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Analytical Methods

Development and validation of a dissolution test for lutein tablets and evaluation of intestinal permeability

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ABSTRACT

Lutein is a carotenoid with antioxidant activity that is present in various dosage forms. The bioavailability of carotenoid from oral dosage formulations depends on their release, dissolution and its permeability through the gastrointestinal tract. Here, a dissolution test was developed for evaluating formulations and the bioavailability was assessed. The test utilized a USP-apparatus II with rotations of 50, 75 and 100 rpm in water with P80 at 1, 2 and 5% (w/v). A non-everted rat intestinal sac model was used in conjunction to assess the intestinal permeability. The most discriminative conditions were 100 rpm in water with 2% polysorbate 80, which showed profile differences between two formulations. The intestinal permeation studies showed a lag-time and apparent permeability coefficient that were characteristic of highly permeable drugs. We suggest that a dissolution test can be an essential quality control tool for formulations containing compounds as lutein, although not mandatory by the regulation agencies.

1. Introduction

The United States Food and Drug Administration (FDA) considers dietary supplements to be orally administered products that contain a dietary ingredient to augment or compensate dietary intake. These ingredients can include vitamins, minerals, herbs or other plants as well as amino acids, enzymes, organ tissues and metabolites (DSHEA, 1994). In 1994, a law was passed in the USA, known as the *Dietary Supplement Health and Education Act* (*DSHEA*), that declassified dietary supplements as medicines and, as a consequence, no longer required FDA registration prior to marketing. The responsibility for ensuring the effectiveness and safety of these products was transferred to the manufacturers along with the guarantee that their marketed products possess the benefits for human health alleged in their claims. In Brazil, regulation of dietary supplements is more stringent than in the USA and requires registration prior to marketing. In 1999, the responsible Brazilian Federal agency, ANVISA (National Health Surveillance Agency of Brazil), approved a number of regulations allowing a product containing substances present in the diet to be registered as a separate category of novel foods that provide health-enhancement. As such, these products are not classified as and do not adhere to regulatory controls enacted for dietary supplements. Furthermore, no regulatory legislation with regard to these products have been implemented to specifically assess physicochemical characteristics and bioavailability of active compounds in marketed products.

An example of a substance marketed with claims of functional properties, and approved by ANVISA, are carotenoids. They are a class of hydrocarbons that can be further divided into carotenes and xanthophylls substances, which are responsible for red or orange coloration of foods such as tomatoes and carrots, respectively (Garcia et al., 2012; Krinsky & Johnson, 2005). Typical diets contain around forty types of carotenoids. Of these, around twenty can be detected in plasma and tissue, whereas only two, zeaxanthin and lutein, accumulate in the retina (Widomska & Subczynski, 2014).

Lutein is a yellow pigment present in the macula lutea of human eyes and is classified as a xanthophyll carotenoid (Azqueta & Collins, 2012; Kijlstra, Yuan, Kelly, & Berendschot, 2012). Xanthophyll biosynthesis occurs only in plants, algae, bacteria and certain fungi (Kijlstra et al., 2012). Therefore, the source of these substances in the bloodstream is from dietary intake of dark green leafy vegetables, fruits, animal products such as eggs, or dietary supplements containing xanthophylls (Gellenbeck, Venzon, Lala, & Chavan, 2012; Kijlstra et al., 2012). Comprised of 40 carbon

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atoms and numerous double bonds, the molecular structure of lutein confers hydrophobicity and susceptible to breakdown by light, heat and oxidation, which contribute to its instability (Ambrósio, Campos & Faro, 2006).

Several studies have shown an association between the consumption of lutein and zeaxanthin with a reduction in chronic diseases such as age-related macular degeneration (Ahmed, Lott, & Marcus, 2005; Huang et al., 2013), anti-inflammatory properties that provide retinal neuroprotection (Sasaki et al., 2009) and a reduction in oxidative stress by eliminating reactive oxygen species (Stahl et al., 1998). After ingestion, lutein becomes incorporated into mixed micelles composed of bile acids, free fatty acids, monoglycerides and phospholipids. Lutein action depends on passive absorption by enterocytes and transportation by plasma lipoproteins (Ambrósio, Campos & Faro, 2006). Retinal tissue demonstrates greater uptake of lutein, which has enhanced abundance compared to plasma (Ahmed et al., 2005; Kijlstra et al., 2012).

In the Brazilian market, twenty-five products are registered as food, but marketed based on lutein content and antioxidant properties that are touted to have a beneficial impact on health. These formulations mainly consist of either a lutein base or ester contained in a capsule or pharmaceutical tablet form. The amount of lutein claimed in these products ranges from 2 to 10 mg per dosage and the daily recommended dosage suggested is one pharmaceutical unit per day (Anvisa, 2016). However, the quality of an oral dosage depends on its ability to release the active substance into aqueous media to facilitate its availability for gastrointestinal absorption (Azarmi, Roa, & Lobenberg, 2007; Davydova, Stippler, Jin, & Giancaspro, 2010).

The dissolution test is an important physicochemical quality control test to assess drugs during development. It has the potential to evaluate the *in vivo* performance of solid oral dosage forms since it assesses the release of the active substance into the dissolution medium over time (Shah et al., 1995; Dressman, Amidon, Reppas, & Shah, 1998; Siewert, Dressman, Brown, & Shah, 2003; Azarmi et al., 2007; USP, 2011, chap. 1092). Lutein is a lipophilic substance with low solubility in water and, in general, these characteristics reflect in low dissolution rate and oral bioavailability (Mitri, Shegokar, Gohla, Anselmi, & Muller, 2011). For these reasons, it is necessary to guarantee the bioavailability of lutein tablets by evaluating its release from dosage forms and its intestinal permeability. The aim of this work was to develop a discriminatory dissolution test to assess the release of lutein from oral tablets and predict the biopharmaceutical classification system to which lutein belongs. The classification of lutein in this study was based on its solubility and permeability during an *in vitro* assay using the non-everted rat intestinal sac model.

2. Methodology

2.1. Tablets of lutein

All tablets used in this study were purchased from drugstores in Brazil. The tablets obtained from different manufacturers of lutein were designated as B and D. Tablet B contained 2.0 mg of lutein and other constituents including: dibasic calcium phosphate dihydrate, calcium carbonate, magnesium oxide, ascorbic acid, niacin, beta-carotene, ferrous fumarate, zinc oxide, manganese sulfate monohydrate, calcium pantothenate, biotin, vitamin A acetate, vitamin E, vitamin D, anhydrous copper sulfate, vitamin K, pyridoxine hydrochloride, thiamine mononitrate, riboflavin, sodium selenate, folic acid, cyanocobalamin, chromium chloridehexahydrate, sodium molybdate, lactose, potassium iodide, microcrystalline cellulose, sodium croscaramellose, insoluble polyvinylpyrrolidone, hydroxypropyl methylcellulose, titanium dioxide, triacetin, polysorbate 80, indigotine, sunset yellow, red 40 and silicon dioxide. Tablet D contained 5.0 mg of lutein and the other constituents are: lactose, vitamin C, vitamin E beta-carotene, zeaxanthin, zinc, polyethylene glycol, riboflavin, copper, selenium, microcrystalline cellulose, polyvinylpyrrolidone, hydroxypropylmethylcellulose, hydroxypropylcellulose, stearic acid, ethyl cellulose, titanium dioxide, brilliant blue and silicon dioxide. The products were stored at room temperature and protected from light. All analyses were performed before the expiration dates of the tablets. Two batches from each manufacturer were selected.

2.2. Chemical products

The lutein chemical standard (Achemo, Hong Kong, China) was 90.03% pure. All solvents used in this study were of chromatographic grade and purchased from Tedia (Rio de Janeiro, RJ, Brazil). Additional reagents used were sodium lauryl sulfate (SLS), polysorbate (P80) and isopropyl alcohol, all of which were analytical grade and purchased from Vetec (Rio de Janeiro, RJ, Brazil). For all filtration procedures, 10 μm polyethylene filters were used and obtained from Water used in this study was purified using the Milli-Q water purification system from Millipore (Bedford, Massachusetts, USA).

2.3. Quantification by HPLC-DAD

The chromatographic method used was modified from Pintea, Bele, Andrei, and Socaciu (2003) and performed using a Merck-Hitachi Elite LaChrom liquid chromatograph (Darmstadt, Hesse, Germany) coupled to a diode-array detector (DAD L-2130), quaternary pump (L-2455), column oven (L-2350) and an autosampler (L-2200) through the control of EzChrom software. A Sunfire column (C_{18}; 4.6 × 250 mm; 5 μm particle size) from Waters (Milford, Massachusetts, USA) was coupled to a guard column from Kromasil (Bohus, Kungälv, Sweden) specific to the stationary phase. The mobile phase consisted of distilled water (A), acetonitrile (B) and ethyl acetate (C) and eluted as follows; 0–9 min: A (9–5%), B (81–45%) C (10–50%); from 9.1 to 15 min: A (5–1%), B (45–9%) C (50–90%); from 15.1 to 18 min: A (1–9%), B (9–81%), C (90–10%). The lutein standard solutions and samples were prepared in ethyl acetate and diluted in the mobile phase. All solutions were filtered through a 0.45 μm PVDF filters (Millex Millipore, São Paulo, SP, Brazil) before injection into the HPLC system. This quantification method was used to analyze the stability, solubility, dissolution and permeation of lutein in the isolated non-everted rat intestinal model.

2.4. Stability

Two saturated lutein solutions containing (1% P80 (w/v) or 1% SLS (w/v) in water were prepared, filtered through a 0.45 μm PVDF membrane and quantified by HPLC-DAD. The concentrations obtained were 0.5 μg/mL with 1% P80 (w/v) and 1.8 μg/mL with 1% SLS (w/v). Multiple 20 mL-aliquots from each solution were placed in tubes, closed, covered with aluminum foil and placed at 37 °C in a water bath. A 2 mL sample was collected from each tube after 24 h. The samples were filtered using 0.45 μm PVDF filters and quantified by HPLC-DAD.

2.5. Solubility

The solubility of lutein was determined in water, simulated gastric fluid (SGF; pH 1.2) and simulated enteric fluid (SEF; pH 6.8), according to USP 34 (USP, 2011, chap. 1092), in the absence of surfactant, and with the addition of P80 at 1, 2 and 5% (w/v).