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DETERMINATION OF ALL-RAC-ALPHA-TOCOPHEROL AND ALL-RAC-ALPHA-TOCOPHERYL ACETATE (SYNTHETIC VITAMIN E) IN COSMETIC PREPARATIONS

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Different forms of tocopherols, together with tocotrienols, are collectively named as vitamin E, (a lipid-soluble vitamin with antioxidation effect in several stages of peroxidation of lipids *in vivo*) and each possesses different degree of medical, biological and physiochemical significance. Among four tocopherols (α-, β-, γ- and δ-tocopherol), alpha-tocopherol has been reported to possess the highest biological activity (1). In order to obtain the larger amounts, vitamin E is synthetized as a racemic mixture of D- and L- forms which are optical isomers, unlike a natural tocopherol that is found only in the D-form. Synthetic vitamin E is derived from petroleum products as all-racemic alpha tocopherol and its ester form all-racemic alpha tocopheryl acetate (or DL-tocopheryl acetate). The tocopheryl esters are more stable for an extended time of use (2).

In the belief that vitamin E is a natural skin conditioner and humidifier, it is used in cosmetic industry in the preparation of lipsticks, shadows, powders, humidifiers, soaps, creams, shampons etc. (3). The recent popularity of vitamin E preparations for topical application, together with reports of adverse dermatological reactions linked to cosmetics containing this vitamin led to concern about the desirability of including these compounds in such products (4). Therefore, a sensitive, accurate and rapid HPLC method was developed for determination of alpha-tocopherol and its ester form in cosmetics.

The chromatographic separation was performed on a column Purospher Star® RP 18 (150 x 4.0 mm i.d., 5μm), with a mobile phase consisted of a methanol - *n*-hexane (90:10, *V/V*) The flow rate was kept at 1.2 ml min^{-1}. Detection of all-racemic alpha-tocopherol and the ester form all-racemic alpha-tocopheryl acetate was carried out at 220 nm.

Effect of various parameters (different concentration of methanol and *n*-hexane in the mobile phase composition and different columns used), and the separation of alpha-tocopherol and alpha-tocopheryl acetate from other numerous ingredients present in cosmetic preparations was studied.

The proposed method was fully validated according to the ICH guidelines in terms of accuracy, precision, linearity, limit of detection and quantification and range (5). The method is sensitive and allows detection of active components in concentrations of 0,007 μg and 0,011 μg for α-tocopherol, and α-tocopherol acetate, respectively, while the lowest concentrations that could be determined were 0,023 μg, and 0,033 μg for α-tocopheryl acetate and α-tocopherol, respectively.

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ОПРЕДЕЛУВАЊЕ НА ALL-RAC-АЛФА-ТОКОФЕРОЛ И ALL-RAC-АЛФА-ТОКОФЕРИЛ АЦЕТАТ (СИНТЕТСКИ ВИТАМИН Е) ВО КОЗМЕТИЧКИ ПРОИЗВОДИ

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Различни форми на токофероли и токотриеноли се нарекуваат со заедничко име - витамин Е (липосолубилен витамин со антиоксидативен ефект во процесот на пероксидација на липиди *in vivo*) и поседуваат различен степен на медицинско, биолошко и физико-хемиско значење. Од сите форми (α-, β-, γ- and δ-токоферол) најголема биолошка активност има алфа-токоферолот (1).

Со цел да се добие поголемо количество на витамин Е, тој се добива синтетски како рацемска смеша од D- and L-форми, кои се оптички изомери, за разлика од природниот токоферол кој се сретнува само во D-форма. Синтетскиот витамин Е се добива од петролеум-базирани производи како *all-rac*-алфа-токоферол и во естерска форма како *all-rac*-алфа-токоферил ацетат (или DL-алфа-токоферил ацетат). Естрите на токоферолот се постабилни за подолг рок на употреба (2).

Познато е дека витаминот Е е природен кондиционер за кожа и навлажнувач, заради што се употребува во козметичката индустрија, во подготвување на кармини, сенки за очи, пудри, навлажнувачи, сапуни, креми, шампони и др. (3). Во поново време во литературата се известува за појава на несакани дерматолошки реакции при употреба на козметички препарати што го содржат овој витамин, што ја доведува во прашање широката употреба на витамин Е во препаратите за локална употреба (4). Заради тоа, ние развивме сензитивен, точен и брз HPLC метод за определување на алфа-токоферол и неговиот естер во козметичките препарати.

Хроматографското раздвојување се изведува на колона Purospher Star® RP 18 (150 x 4.0 mm i.d., 5μm), со мобилна фаза од метанол-*n*-хексан (90:10, *V/V*) изократно, со брзина на проток од 1, 2 ml min-1. Детекцијата на *all-rac*-алфа-токоферол и *all-rac*-алфа-токоферил ацетат се изведува на бранова должина од 220 nm. Се испитува ефектот на различните параметри (различна концентрација на метанол и *n*-хексан во мобилната фаза, како и различни хроматографски колони) на разделувањето на алфа-токоферол и алфа-токоферил ацетат од останатите бројни состојки присутни во козметичките препарати.

Предложениот метод е целосно валидиран според ICH упатствата преку испитување на точност, прецизност, линеарност, лимит на детекција и квантификација и опсег (5). Методот е сензитивен и овозможува детекција на активната компонента во концентрација од 0,007 μg алфа-токоферил ацетат и 0,011 μg алфа-токоферол, додека најниска концентрација што може да се определи беше

0,023 μg алфа-токоферил ацетат и 0,033 μg алфа-токоферол.

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DEGRADATION OF MYCOPHENOLATE MOFETIL UNDER OXIDATIVE STRESS CONDITIONS

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INTRODUCTION

Inherent chemical stability of the active pharmaceutical ingredient (API) largely determines the stability of the final drug product (DP), and regulatory authorities require novel chemical entities to undergo extensive chemical stability evaluation through stress testing (1,2).
Mycophenolate mofetil (MMF) is an immunosuppressive agent, which is mostly used in solid organ transplant patients to prevent organ rejection. MMF is pro drug that is rapidly absorbed and hydrolyzed to form free Mycophenolate mofetil (MPA), the active metabolite, which is considered as impurity in the final DP (3).

OBJECTIVES

The main objectives of this investigation were determination of the influence of oxidative stress agent on MMF stability and identification of unknown key-degradation products, if any.

MATERIALS AND METHODS

MMF was purchased from Sigma. HPLC analysis was performed with an Agilent Technologies (Palo Alto, CA, USA) HP 1200 chromatograph equipped with on-line degasser, binary pump, column oven, diode array detector, and Rheodyne 20-mL loop injector. Data was acquired by means of HP ChemStation software. Before HPLC performing, all mobile phases were degassed and vacuum filtered through 0.45 mm nylon membranes. The chromatographic conditions included Chromolith RP-18e (100 mm x 4.6 mm, macropore size 2 μm, mesopore size 13 nm) column, flow rate of the mobile phase of 1 mL min^{-1}, the chosen temperature of the column of 40°C and composition of the mobile phase of acetonitrile – 0.015 M ammonium acetate (pH 4.4) (28:72, v/v). The pH of the water phase was adjusted to 4.4 with glacial acetic acid, and oxidation product was analyzed in the positive ionization (PI) mode, though MPA was detected in the negative ionization (NI) mode with the proposed mobile phase. The mass spectrometer operated in full scan mode and for all the experiments spray needle voltage was set at 4.5 kV. Nitrogen was used as a sheath and auxiliary gas, and values (a scale of arbitrary units in the 0–100 range defined by the LCQ Advantage system) of their flow rate were 50 and 0, respectively. The capillary temperature was maintained at 290 °C and Helium was used as the collision gas.

RESULTS AND DISCUSSION

The influence of 3%, 10% and 15% H_2O_2, as oxidation agent, on MMF was investigated in duration of 1h. Adding 10% H_2O_2 resulted in degradation of 17.90% of drug amount and MPA, first (DP I) and second oxidation products (DP II) were formed.
The importance of all degradation products was estimated considering key-degradants rule (4). Therefore, the main degradation product was DP II with 41.67% of total degradation followed by MPA with 14.32% of total degradation and 34% of the largest degradant. On the other side, DP I appeared in 3% of total degradation and 7.09% of the largest degradant. Considering these results DP II and MPA could be noted as key-degradants, whereas DP I is not significant. In order to identify key-degradant denoted as DP II LC–MS^n nalysis was performed. In full scan mode were observed positive ions *m/z* 434 corresponding to MMF, *m/z* 336 corresponding to DP I, *m/z* 450 corresponding to DP II and negative ion *m/z* 319 corresponding to MPA. Along with *m/z* 450 adduct ions [M+Na]+ (*m/z* 472) and [M+K]+ (*m/z* 488) have been formed. DP II has *m/z* 450 and is 16 units higher than *m/z* 434 of MMF, indicating oxidation of MMF and arisen of its N-oxide. To confirm the identity of DP II the MS^n analysis of all analytes has been conducted. The MS^n analysis was performed by infusing sample subjected to 15% H_2O_2 directly to the mass spectrometer, with the syringe pump at the 5 μl min^{-1}. Obtained fragments for mycophenolate mofetil, mycophenolic acid and DP II, all in positive ionization mode, together with their relative abundances confirmed the structure of DP II. The identical fragmentation pattern for both MMF and DP II strongly indicates the same main structure of these compounds.

CONCLUSION

Mycophenolate mofetil showed to be instable towards oxidation agents and tree degradation products have been formed. LC-MS^n analysis was used to resolve the structure of key-oxidation product N-oxide of MMF.

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