correction is necessary [7]. In these conditions, the maximum of emission for vitamin E was observed at 306 nm, about 20 nm hypsochrom shifted compared to older literature data; this maximum emission was further used in analysis.

As fluorescence is observed for α-tocopherol only when dissolved in n-hexane, together with the specific excitation and emission wavelength of the compound and the fact that corrected spectra for the blank were used in both methods, the methods can be considered specific. In analyzing pharmaceutical oily vitamins neither the other vitamins, nor the usual excipients do not spectrally interfere with the n-hexane fluorescence of vitamin E. However, in biological matrices, even if rather improbable, the specificity of the method has to be determined for each kind of a matrix.

Table 1. Instrumental used parameters

Parameter	Excitation	Emission
Fixed wavelength	290 nm	–
Slits	2.5 nm	2.5 nm
Registered domain	–	300–410 nm
Scanning speed	–	600 nm/min

It can be noticed that vitamin E presents an inhibition of fluorescence effect (inner-cell effect) at great concentration values, marked at concentrations higher than 100 μg/mL (Fig. 1). That is why the calibration graph was plotted only in the 1-100 μg/mL domain.

The linearity domain (Fig. 2), detection and quantification limits were established following the ICH recommendations [8].

The linearity parameters are listed in Table 2; each value of the used points is a mean of 5 replicates.

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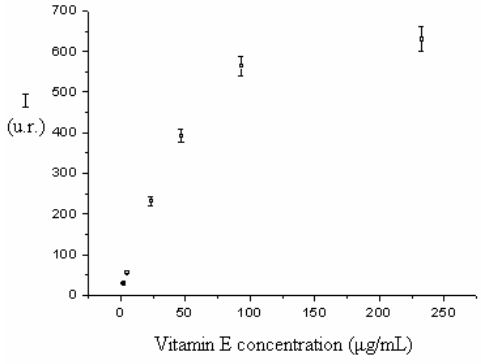

Fig. 1 The evolution of the fluorescence intensity with vitamin E concentration

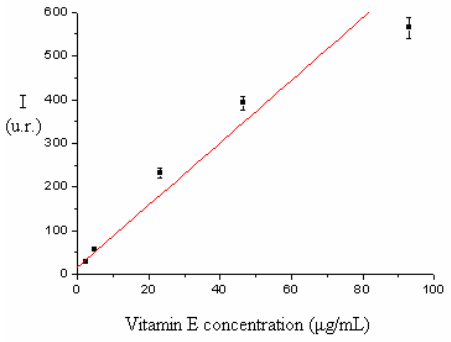

Fig. 2 The calibration curve of vitamin E in n-hexane spectrofluorimetric determination (method 1)

Table 2. Parameters values obtained from calibration curve of vitamin E spectrofluorimetric determination in hexane (method 1)

Parameter		Value	Error
A		16.34915	7.40974
B		7.14056	0.90248
R	SD	N	p
0.97687	4.45261	5	0.00421

where: A = intercept, B = slope, R = correlation coefficient, SD = standard deviation of experimental points from the regression line, N = number of points, p = probability

The detection and quantification limits were established according to ICH recommendations [8], based on visual evaluation. The obtained values were 1 µg/mL for the detection limit of and 2 µg/mL for the quantification limit.

The recovery coefficient [7] calculated by using the specifications of the vitamin E soft jellylike capsules was of 101.5%. The greater amount found compared to that one declared can be due to overdosing of the product.

Instrumental parameters used for the spectra collection in the second method are listed in Table 3.

When plotting the spectra, Raman band of solvent (n-hexane) appears at 315 nm, so, the spectra have to be corrected for the blank [7].

Table 3. Instrumental parameters used

Parameter	Excitation	Emission
Fixed wavelength	290 nm	-
Slits	3 nm	3 nm
Registered domain	-	300–410 nm
Scanning speed	-	1000 nm/min

The calibration curve of vitamin E following n-hexane extraction was made using the corrected values of emission peaks intensity (Fig. 3).

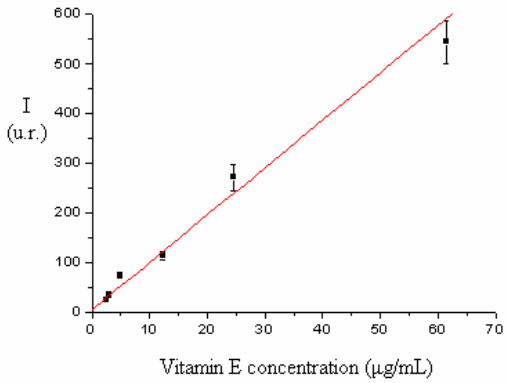

Fig. 3 The calibration curve of vitamin E spectrofluorimetric determination following n-hexane extraction (method 2)

The calibration graph parameters are summarised in Table 4.

The method is linear in the range 2 – 50 µg/mL, with a 0.68 µg/mL detection limit and a 2.27 µg/mL quantification limit, computed following the ICH rules, based on the standard deviation of the response and the slope [8].

The recovery coefficient [7] calculated by using the specifications of the *vitamin E* soft jellylike capsules was 92.3%, and for the samples spiked with standard solutions of DL-α-tocopherol acetate was 86.6 %.

Table 4. Parameters values obtained from calibration curve of vitamin E spectrofluorimetric determination following n-hexane extraction (method 2)

Parameter	**Value**	**Error**
A	5.7633	3.96087
B	9.54454	1.17722

R	**SD**	**N**	**p**
0.9709	2.28901	6	0.00126

where: A = intercept, B = slope, R = correlation coefficient, SD = standard deviation of experimental points from the regression line, N = number of points, p = probability

The differences between the two methods in what concerns the recovery coefficients can be due to extraction looses.

4. Conclusions

The spectrofluorimetric methods are generally used because of their specificity, selectivity and low detection limits.

As vitamin E becomes fluorescent only when solubilised in n–hexane, two methods were developed for the spectrofluorimetric assay of vitamin E: one using the direct solubilisation of the oily content of soft jellylike capsules, and the second by hexane extraction from aqueous matrix using ethanol as a carrier. Both methods use background corrected emission spectra, with excitation at 290 nm and emission at 306 nm.

The first method was linear in the range of 1-100 μg/mL (R=0.97687), having a detection limit of 1 μg/mL and a quantification limit of 2 μg/mL. To above 100 μg/mL, the inhibition of fluorescence with concentration effect appears.

The second method was linear in field 2-50 μg/mL (R=0.9709), having a computed detection limit of 0.68 μg/mL and a quantification limit of 2.27 μg/mL.

Prerequisite steps for the methods validation have been also presented.

5. References

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