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Testing the content of vitamin A in dietary supplements

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Summary

Vitamins in the human body perform regulatory functions, determine the development, health and physical performance of the body. Most of them are not synthesised in the body, therefore they must be supplied with food. They interact with enzymes, which is why they are often called coenzymes or catalysts. Vitamins regulate many life processes. Their deficiency may cause disturbances in metabolic processes and promote the development of various types of diseases.

The aim of the work was to analyse the level of vitamin A content available on the Polish pharmaceutical market and dietary supplements.

Key words: vitamin A, supplements, content designation , HPLC method

Introduction

The term of vitamin A refers to a group of substances with an analogous chemical structure, characterised by similar activity [1]. There are natural and synthetic derivatives of retinol (retinoids), which are characterised by the activity of vitamin A [1,4]. Due to the large number of such compounds, an attempt was made to systematise them. The committees on nomenclature participated in these works: IUPAC (*International Union of Pure and Applied Chemistry*) and IUB (*International Union of Biochemistry and Molecular Biology*) [4].

✓ Generation I includes natural compounds with a non-aromatic β-ionone fragment in their molecule. They are maintained by modification of groups at the polar end and polyene side chain of vitamin A [1,4]. The compounds of this type include:

• retinol - long-chain alcohol, considered as the basic form of vitamin A [4]. It is the most often used in the form of esters - palmitate (*retinyl palmitate*) and acetate (*retinylacetate*) [1].

• retinal aldehyde (retinal) – it obtained in the process of oxidation of retinol [4,1]. Some of the retinol metabolites have specific biological functions: 11-*cis* retinal necessary for normal vision [6].

• retinoic acid – it obtained in the process of oxidation of retinol, it is its most oxidised derivative [4]. Retinoic acid has become the basic compound for obtaining subsequent generations of retinoids [1]. It occurs in the form of three geometrical isomers: a completely transretinic acid (all-*trans*, customarily tretinoin), 9-*cis* retin (usually isotretinoin) and 13-cisretin (usually alitretinoin) [4]. Tretinoin was the first synthetic retinoid in the following years was synthetised isotretinoin (1955) [1].

✓ Generation II is synthetic synthesis of monoaromatic compounds that resemble natural retinoids in their structure [4]. In these compounds (e.g. acitretin), the cyclohexene ring was replaced with a benzene ring. This compound was synthesised for the first time in 1972 [1].

✓ Generation III – it includes compounds with a structure significantly different from the naturally occurring retinoids [4]. They arise as a result of cyclisation of the polyene side chain [1].

Retinoids are lipophilic compounds that are hardly soluble in hydrophilic fluids, therefore the transport of these compounds and the control of their activity takes place with the participation of various types of proteins. These include RBP plasma proteins (*retinoidbindingproteins*) and CRBP (*cellularretinoidbindingproteins*) proteins present in the cytoplasm.

There are two types of receptors in the cell nucleus that are capable of binding retinoids:

- RAR (*retinoicacidreceptors*) - receptors for retinoic acid binding

with ligands: tretinoin, isotretinoin, tazarotene and alitretinoin,

- RXR (*retinoid X receptors*) - X retinoid receptors binding to the ligands: alitretinoin, bexarotene [4].

The retinoid receptors are located in the cells of the epidermis, hair follicles, sebaceous glands and on Langerhans cells, and their number depends on many factors, including the presence of inflammation and the stage of the cell development cycle [4].

Vitamin A stimulates the formation of new cells, which affects the state of the epidermis by regulating the processes of exfoliation and regeneration of epithelia, inhibition of the action of metallopreteinases, functions of melanocytes. In addition, it has antioxidant properties, affects the vision process, immune processes and the immune system, and plays an important role in embryonic development processes [3,4,6]. Provitamin A and vitamin A neutralise the action of free radicals and lipid peroxides [7].

Due to its antioxidative properties, vitamin A is mentioned among the essential components of functional foods [2]. Vitamin A, along with other factors, affects the development of dental germs, and its deficiency may lead to enamel hypoplasia and odontoblast atrophy with atypical dentine production [8]. In pregnant women, vitamin A deficiency can lead to fetal growth disorders, eclampsia, premature membrane rupture. fetal [3]. Also during lactation among other nutrients, mineral should be taken into account the increased need for vitamin A [8].

Retinoids are also used in the treatment of certain cancers, such as: acute promyelocytic leukemia, Kaposi's sarcoma [4]. Bexarotene (the third generation of retinoids)

is used in cutaneous forms of T-cell lymphoma [1]. In addition, retinoids have found use in the treatment of many diseases such as acne and rosacea, lupus erythematosus, purulent dermatitis, bacterial folliculitis, ichthyosis, psoriasis [4].

Aim of the work

The aim of the work was to analyse and quantify the level of vitamin A in 10 different dietary supplements, available directly on the Polish pharmaceutical market.

Research material and methods

The research material consisted of 10 samples of randomly selected supplements from various manufacturers containing vitamin A that were purchased in the fourth quarter of 2017. The study was subjected to products that included only vitamin A, vitamins A + E, or a set of many vitamins, of which vitamin A was only as a part.

The test was carried out at the Regional Center for Research EKO-AGRO-TECH of Pope John Paul II State School of Higher Education in Biała Podlaska, in the laboratory of biological and food analyses, at the turn of October and November 2017.

The manufacturers provided a declaration regarding the content of vitamin A on the packaging of the labeled preparations. The content of vitamin A was determined by HPLC method according to the developed test procedure, in accordance with the PN-EN 12823-1: 2002 standard [5]. For analysis, a liquid chromatograph from Dionex was used, equipped with a UV detector using a wavelength of 325 nm. AQUASIL C18 inverted column from Thermo Scientific company with dimensions 250 mm x 4.6 mm 5 μm was used for the separation together with the protection column LC-18 and the mobile phase methanol / water in a ratio of 93: 7 (v / v).

Before the analysis, the samples were saponified with methanolic potassium hydroxide in the presence of BHT as an antioxidant, followed by extraction with hexane. The extracts were evaporated and the residue was dissolved in methanol and subjected to chromatographic analysis. Each sample was tested twice. The content of vitamin A was determined on the basis of a standard curve. The standard was RETINYL ACETATE by Sigma Aldrich [5].

Results

In the analytical study, conducted at the turn of October and November 2017, the measurements of the vitamin A content in capsules of 10 different dietary supplements available on the Polish pharmaceutical market were made. The research was carried out on products containing vitamin A + E, vitamin A alone or a set of many vitamins, of which vitamin A was only as a part.

The results of the analysis are presented in the table below:

Table I. Declared and actual content of vit. A in the capsule of the dietary supplement

Sample	Content declared in J.M.	Content declared by the manufacturer in mg / capsule	marked content in μg / capsule
A01	2666,7	800	745,470
A02	30000	9000	4012,873
A03	2500	750	536,318
A04	2500	750	446,353
A05	2500	750	171,658
A06	2500	750	333,958
A07	1670	500	274,152
A08	2000	600	317,958
A09	2000	600	66,716
A10	888	267	68,000

Source: a study based on research which was done with the help of EAT employees, as a part of the statutory theme (2017) entitled: "Qualitative and quantitative analysis of selected food products and cosmetics in terms of the content of fat-soluble vitamins and micro and macro elements".

Discussing the results

As it can be seen from the table above, the vitamin A content declared by the producers deviated from the actual values described in the contents of the dissolved capsule of the dietary supplement.

From supplements with the main composition of vitamins A + E, the most consistent with the declared content of vitamin A in the sample taken from the supplement specified by the code A01. The determined content was 745.470μg, declared by a pharmaceutical company 800μg in one capsule. In preparations that had codes A06, A07, A08, the content of vitamin A was about half that declared by the producers. Supplements with these codes contained only vitamin A. In the supplement marked with the code A02, the content of vitamin A was 4012, 873μg and in comparison with the declared 9000μg it was less than half.

The worst results were obtained by products from the multivitamin group. In the case of A09, the content of vitamin A was 10 times lower than declared by the producer (66,716 μg with 600 μg / capsule). In the multivitamin preparation marked with the A010 code, the declared content was 4 times higher than that obtained in the study (68 μg with 267 μg / capsule)

Conclusions

1. The content of vitamin A declared by the manufacturers of dietary supplements in one capsule deviated from the actual content measured during own research and fluctuated from about 93.2% to about 11.1%.

2. It is astonishing that the differences in preparations containing only vitamin A in their composition were so significant in comparison to the value declared by the manufacturers and obtained in the test results.

3. Definitely the biggest difference between the declared value and received as a result of the tests occurred in multivitamin preparations. In one, it was a 10-fold difference in the second 4- fold.

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