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Solid state compatibility study and characterization of a novel degradation product of tacrolimus in formulation

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ABSTRACT

Tacrolimus is macrolide drug that is widely used as a potent immunosuppressant. In the present work compatibility testing was conducted on physical mixtures of tacrolimus with excipients and on compatibility mixtures prepared by the simulation of manufacturing process used for the final drug product preparation. Increase in one major degradation product was detected in the presence of magnesium stearate based upon UHPLC analysis. The degradation product was isolated by preparative HPLC and its structure was elucidated by NMR and MS studies. Mechanism of the formation of this degradation product is proposed based on complementary degradation studies in a solution and structural elucidation data. The structure was proven to be alpha-hydroxy acid which is formed from the parent tacrolimus molecule through a benzilic acid type rearrangement reaction in the presence of divalent metallic cations. Degradation is facilitated at higher pH values.

1. Introduction

Tacrolimus (TAC) (Fig. 1) is a 23-memberd macrolide lactone, originally isolated from the bacterium *Streptomyces tsukubaensis* [1–3]. As a potent immunosuppressant it is widely used for graft rejection prevention after organ transplantation [4–6]. TAC is also used in the treatment of autoimmune diseases [7–11] and atopic dermatitis [12–14].

TAC is a highly functionalized molecule with macrocylic ring containing both masked tricarbonyl and pipecolic acid moiety. Two kinds of conformational heterogeneity of TAC have been reported in the literature [15]. The first one is restricted rotation of the amide bond in the pipecolic acid moiety which results in a solvent-dependent equilibrium between *cis* and *trans* rotamers in the solution. The cyclic ketal moiety of TAC is the second cause for dynamic equilibria in solution. Especially in polar solvents, TAC undergoes a hydration reaction to diol which is in turn converted into C-10 epimer (Fig. 1). An equilibrium containing all three forms is thus established [15,16]. The diol and the C-10 epimer are equilibrium compounds directly related to the drug substance and are in vivo considered equivalent to TAC [17,18]. So far in the published literature, the diol and C-10 epimer were erroneously denoted as tautomer I and tautomer II, respectively [16].

Attempts by the International Conference on Harmonization (ICH) with regard to impurities in drug substances and drug products have brought an increased regulatory scrutiny of impurities, requiring identification and toxicological qualification at very low levels [19,20]. Stress testing is the main tool that is used to predict stability problems and identify degradation pathways. Among the various types of stress testing drug–excipient compatibility studies often lead to new, unknown degradation products. It is not uncommon that drug substance is stable as bulk drug but unstable when formulated with the excipients required for final dosage forms. Therefore, the development of a stable formulation is greatly aided by chemical understanding of the reactions leading to degradation [21,22].

Although there is extensive literature on the determination and characterization of tacrolimus process related compounds and some potential degradation products [17,18,23–29], practically no attention has been paid to the impurities that originate from drug–excipient interactions. The main purpose of this work was to investigate influence of commonly used excipients on degradation of TAC. Compatibility study revealed that presence of magnesium stearate can deteriorate stability of TAC when formulated in a form of solid dispersion. Moreover, in the present work we have identified and characterized a new degradation product, tacrolimus alpha-hydroxy acid impurity (TAC-H1) (Fig. 2). The possible mechanism of degradation product formation has been proposed based on the comprehensive investigation of factors responsible for the increase of this degradation product in the final formulation.

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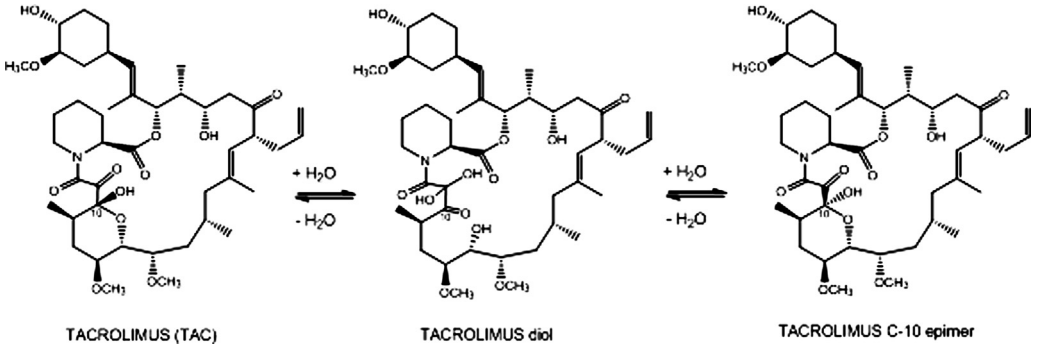

Fig. 1. Chemical structure of tacrolimus (TAC) and its equilibrium compounds TAC diol and TAC C-10 epimer in the solution.

2. Experimental

2.1. Chemicals, reagents and samples

Tacrolimus monohydrate drug substance was available from Lek Pharmaceuticals (Slovenia). Marketed product Prograf® 5 mg (batch no. 044012) was obtained from Japan market (Astellas Pharma, Japan). The excipients used for the preparation of compatibility mixtures were of pharmaceutical grade. The following excipients were used: lactose monohydrate (200 MESH, Friesland-Campina DMV BV, The Netherlands), magnesium stearate (FACI S.p.A., Italy), hypromellose (substitution type 2910, Pharmacoat 606, Shin-Etsu Chemicals Co., Japan), croscarmellose sodium (Acdi-sol, FMC BioPolymer International, Ireland) and stearic acid (Merck, Darmstadt, Germany). All excipients were used as received. Acetonitrile was HPLC grade (J.T. Baker, USA). All other chemicals were of analytical grade or pure grade quality. The following chemicals purchased from Merck (Darmstadt, Germany) were used: 85% phosphoric acid, methyl tert-butyl ether, absolute ethanol, magnesium acetate tetrahydrate, magnesium sulfate heptahydrate, magnesium citrate, acetic acid, magnesium oxide, calcium acetate hydrate, calcium hydroxide, sodium hydroxide solution Titrisol®. Brij 35 P, magnesium chloride hexahydrate and sodium acetate anhydrous were purchased from Sigma–Aldrich (St Lois, MO, USA) Ultra-pure water was obtained with a Millipore Milli-Q system (Bedford, MA, USA).

2.2. Ultra high performance liquid chromatography (UHPLC)

The UHPLC analysis was performed with a Waters ACQUITY UPLC equipped with binary solvent manager, sample manager, column-heating compartment, and photodiode array detector (PDA) (Milford, USA). Waters Empower 3 software (Build 3471) was used for the data acquisition and processing. An ACQUITY UPLC™ BEH C_{18} column, 100 mm × 2.1 mm, 1.7 μm (Waters, Milford, USA) was employed for chromatographic separation. The column was kept at 65 °C. Mobile phase A consisted of 0.1% water solution of phosphoric acid. Mobile phase B was a mixture of acetonitrile and methyl tert-butyl ether in the ratio of 850:80 (v/v). Run time was 13 min. The separation was achieved by gradient elution at a flow-rate of 0.75 mL min^{-1} according to the following program: mobile phase A–B (v/v)/time (min); 63–37/0 min, 63–37/1.0 min, 52–48/9.0 min, 30–70/11.0 min, 30–70/13.0 min; the ratio was changed linearly. The system came back to initial ratio and continued at the same ratio for 2 min. The mobile phase was

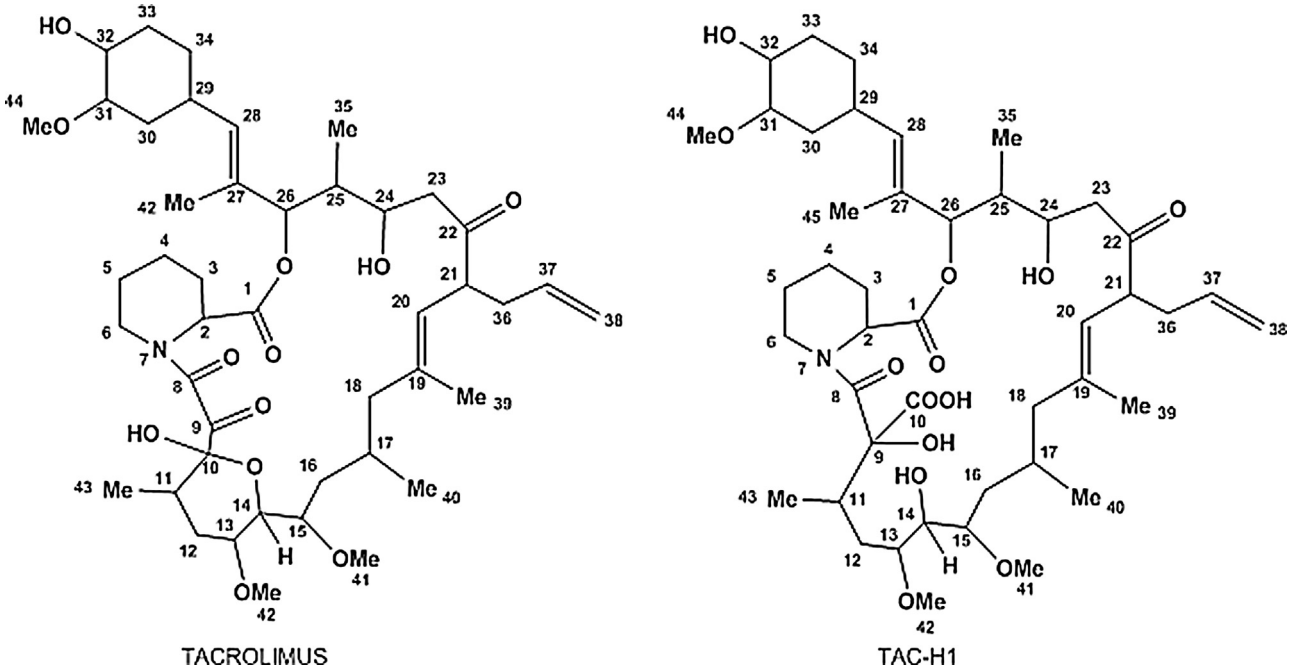

Fig. 2. Chemical structure of tacrolimus and tacrolimus alpha-hydroxy acid impurity (TAC-H1) with numbering.