ANALYTICAL CHARACTERIZATION OF STABILITY FOR SOME FAT-SOLUBLE VITAMINS IN PHARMACEUTICAL PRODUCTS

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abstract: Vitamins are generally sensitive to light, high temperatures and moisture. They should be kept in properly closed containers, protected from humidity and light at temperature of maximum 21^0C.The aim of this work is to analyzed the stability of some fat-soluble vitamins (A and E). The analyzed technique used was the HPLC method for analytical characterization of these.

Introduction

Most of the fat-soluble vitamins present in pharmaceutical preparations or in natural products are accompanied by a number of closely related compounds. This explains why chromatographic methods are so frequently used in analysis of these compounds. HPLC is almost ideally suited for these compounds because of its simplicity, speed, selectivity and sensitivity [1-6].

Vitamin A (formula 1) was chromatographed in the form of the alcohol, the acid or the respective ester in the presence of its isomers or other vitamins, especially D_2, D_3 and tocopherol [7-10]. Reverse-phase chromatography with a mobile phase of methanol and water (96:4 or 95:5) was used for the quantitative determination of vitamins A and E [11-14]. Retinol and α-tocopherol were determined in serum with high sensitivity over a wide linear range using UV detection at 340 or 280 nm and using retinyl acetate as the internal standard.

A number of HPLC modifications for the simultaneous analysis of vitamins A and E (formula 2) in pharmaceutical preparations and biological material have been worked out. These methods usually used UV, fluorimetric or electrochemical detection [15,16]. The two vitamins were determined using elution with aqueous methanol and detection at 325 nm (retinol) or 292 nm (tocopherol) [17].

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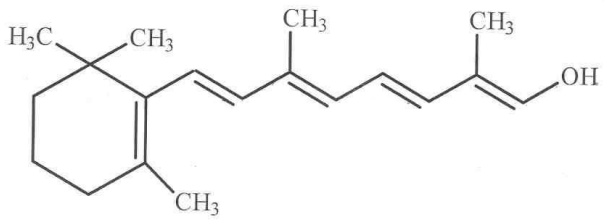
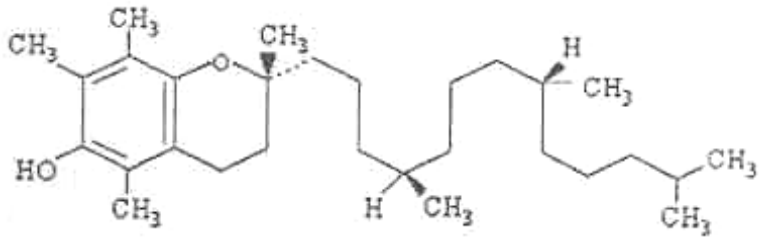


Formula 1



Formula 2

In this paper the influence of temperature on the stability of these vitamins fat-soluble is analysed using the HPLC method, during 30 months. The stability of these is influenced in principal by temperature, light and moisture and also by the preservation conditions.

Experimental part

Investigations were carried out with a Spectra-Physics Analytical Model P 4000 liquid chromatograph with a Model 2000 UV detector (Spectra-Physics Analytical). This system was connected to a computer Pentium III 800MHz.

The pump P 4000 is conceived to reach the performances and the maximal capacities. It presents features of vanguard: four solvents, eleven files users of the names of files and solvents, and a waiting line for the link of the files.

The UV 2000 is a detecting UV/VIS doubles length of wave, programmable, entirely equipped. It functions as well in simple fashion that length of wave doubles in fashion, in the UV ranges and visible. The UV 2000 also offers the spectral sweep, a file develop (for the development of the methods), the storage of several files, a waiting line (for the link of the files), and more. We place the detector to a length of wave λ = 326 nm for vitamin A and for vitamin E to λ = 292 nm.

A mobile phase composed of methanol and water (98/2) allowed a very good separation of vitamins A and E on LC 18 column.

The vials of oral solution Biosol (Biokim Istanbul Turkey) were stored in special climate shelves. The vials were kept at temperatures of $+5^0$C, $+15^0$C, $+21^0$C and $+37^0$C.

The following tests have been executed:

– optical appearance of the product;

– qualitative and quantitative analysis of the active ingredients (vitamins A and E).

Results and discussion

For vitamins A and E, the European Pharmacopoeia gives the following storage instructions: "store in a well-closed, well-filled container, protected from light, between 8^0C and 15^0C. When the container has been opened, its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas (e.g. nitrogen)".

The limits for the contain in vitamins for each vial are 45000-55000 U.I./mL vitamin A and 27 – 33 mg/mL vitamin E. The results of the determinations for each temperature, test period 30 months, are presented below in Tables 1-4.

Table 1. The results obtain at temperature $+5^0$C (refrigerator).

Assay	Vitamin A (U.I./mL)	Vitamin E (mg/mL)
Nominal value	50000	30
Date of manufacture	55000	33
6 months	54000	33
12 months	54000	32
18 months	53000	32
24 months	51000	31
30 months	50000	31

Table 2. The results obtain at temperature $+15^0$C (cool cabinet).

Assay	Vitamin A (U.I./mL)	Vitamin E (mg/mL)
Nominal value	50000	30
Date of manufacture	55000	33
6 months	54000	33
12 months	53000	32
18 months	51000	31
24 months	50000	31
30 months	49000	30

Table 3. The results obtain at temperature $+21^0$C (room temperature).

ssay	Vitamin A (U.I./mL)	Vitamin E (mg/mL)
Nominal value	50000	30
Date of manufacture	55000	33
6 months	54000	33
12 months	52800	32
18 months	51000	31
24 months	49500	30
30 months	48000	29

Table 4. The results obtain at temperature +37^{0}C (climate cabinet).

Assay	Vitamin A (U.I./mL)	Vitamin E (mg/mL)
Nominal value	50000	30
Date of manufacture	55000	33
6 months	52000	31
12 months	49000	30
18 months	47000	29
24 months	41000	27
30 months	37000	25

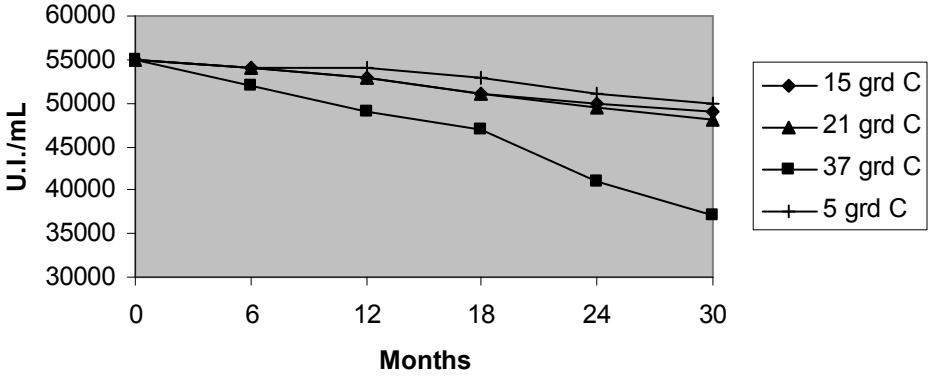


Fig. 1. *Variation of content in vitamin A with time at different temperatures.*

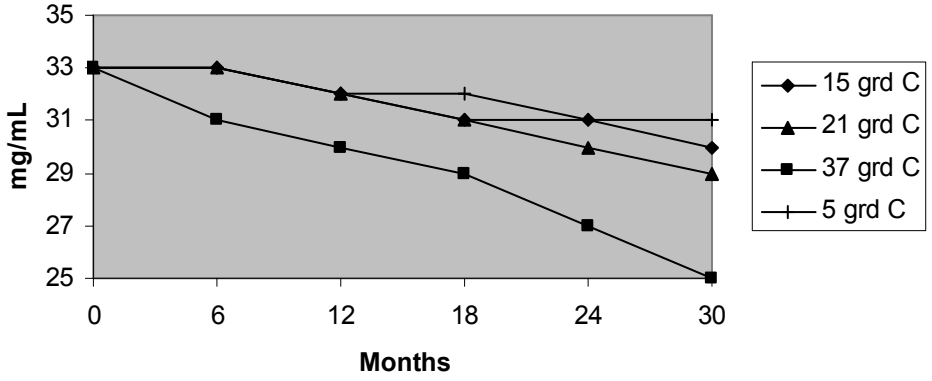


Fig. 2. *Variation of content in vitamin E with time at different temperatures.*

The oral solution of Biosol in the original vial is stable and retains its activity when stored at room temperature (18-21^{0}C). Under high temperatures (37^{0}C climate cabinet), the solution is showing an intensifying colouring after longer storage and looses activity, which are even below the tolerances of the declared value.

Conclusions

The stability tests show that during a storage period of 2 years an adequate stability is provided, when the product is stored at room temperature (< 21^0C) and protected from light. The choice of the storage temperature plays an important roll for the stability of the pharmaceutical products of vitamins.

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