Title: PHARMACEUTICAL COMPOSITIONS COMPRISING TACROLIMUS AND A CYP3A4 INHIBITOR

Abstract: A pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the composition upon administration to a human in need thereof results in a relative AUC_{intrinsic}/AUC_{separate} value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the AUC_{separate} value is determined after administration of tacrolimus and CYP3A4 inhibitor in separate dosage forms.
Pharmaceutical compositions comprising tacrolimus and a CYP3A4 inhibitor

The present invention relates to a pharmaceutical composition comprising tacrolimus or an analogue thereof and a CYP3A4 inhibitor, oral solid dosage forms comprising the pharmaceutical composition such as tablets, methods for preparing the pharmaceutical composition and oral dosage forms and use of the pharmaceutical composition for preparing a medicament. The CYP3A4 inhibitor is preferably a compound naturally occurring in citrus juice, for example grapefruit juice, preferably a spiro ortho ester compound.

Background of the invention

Tacrolimus, also known as FK-506 or FR-900506, has the chemical tricyclic structure shown below:

![Chemical structure of tacrolimus]

Corresponding to C_{44}H_{69}NO_{12}. Tacrolimus appears in the form of white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol and very soluble in methanol and chloroform.

The preparation of tacrolimus is described in EP-A-0 184 162 and analogues of tacrolimus are disclosed e.g. in EP-A-0 444 659 and US 6,387,918, which are both hereby incorporated by reference. Improved pharmaceutical formulations of tacrolimus with improved bioavailability, for example solid solutions of tacrolimus, are disclosed in WO2005/020993 and WO2005/020994 both of which are hereby incorporated by reference.

Tacrolimus is a macrolide compound with useful immunosuppressive activity, antimicrobial activity and other pharmacological activities and is of value for the treatment or prevention of rejection reactions by transplantation of organs or tissues, graft versus host
diseases, autoimmune diseases and infectious diseases. Tacrolimus prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow and small bowel and pancreas, lung and trachea, skin, cornea and limb.

In animals, tacrolimus has been demonstrated to suppress some humoral immunity and, to a greater extent, cell-mediated reactions such as allograft rejection, delayed type hypersensitivity, collagen-induced arthritis, experimental allergic encephalomyelitis and graft-versus-host disease.

Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is unknown. Experimental evidence suggest that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells, a nuclear component thought to initiate gene transcription for the formation of lymphokines. The net result is the inhibition of T-lymphocyte activation, i.e. immunosuppression.

Usually tacrolimus is administered orally and is therefore absorbed from the gastrointestinal tract. It has been observed that the absorption is negatively influenced by the simultaneous ingestion of food. Thus, the rate and extent of tacrolimus absorption were greatest under fasted conditions.

In general, it is known that the absorption and bioavailability of a therapeutically active substance can be affected by a variety of factors when administered orally. Such factors include the presence of food in the gastrointestinal tract and, in general, the gastric residence time of a drug substance is significantly longer in the presence of food than in the fasted state. If the bioavailability of a drug substance is affected beyond a certain point due to the presence of food in the gastrointestinal tract, the drug substance is said to exhibit a food effect. Food effects are important because absorption and hence the plasma levels becomes highly variable depending on food intake. Absorption into the bloodstream may be adversely affected to the point that the patient risks insufficient absorption to remedy the condition for which the drug was administered. On the other hand, the very high peak concentrations occasionally observed at fasted conditions may very well induce significant side effects of nephro- or neuro-toxic origin as well as GI side-effects and others.

Absorption of tacrolimus from the gastrointestinal tract after oral administration is rapid with a mean time-to-peak concentration (t_{max}) of approximately 1-2 hours after administration to healthy subjects or kidney or liver transplanted patients, but incomplete and variable. The bioavailability is generally as low as at the most about 20% after oral administration. This phenomenon is sometimes also denoted the "first-pass effect", i.e. the process of drug degradation during a drug's transition from initial ingestion to circulation in the blood stream.
Frequently observed side effects are vomiting and nausea but side effects like tremor, headache, hypertension, renal dysfunction, hyperkalemia, hypomagnesaemia, hyperglycemia, insomnia, diarrhea, constipation, abdominal pain, nephrotoxicity and neurotoxicity are also observed.

For oral administration, tacrolimus is currently formulated and marketed as soft gelatine capsules comprising the equivalent of 0.5, 1 or 5 mg anhydrous tacrolimus and marketed under the trade name Prograf® and Protropic®. The recommended initial oral dose is from about 0.1 to 0.2 mg/kg/day in patients. The dose aims at a certain trough plasma level from about 5 to about 20 ng/ml. Prograf® is indicated for the prophylaxis of organ rejection in patients receiving allogeneic liver or kidney transplants.

Tacrolimus is extensively metabolized by the CYP3A4 isoenzyme in the gut wall and liver. Therefore, drugs that affect this isoenzyme may influence absorption and the subsequent elimination of systemically absorbed tacrolimus. Inhibitors of CYP3A4 may increase tacrolimus levels, while inducers of CYP3A4 may increase the metabolism of tacrolimus and decrease tacrolimus levels. It has been suggested to administer tacrolimus together with one or more CYP3A4 inhibitors in order to improve the overall bioavailability.

In order to increase the bioavailability of tacrolimus, prior art has focused on agents capable of inhibiting the cytochrome P450 system. Inhibition of the P450 system is a model for in vitro determination of in vivo bioavailability enhancement. See, e.g., U.S. Pat. Nos. 5,478,723 and 5,567,592, both incorporated herein by reference, for a more full description of the P450 system. It has been found that the following compounds may be inhibitors of the cytochrome P450 system, i.e. are CYP3A4 inhibitors: ketoconazole, fluconazole, ritonavir, itraconazole, miconazole, erythromycin and troleandomycin. In addition hereto, it is well established that the intake of grapefruit juice may also enhance the bioavailability of certain drugs. Based on this known effect, a number of compounds present in citrus juice including grapefruit juice have been identified and synthesized as disclosed in the international publications WO98/53658, WO99/09976, WO00/54768, WO00/00042 and WO2004/037827 which are all incorporated herein by reference. Especially, these publications disclose a number of spiro ortho esters and spiro ortho carbonates as CYP3A4 inhibitors.

Despite these findings, there is an unmet need for novel pharmaceutical compositions and/or dosage forms comprising tacrolimus exhibiting enhanced bioavailability. An increased bioavailability may allow a reduction in the dosage units taken by a patient, e.g. down to a single dose daily, and may also reduce or negate the need for food to be taken simultaneously with the dosage form thereby allowing patients more freedom on when the drug is taken. Furthermore, it is contemplated that fluctuations in the plasma concentration versus time profile may be significantly reduced. Further, enhanced bioavailability may also result in a more reproducible (i.e. less variable compared to that of Prograf®) release profile.
Summary of the invention

The inventors have surprisingly found that co-administration of tacrolimus and a CYP3A4 inhibitor in a single oral dosage form results in an improvement of the bioavailability of tacrolimus when compared with administration in separate dosage forms, i.e. a synergistic effect.

Accordingly, in a first aspect the present invention relates to a pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the composition upon administration to a human in need thereof results in a relative $\frac{AUC_{invention}}{AUC_{separate}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the $AUC_{separate}$ value is determined after administration of tacrolimus and CYP3A4 inhibitor in separate dosage forms. It is to be understood, that the invention also encompasses a pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the composition upon administration to a human in need thereof results in a relative $\frac{AUC_{invention}}{AUC_{per se}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the $AUC_{per se}$ value is determined after administration of tacrolimus per se (without CYP3A4 inhibitor).

In a second aspect, the invention relates to a dosage form, typically a solid unit dosage form for oral administration, comprising the pharmaceutical composition of the invention.

In a further aspect, the invention relates to a single solid dosage form for oral administration comprising a first solid pharmaceutical composition containing tacrolimus or an analogue thereof as the active substance and second solid pharmaceutical composition containing a CYP3A4 inhibitor as the active substance, wherein the first and the second pharmaceutical composition are present in separate entities. This single solid dosage form may be prepared by a method comprising the steps of: i) preparing the first solid pharmaceutical composition, ii) preparing the second solid pharmaceutical composition, and iii) compressing the first and second compositions into a multilayer tablet, the first and second compositions being present in separate layers.

In a further aspect, the invention relates to a single solid dosage form suitable for oral administration comprising tacrolimus or an analogue thereof as the active substance and second solid pharmaceutical composition containing a CYP3A4 inhibitor as the active substance, wherein the tacrolimus is present as component of an immediate release pharmaceutical formulation and the CYP3A4 inhibitor is present as component of either an immediate release pharmaceutical formulation or a delayed release pharmaceutical formulation.
In yet another aspect, the invention relates to a method for the preparation of the pharmaceutical composition invention, the method comprising the steps of dissolving or dispersing tacrolimus in a solid, hydrophilic or water-miscible vehicle to obtain a solid dispersion or a solid solution or a mixture thereof, followed by mixing the CYP3A4 inhibitor with the solid dispersion or solid solution.

In yet another aspect, the invention relates to use of the pharmaceutical composition of the invention for preparing a medicament.

Detailed description of the invention

Definitions

As used herein, the term "active substance", "active pharmaceutical substance", "active ingredient" or "active pharmaceutical ingredient" means any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and are present in the drug product in a modified form intended to furnish the specified activity or effect.

In the present context, the term "hydrophilic" describes that something 'likes water', i.e. a hydrophilic molecule or portion of a molecule is one that typically is electrically polarized and capable of forming hydrogen bonds with water molecules, enabling it dissolve more readily in water than in oil or other "non-polar" solvents.

In the present context, the term "amphiphilic" describes a molecule (as a surfactant) having a polar water-soluble group attached to a water-insoluble hydrocarbon chain. Thus, one end of the molecule is hydrophilic (polar) and the other is hydrophobic (non-polar).

In the present context, the term "hydrophobic" denotes a compound tending to be electrically neutral and non-polar, and thus preferring other neutral and nonpolar solvents or molecular environments.

As used herein, the term "vehicle" means any solvent or carrier fluid in a pharmaceutical product that has no pharmacological role. For example, water is the vehicle for xilocaine and propylene glycol is the vehicle for many antibiotics.

In the present context, the term "solid dispersion" denotes a drug or active ingredient or substance dispersed on a particulate level in an inert vehicle, carrier, diluent or matrix in the solid state, i.e. usually a fine particulate dispersion.

In the present context, the term "solid solution" denotes a drug or active ingredient or substance dissolved on a molecular level in an inert vehicle, carrier, diluent or matrix in the solid state.
As used herein, the term "analogue" means a chemical compound that is structurally similar to another.

The term "drug" means a compound intended for use in diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals.

In this context, the term "dosage form" means the form in which the drug is delivered to the patient. This could be parenteral, topical, tablet, oral (liquid or dissolved powder), suppository, inhalation, transdermal, etc.

As used herein, the term "bioavailability" denotes the degree means to which a drug or other substance becomes available to the target tissue (blood stream) after administration.

As used herein, the term "bioequivalency" denotes a scientific basis on which generic and brand name drugs are compared with one another. For example, drugs are bioequivalent if they enter circulation at the same rate when given in similar doses under similar conditions. Parameters often used in bioequivalence studies are $t_{\text{max}}$, $c_{\text{max}}$, $\text{AUC}_0-\infty$, $\text{AUC}_{0-t}$ (Area Under Curve). Other relevant parameters may be $W_{50}$, $W_{75}$ and/or MRT.

Accordingly, at least one of these parameters may be applied when determining whether bioequivalence is present. Furthermore, in the present context, two compositions are regarded as bioequivalent if the value of the parameter used is within 80-125% of that of Prograf® or a similar commercially available tacrolimus-containing product used in the test.

In the present context $t_{\text{max}}$ denotes the time to reach the maximal plasma concentration ($c_{\text{max}}$) after administration; $\text{AUC}_0-\infty$ denotes the area under the plasma concentration versus time curve from time 0 to infinity; $\text{AUC}_{0-t}$ denotes the area under the plasma concentration versus time curve from time 0 to time $t$; $W_{50}$ denotes the time where the plasma concentration is 50% or more of $C_{\text{max}}$; $W_{75}$ denotes the time where the plasma concentration is 75% or more of $C_{\text{max}}$; and MRT denotes mean residence time for tacrolimus (and/or an analogue thereof).

In this context, the term "medicine" means a compound used to treat disease, injury or pain. Medicine is justly distributed into "prophylactic," i.e. the art of preserving health, and "therapeutic", i.e. the art of restoring health.

In the present context, the terms "controlled release" and "modified release" are intended to be equivalent terms covering any type of release of tacrolimus from a composition of the invention that is appropriate to obtain a specific therapeutic or prophylactic response after administration to a subject. A person skilled in the art knows how controlled release/modified release differs from the release of plain tablets or capsules. The terms "release in a controlled manner" or "release in a modified manner" have the same meaning as stated above. The terms include slow release (that results in a lower $C_{\text{max}}$ and later $t_{\text{max}}$, but $t_{1/2}$ is unchanged), extended release (that results in a lower $C_{\text{max}}$, later $t_{\text{max}}$, but apparent $t_{1/2}$ is longer); delayed release (that result in an unchanged $C_{\text{max}}$, but lag time and,
accordingly, $t_{\text{max}}$ is delayed, and $t_{\tfrac{1}{2}}$ is unchanged) as well as pulsatile release, burst release, sustained release, prolonged release, chrono-optimized release, fast release (to obtain an enhanced onset of action) etc. Included in the terms is also e.g. utilization of specific conditions within the body e.g. different enzymes or pH changes in order to control the release of the drug substance.

In this context, the term "erosion" or "eroding" means a gradual breakdown of the surface of a material or structure, for example of a tablet or the coating of a tablet.

CYP3A4 inhibitors

It is contemplated that any known and pharmacologically acceptable compound capable of inhibiting cytochrome P450, i.e. inhibit CYP3A4 isoenzyme, is useful in the present invention.

Examples of useful CYP3A4 inhibitors are diethyl dithiocarbamate, ketoconazole, itraconazole, erythromycin, ritonavir, lansoprazol, saffrole, rutaecarpine, limonin, dipiperamide A (from white pepper), gomisin C (from schisandra fruit), paradisin A and paradisin B (from grape fruit juice).

Further useful compounds are those according to the following Formulae I-IV:

![Chemical structures I-IV](image)

In each of the above structures, R is, independently, H or an optionally substituted C$_1$-C$_{15}$ alkyl group, L is an optionally substituted C$_1$-C$_{15}$ linear or branched, saturated,
monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen, HAr is an optionally substituted C₆₋C₂₄ aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P, and E is -OH, -COOH, -COOR, or an optionally substituted C₁₋C₉ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C₃₋C₉ optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr; and the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C₁₋C₉ linear, branched or cyclic alkyl group, -OH, halogen, a C₁₋C₅ alkoxy group, a C₁₋C₅ alkyl carbonyloxy group and a C₁₋C₅ alkoxy carbonyl group.

Preferably, the compounds of Formulae I-IV as well as those described below do not contain a peroxide (O-O) group. Disulfide groups (S-S) are not preferred, but may be present. Preferably E is an epoxide or dihydroxy radical such as -CH(OH)₂. E may also be an acid-opened epoxide group.

These compounds are unlimited with regard to stereochemistry, E-Z isomerism and all possibilities are included. Racemic mixtures are included as are each and every enantiomer and diastereomer.

The groups R, L, HAr, and E may optionally be substituted with a C₁₋C₆ linear, branched or cyclic alkyl group, -OH, a halogen atom, a C₁₋C₅ alkoxy group, a C₁₋C₅ alkyl carbonyloxy group, a C₁₋C₅ alkoxy carbonyl group, etc. Such substituents also may be optionally substituted directly on the ring structures of Formulae I-IV regardless of whether such substituents appear on R, L, HAr or E.

Further useful compounds are:

In each of the above structures, R is, independently, H or an optionally substituted C₁₋C₁₅ alkyl group, L is an optionally substituted C₁₋C₁₅ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen, HAr is an optionally substituted C₆₋C₂₄ aromatic group or heteroaromatic group.
optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P, and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C<sub>1</sub>-C<sub>8</sub> linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C<sub>3</sub>-C<sub>8</sub> optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr; and the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C<sub>1</sub>-C<sub>6</sub> linear, branched or cyclic alkyl group, -OH, halogen, a C<sub>1</sub>-C<sub>6</sub> alkoxy group, a C<sub>1</sub>-C<sub>6</sub> alkyl carbonyloxy group and a C<sub>1</sub>-C<sub>5</sub> alkoxy carbonyl group.

Further useful compounds are compounds of the formulae V-X:
As noted above for Formulae I-IV, the compounds of Formulae V-X are unlimited with regard to stereochemistry, E-Z isomerism, etc.

The most preferred CYP3A4 inhibitor compounds are those of Formulae XI-XVI:
The compositions of the present invention contain at least one CYP3A4 inhibiting compound in an amount effective for enhancing the bioavailability of tacrolimus or an analogue thereof. It is to be understood that the composition of the invention may also, in addition to or as a substitute for the mentioned compounds of formulae V-XVI, comprise a citrus-derived extract, concentrate, peel, juice, oil, by-product, etc., (hereinafter referred to as the citrus-derived substance) and may be provided by any combination of these forms and may be derived from more than one citrus fruit. Useful citrus fruits herein include grapefruit, lemon, lime and, preferably, any citrus fruit naturally containing an invention first-pass effect inhibiting compound or mixture of such compounds.

It is contemplated that an effective amount of a CYP3A4 inhibitor is from about 2 mg to about 2000 mg, for example 2 mg, 5 mg, 10 mg, 20 mg, 40 mg, 60 mg, 80 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1500 mg.

**Tacrolimus**

In a preferred embodiment of the present invention, the active ingredient tacrolimus or an analogue thereof is used in the form of a solid dispersion or a solid solution or a mixture of a solid dispersion and a solid solution. These forms are disclosed in the international publications WO2005/020993 and WO2005/020994. Accordingly, the active ingredient is dispersed or dissolved in a hydrophilic or water-miscible vehicle having a melting point (freezing point or pour point) of at least 20°C in a concentration of between about 0.01 w/w% and about 15 w/w%, and which dispersion is forming a solid dispersion or solid solution at ambient temperature (room temperature).

The active ingredient is preferably tacrolimus or any analogue or derivative of tacrolimus, which exhibits either a pharmacological or a therapeutical activity, which is equivalent to that of tacrolimus (FK-506 or FR-900506). However, within the scope of the present invention is tacrolimus in any physical form (crystals, amorphous powder, any
possible polymorphs, any possible solvates including the hydrate, anhydrate, complexes thereof etc.). Included is also any analogue, derivative or active metabolite of tacrolimus, pharmaceutically acceptable salts, solvates, complexes and prodrugs thereof.

The concentration of the active ingredient in the hydrophilic or water-miscible vehicle is at the most 15w/w%, preferably at the most 10w/w%, preferably at the most 8w/w%, more preferably at the most 6w/w%, even more preferably at the most 5w/w%, at the most 4%w/w, especially at the most 3w/w%, in particular at the most 2% w/w; and/or is at least about 0.05w/w%, preferably at least about 0.1w/w%, more preferably at least about 0.5w/w%, especially at least about 0.7w/w%, in particular at least about 1w/w%.

Physically, the combination of active ingredient and vehicle may either form a solid dispersion, i.e. the active ingredient is dispersed in the vehicle in particulate form, or may form a solid solution, i.e. the active ingredient is dissolved in the vehicle at a molecular level. The active ingredient and the vehicle may also form a solid dispersion having therein a part of the active ingredient dissolved at a molecular level. The physical state of the dispersion and/or solution may be determined by using various techniques such as Hot Stage Microscopy (HSM), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) optionally in combination with Energy Dispersive X-ray (EDX), and X-ray powder diffraction. In a preferred embodiment, the active ingredient is fully dissolved in the vehicle to form a solid solution at ambient temperature.

In a preferred embodiment, at least 80 w/w%, or at least 90 w/w%, or at least 99 w/w% of the tacrolimus is present in the composition as a solid dispersion or a solid solution in a hydrophilic or water-miscible vehicle.

Pharmaceutical composition

The pharmaceutical composition of the invention comprises tacrolimus, preferably in the form of a solid dispersion or solid solution, a CYP3A4 inhibitor and optionally one or more pharmaceutically acceptable excipients, for example one or more excipients useful as fillers, disintegrants, binders and/or lubricants.

Preferably, the pharmaceutical composition of the invention is in particulate form, for example in powder form. Preferably, the particulate material obtained is a free-flowing powder and therefore readily processable into e.g. solid dosage forms such as tablets, capsules or sachets. Normally, the particulate material has properties that are suitable in order to manufacture tablets by direct compression without addition of large amounts of further additives. A suitable test for testing the flowability of the particulate material is the method described in Ph.Eur. and measuring the flow rate of the material out of a funnel with a nozzle (orifice) diameter of 10.0 mm.
The particles may have a geometric weight mean diameter $d_{gw}$ from about 10 μm to about 2000 μm, preferably from about 20 μm to about 2000 μm, more preferably from about 30 μm to about 2000 μm, more preferably from about 50 μm to about 2000 μm, more preferably from about 60 μm to about 2000 μm, more preferably from about 75 μm to about 2000 μm, more preferably from about 100 μm to about 1500 μm, more preferably from about 100 μm to about 1000 μm, more preferably from about 100 μm to about 700 μm, more preferably from about 50 μm to about 400 μm, more preferably from about 50 μm to about 350 μm, even more preferably from about 50 μm to about 300 μm, especially from about 50 μm to about 250 μm or, in particular, from about 100 μm to about 300 μm. In a preferred embodiment of the invention, the particles have a geometric weight mean diameter $d_{gw}$ from about 50 μm to about 300 μm.

It is contemplated that the pharmaceutical composition of the invention, when administered to a human in need thereof, results in a relative $\text{AUC}_{\text{invention}}/\text{AUC}_{\text{separate}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the $\text{AUC}_{\text{separate}}$ value is determined after administration of tacrolimus and CYP3A4 inhibitor in separate dosage forms. In other words, there is observed at synergistic effect of administering the two active compounds simultaneously in the very same pharmaceutical formulation. Preferably, the relative $\text{AUC}_{\text{invention}}/\text{AUC}_{\text{separate}}$ value is at least about 1.2, or at least about 1.3, or at least about 1.4, or at least about 1.5 or at least about 1.6.

It is to be understood that the pharmaceutical composition of the invention, when administered to a human in need thereof, also results in a relative $\text{AUC}_{\text{invention}}/\text{AUC}_{\text{per se}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the $\text{AUC}_{\text{per se}}$ value is determined after administration of tacrolimus per se (i.e. without any administration of a compound having effect as CYP3A4 inhibitor). Preferably, the relative $\text{AUC}_{\text{invention}}/\text{AUC}_{\text{per se}}$ value is at least about 1.2, or at least about 1.3, or at least about 1.4, or at least about 1.5 or at least about 1.6.

The pharmaceutical composition of the invention is stable, i.e. the CYP3A4 inhibitor and/or the tacrolimus or an analogue of tacrolimus is present in the composition in an amount of at least 90%, or at least 95%, or at least 100%, relative to the amount prior to storage, when assayed after 3 months of storage at a temperature of about 25°C and a relative humidity of about 60%. In a preferred embodiment, at least 95 w/w% of each of the active compounds are present after 3 months of storage at a temperature of about 40°C and a relative humidity of about 75%.

In a preferred embodiment of the invention, the CYP3A4 inhibitor is the active ingredient of an immediate release (IR) pharmaceutical formulation, i.e. release of the active ingredient from the pharmaceutical formulation begins immediately after administration to a patient; or the CYP3A4 inhibitor is the active ingredient of a modified release pharmaceutical
formulation, for example a formulation which releases less than 20% w/w of the active ingredient during the first 5 hours after administration to a patient - corresponding to delaying the release of at least 80% w/w of the active ingredient for at least 5 hours after administration to the patient, that is to obtain a delayed therapeutic or prophylactic response.

Tacrolimus may be provided as the active ingredient of an immediate release (IR) pharmaceutical formulation or of an entero-coated immediate release formulation (IR-enteric), either co-formulated with the CYP3A4 inhibitor or formulated separately, i.e. in a separate formulation. Tacrolimus may also be provided as the active ingredient of a modified release pharmaceutical formulation, for example a formulation which releases less than 20% w/w of the active ingredient throughout the first 5 hours after administration to a patient (corresponding to delaying the release of at least 80% w/w of the active ingredient for at least 5 hours after administration to the patient). The modified release formulation may be co-formulated, i.e. comprises tacrolimus as well as CYP3A4 inhibitor as active ingredients; or tacrolimus may be formulated separate from CYP3A4, i.e. in a separate formulation.

Examples of useful formulations are provided in the examples herein.

Dosage forms
Useful dosage forms of the invention are solid oral dosage forms comprising the solid dispersion and/or solid solution and one or more pharmaceutically acceptable excipients, preferably unit dosage forms.

The pharmaceutical composition according to the invention is in particulate form and may be employed as such. However, in many cases it is more convenient to present the composition in the form of granules, pellets, microspheres, nanoparticles and the like or in the form of solid dosage forms including tablets, capsules and sachets and the like.

A solid dosage form according to the invention may be a single unit dosage form or it may in the form of a polydepot dosage form contain a multiplicity of individual units such as, e.g., pellets, beads and/or granules.

The dosage forms may further comprise pharmaceutically acceptable additives such as flavoring agents, coloring agents, taste-masking agents, pH-adjusting agents, buffering agents, preservatives, stabilizing agents, anti-oxidants, wetting agents, humidity-adjusting agents, surface-active agents, suspending agents, absorption enhancing agents and release modifying agents.

In a preferred embodiment, the dosage form comprises silica acid or a derivative or salt thereof including silicates, silicon dioxide and polymers thereof; and/or magnesium aluminosilicate and/or magnesium alumino-metasilicate, bentonite, kaolin, magnesium trisilicate, montmorillonite and/or saponite. A particularly useful excipient to be included in the
dosage forms is any silicon dioxide product having properties corresponding to Aeropen® 300 (available from Degussa, Frankfurt, Germany).

A solid dosage form according to the present invention comprises a pharmaceutical composition in particulate form as described above. The details and particulars disclosed under this main aspect of the invention apply mutatis mutandis to the other aspects of the invention. Accordingly, the properties with respect to increase in bioavailability, changes in bioavailability parameters, reduction in adverse food effect as well as release of tacrolimus and/or an analogue thereof etc. described and/or claimed herein for pharmaceutical compositions in particulate form are analogues for a solid dosage form according to the present invention.

Normally, the concentration of the pharmaceutical composition in particulate form is in a range of from about 5 to 100% w/w such as, e.g., from about 10% to about 90% w/w, from about 15% to about 85% w/w, from about 20% to about 80% w/w, from about 25% to about 80% w/w, from about 30% to about 80% w/w, from about 35% to about 80% w/w, from about 40% to about 75% w/w, from about 45% to about 75% w/w or from about 50% to about 70% w/w of the dosage form. In an embodiment of the invention, the concentration of the pharmaceutical composition in particulate form is 50% w/w or more of the dosage form.

A solid dosage form according to the invention is obtained by processing the particulate material according to the invention by means of techniques well-known to a person skilled in the art. Normally, it involves further addition of one or more of the pharmaceutically acceptable excipients mentioned herein.

The composition or solid dosage form according to the invention may be designed to release tacrolimus and/or an analogue thereof in any suitable manner provided that the increase in bioavailability is present. Thus, the active substance may be released relatively fast in order to obtain an enhanced on-set of action, it may be released so as to follow zero or first order kinetics or it may be released in a modified manner in order to obtain a predetermined pattern of release. All of these ways are considered controlled manners. Plain formulations are also within the scope of the present invention.

The contemplated dosage recommendation for products of the present invention will be from 0.02 mg tacrolimus/kg/day to 0.15 mg tacrolimus/kg/day, dosed once a day.

In a preferred embodiment of the invention, the dosage form is provided as a single solid dosage form for oral administration comprising a first solid pharmaceutical composition containing tacrolimus or an analogue thereof as the active substance and second solid pharmaceutical composition containing a CYP3A4 inhibitor as the active substance, wherein the first and the second pharmaceutical composition are present in separate entities. By separating the active ingredients, any undesired interfering reactions and possible
degradations are avoided, while the advantage of having a single unit dosage form is maintained.

Prior to combining the active ingredients into the single dosage form, each of these may be provided is in the form of granulate, granules, grains, beads or pellets which are optionally enterico-coated or coated with a protective coating.

Preferably, the single solid dosage form is a tablet. Other useful forms are capsules and sachets.

In a tablet, the two active ingredients may be present in at least two separate layers, optionally separated by an intermediate, inactive layer. The tablet may also be prepared for example by direct compression of a granulate comprising tacrolimus, optionally enterico-coated or coated with a protective coating, and a granulate comprising CYP3A4 inhibitor; or by direct compression of a granulate comprising both active ingredients.

**Vehicle**

The hydrophilic or water-miscible vehicle to be used according to the invention, i.e. in the solid solution of tacrolimus, is preferably one having a melting point (freezing point or pour point) of at least 20°C, more preferably at least 30°C, more preferably at least 40°C, more preferably at least 50°C, even more preferably at least 52°C, even more preferably at least 55°C, even more preferably at least 59°C, especially at least 61°C, in particular at least 65°C.

Examples of useful hydrophilic or water-miscible vehicles to be used according to this invention are selected from the group consisting of polyethylene glycols, polyoxyethylene oxides, poloxamers, polyoxyethylene stearates, poly-epsilon caprolactone, polyglycolized glycerides such as Gelucire®, and mixtures thereof.

It is also contemplated that certain amphiphilic vehicles may be useful in the present invention, including those vehicles disclosed herein which may be amphiphilic in addition to being water-miscible.

In a preferred embodiment of the invention, the vehicle is a polyethylene glycol (PEG), in particular a PEG having an average molecular weight of at least 1500, preferably at least 3000, more preferably at least 4000, especially at least 6000. The polyethylene glycol may advantageously be mixed with one or more other hydrophilic or water-miscible vehicles, for example a poloxamer, preferably in a proportion (on a weight/weight basis) of between 1:3 and 10:1, preferably between 1:1 and 5:1, more preferably between and 3:2 4:1, especially between 2:1 and 3:1, in particular about 7:3. A specific example of a useful mixture is a mixture of PEG6000 and poloxamer 188 in the ratio 7:3.

For polyethylene glycols (PEG), the melting point (freezing point or pour point) increases as the average molecular weight increases. For example, PEG 400 is in the range
of 4-8°C, PEG 600 is in the range of 20-25°C, PEG1500 is in the range of 44-48°C, PEG2000 is about 52°C, PEG 4000 is about 59°C, PEG 6000 is about 65°C and PEG 8000 is about 61°C.

Useful poloxamers (also denoted polyoxypropylene-polyoxyethylene block copolymers) are for example poloxamer 188, poloxamer 237, poloxamer 338 or poloxamer 407 or other block copolymers of ethylene oxide and propylene oxide such as the Pluronic® and/or Tetronic® series. Suitable block copolymers of the Pluronic® series include polymers having a molecular weight of about 3,000 or more such as, e.g., from about 4,000 to about 20,000 and/or a viscosity (Brookfield) from about 200 to about 4,000 cps such as, e.g., from about 250 to about 3,000 cps. Suitable examples include Pluronic® F38, P65, P68LF, P75, F77, P84, P85, F87, F88, F98, P103, P104, P105, F108, P123, F123, F127, 10R8, 17R8, 25R5, 25R8 etc. Suitable block copolymers of the Tetronic® series include polymers having a molecular weight of about 8,000 or more such as, e.g., from about 9,000 to about 35,000 and/or a viscosity (Brookfield) of from about 500 to about 45,000 cps such as, e.g., from about 600 to about 40,000. The viscosities given above are determined at 60°C for substances that are pastes at room temperature and at 77 °C for substances that are solids at room temperature.

In a preferred embodiment of the present invention, the poloxamer is poloxamer 188, which has an average molecular weight of about 8400 and a melting point of about 50-54°C.

Other useful hydrophilic or water-miscible vehicles may be polyvinylpyrrolidones, polyvinyl-polyvinylacetate copolymers (PVP-PVA), polyvinyl alcohol (PVA), polymethacrylic polymers (Eudragit RS; Eudragit RL, Eudragit NE, Eudragit E), cellulose derivatives including hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), methylcellulose, sodium carboxymethylcellulose, hydroxyethyl cellulose, pectins, cyclodextrins, galactomannans, alginates, carragenates, xanthan gums and mixtures thereof.

"Polyglycolized glycerides" denotes a mixture of mono-, di- and triglycerides and polyethylene glycol (PEG) mono- and diesters, preferably of molecular weight between 200 and 600, where appropriate of free glycerol and free PEG, whose HLB value is adjusted by the length of the PEG chain, and whose melting point is adjusted by the length of the chains of the fatty acids, of the PEG and by the degree of saturation of the fatty chains, and hence of the starting oil; examples of such mixtures are Gelucire®. Gelucire® compositions are inert semi-solid waxy materials which are amphiphilic in character and are available with varying physical characteristics. They are surface active in nature and disperse or solubilize in aqueous media forming micelles, microscopic globules or vesicles. They are identified by their melting point/HLB value. The melting point is expressed in degrees Celsius and the HLB (Hydrophile-Lipophile Balance) is a numerical scale extending from 0 to approximately 20. Lower HLB values denote more lipophilic and hydrophobic substances, and higher
values denote more hydrophilic and lipophobic substances. The affinity of a compound for water or for oily substances is determined and its HLB value is assigned experimentally. One or a mixture of different grades of Gelucire® excipient may be chosen to achieve the desired characteristics of melting point and/or HLB value. They are mixtures of monoesters, diesters and/or triesters of glycerides of long chain (C₁₂ to C₁₈) fatty acids, and PEG (mono- and/or di) esters of long chain (C₁₂ to C₁₈) fatty acids and can include free PEG. Gelucire® compositions are generally described as fatty acid esters of glycerol and PEG esters or as polyglycolized glycerides. Gelucire® compositions are characterized by a wide range of melting points of from about 33°C to about 64°C and most commonly from about 35°C to about 55°C, and by a variety of HLB values of from about 1 to about 14, most commonly from about 7 to about 14. For example, Gelucire® 50/13 designates a melting point of approximately 50°C and an HLB value of about 13 to this grade of Gelucire®.

Pharmaceutically acceptable excipients

Examples of suitable excipients for use in a composition or solid dosage form according to the present invention include fillers, diluents, disintegrants, binders, lubricants and the like and mixtures thereof. As the composition or solid dosage form according to the invention may be used for different purposes, the choice of excipients is normally made taken such different uses into considerations. Other pharmaceutically acceptable excipients for suitable use are e.g. acidifying agents, alkalizing agents, preservatives, antioxidants, buffering agents, chelating agents, coloring agents, complexing agents, emulsifying and/or solubilizing agents, flavors and perfumes, humectants, sweetening agents, wetting agents and the like.

Examples of suitable fillers, diluents and/or binders include lactose (e.g. spray-dried lactose, α-lactose, β-lactose, Tabletose®, various grades of Pharmatose®, Microtose® or Fast-Floc®), microcrystalline cellulose (various grades of Avicel®, Eclema®, Vivace®), Ming Tai® or Solka-Floc®), hydroxypropylcellulose, L-hydroxypropylcellulose (low substituted), hydroxypropyl methylcellulose (HPMC) (e.g. Methocel E, F and K, Metolose SH of Shin-Etsu, Ltd, such as, e.g. the 4,000 cps grades of Methocel E and Metolose 60 SH, the 4,000 cps grades of Methocel F and Metolose 65 SH, the 4,000, 15,000 and 100,000 cps grades of Methocel K; and the 4,000, 15,000, 39,000 and 100,000 grades of Metolose 90 SH), methylcellulose polymers (such as, e.g., Methocel A, Methocel A4C, Methocel A15C, Methocel A4M), hydroxyethylcellulose, sodium carboxymethylcellulose, carboxymethylene, carboxymethylhydroxyethylcellulose and other cellulose derivatives, sucrose, agarose, sorbitol, mannitol, dextrits, maltodextrins, starches or modified starches (including potato starch, maize starch and rice starch), calcium phosphate (e.g. basic calcium phosphate,
calcium hydrogen phosphate, dicalcium phosphate hydrate), calcium sulfate, calcium carbonate, sodium alginate, collagen etc.

Specific examples of diluents are e.g. calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextran, dextrin, dextrose, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, sugar etc.

Specific examples of disintegrants are e.g. alginic acid or alginates, microcrystalline cellulose, hydroxypropyl cellulose and other cellulose derivatives, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, starch, pregelatinized starch, carboxymethyl starch (e.g. Primogel® and Explotab®) etc.

Specific examples of binders are e.g. acacia, alginic acid, agar, calcium carrageenan, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methylcellulose, pectin, PEG, povidone, pregelatinized starch etc.

Gildants and lubricants may also be included in the composition. Examples include stearic acid, magnesium stearate, calcium stearate or other metallic stearate, talc, waxes and glycerides, light mineral oil, PEG, glyceryl behenate, colloidal silica, hydrogenated vegetable oils, corn starch, sodium stearyl fumarate, polyethylene glycols, alkyl sulfates, sodium benzoate, sodium acetate etc.

Other excipients which may be included in a composition or solid dosage form of the invention are e.g. flavoring agents, coloring agents, taste-masking agents, pH-adjusting agents, buffering agents, preservatives, stabilizing agents, anti-oxidants, wetting agents, humidity-adjusting agents, surface-active agents, suspending agents, absorption enhancing agents, agents for modified release etc.

Other additives in a composition or a solid dosage form according to the invention may be antioxidants like e.g. ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, potassium metabisulfite, propyl gallate, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, tocopherol acetate, tocopherol hemisuccinate, TPGS or other tocopherol derivatives, etc. The carrier composition may also contain e.g. stabilising agents. The concentration of an antioxidant and/or a stabilizing agent in the carrier composition is normally from about 0.1 % w/w to about 5% w/w.

A composition or solid dosage form according to the invention may also include one or more surfactants or substances having surface-active properties. It is contemplated that such substances are involved in the wetting of the slightly soluble active substance and thus, contributes to improved solubility characteristics of the active substance.
Suitable excipients for use in a composition or a solid dosage form according to the
invention are surfactants such as, e.g., amphiphilic surfactants as those disclosed in WO
00/50007 in the name of Lipocine, Inc. Examples of suitable surfactants are

i) polyethoxylated fatty acids such as, e.g. fatty acid mono- or diesters of
polyethylene glycol or mixtures thereof such as, e.g. mono — or diesters of
polyethylene glycol with lauric acid, oleic acid, stearic acid, myristic acid, ricinoleic
acid, and the polyethylene glycol may be selected from PEG 4, PEG 5, PEG 6,
PEG 7, PEG 8, PEG 9, PEG 10, PEG 12, PEG 15, PEG 20, PEG 25, PEG 30,
PEG 32, PEG 40, PEG 45, PEG 50, PEG 55, PEG 100, PEG 200, PEG 400, PEG
600, PEG 800, PEG 1000, PEG 2000, PEG 3000, PEG 4000, PEG 5000, PEG
6000, PEG 7000, PEG 8000, PEG 9000, PEG 1000, PEG 10,000, PEG 15,000,
PEG 20,000, PEG 35,000

ii) polyethylene glycol glycerol fatty acid esters, i.e. esters like the above-mentioned
but in the form of glyceryl esters of the individual fatty acids;

iii) glycerol, propylene glycol, ethylene glycol, PEG or sorbitol esters with e.g.
vegetable oils like e.g. hydrogenated castor oil, almond oil, palm kernel oil, castor
oil, apricot kernel oil, olive oil, peanut oil, hydrogenated palm kernel oil and the
like,

iv) polyglycerized fatty acids like e.g. polyglycerol stearate, polyglycerol oleate,
polyglycerol ricinoleate, polyglycerol linoleate,
v) propylene glycol fatty acid esters such as, e.g. propylene glycol monolaurate,
propylene glycol ricinoleate and the like,

vi) mono- and diglycerides like e.g. glyceryl monooleate, glycercyl dioleae, glycercyl
mono- and/or dioleate, glycercyl caprylate, glycercyl caprate etc.;

vii) sterol and sterol derivatives;

viii) polyethylene glycol sorbitan fatty acid esters (PEG-sorbitan fatty acid esters) such
as esters of PEG with the various molecular weights indicated above, and the
various Tween® series;

ix) polyethylene glycol alkyl ethers such as, e.g. PEG oleyl ether and PEG lauryl
ether;

x) sugar esters like e.g. sucrose monopalmitate and sucrose monolaurate;

xi) polyethylene glycol alkyl phenols like e.g. the Triton® X or N series;

xii) polyoxyethylene-polyoxypropylene block copolymers such as, e.g., the Pluronic®
series, the Synermonic® series, Emkalyx®, Lutrol®, Supronic® etc. The generic
term for these polymers is "poloxamers" and relevant examples in the present
context are Poloxamer 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188,
212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338,
401, 402, 403 and 407;

xiii) sorbitan fatty acid esters like the Span® series or Ariacel® series such as, e.g.
sorbitan monolaurate, sorbitan monopalmitate, sorbitan monooleate, sorbitan
monostearate etc.;

xiv) lower alcohol fatty acid esters like e.g. oleate, isopropyl myristate, isopropyl
palmitate etc.;

xv) ionic surfactants including cationic, anionic and zwitterionic surfactants such as,
e.g. fatty acid salts, bile salts, phospholipids, phosphoric acid esters,
carboxylates, sulfates and sulfonates etc.

When a surfactant or a mixture of surfactants is present in a composition or a solid
dosage form of the invention, the concentration of the surfactant(s) is normally in a range of
from about 0.1 – 80% w/w such as, e.g., from about 0.1 to about 20% w/w, from about 0.1 to
about 15% w/w, from about 0.5 to about 10% w/w, or alternatively, from about 0.10 to about
80% w/w such as, e.g. from about 10 to about 70% w/w, from about 20 to about 60% w/w or
from about 30 to about 50% w/w.

Release profiles

In the present context "t_{max}" denotes the time to reach the maximal plasma
concentration (c_{max}) after administration.

Thus, the dosage form of the invention may further comprise one or more release
modifying agents selected from the group consisting of water-miscible polymers, water-
insoluble polymers, oils and oily materials.

The water-insoluble polymer may be ethyl cellulose, cellulose acetate, cellulose
nitrate, and mixtures thereof. The water-miscible polymer may also be a cellulose derivative
selected from the group consisting of hydroxypropyl methylcellulose (HPMC), hydroxypropyl
cellulose (HPC), methylcellulose, sodium carboxymethylcellulose, hydroxyethyl cellulose,
poloxamers, polyoxyethylene stearates, poly-ε-caprolactone, polyvinylpyrrolidone (PVP),
polyvinylpyrrolidone-polyvinylacetate copolymer PVP-PVA, polymethacrylic polymers and
polyvinyl alcohol (PVA), poly(ethylene oxide) (PEO) and mixtures thereof. Examples of
especially useful polymethacrylic polymers are Eudragit® RS, Eudragit® RL, Eudragit® NE
and Eudragit® E.

The oil or oily material may be hydrophilic and hydrophobic oils or oily materials.
Hydrophilic oil or oily material may be polyether glycols such as polypropylene
glycols; polyoxyethylenes; polyoxypropylenes; poloxamers; polyglycolized glycerides such as
Gelucire®, for example Gelucire® 50/13, Gelucire® 44/14, Gelucire® 50/10, Gelucire® 62/05 and mixtures thereof.

Hydrophobic oil or oily material may have a melting point of at least about 20°C. Useful examples are straight chain saturated hydrocarbons, sorbitan esters, paraffins; fats and oils such as cacao butter, beef tallow, lard, polyether glycol esters; higher fatty acid such as stearic acid, myristic acid, palmitic acid, higher alcohols such as cetanol, stearyl alcohol, low melting point waxes such as glyceryl monostearate, glyceryl monooleate, hydrogenated tallow, myristyl alcohol, stearyl alcohol, substituted and/or unsubstituted monoglycerides, substituted and/or unsubstituted diglycerides, substituted and/or unsubstituted triglycerides, yellow beeswax, white beeswax, carnauba wax, castor wax, japan wax, acetylated monoglycerides; NVP polymers, PVP polymers, acrylic polymers, and mixtures thereof.

The oil or oily-like material may also be a sorbitan ester such as, e.g., sorbitan diisostearate, sorbitan dioleate, sorbitan monolaurate, sorbitan monoisostearate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, sorbitan sesqui-isostearate, sorbitan sesquioleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate or mixtures thereof.

The oil or oily-like material may of course comprise a mixture of different oils or oily-like materials such as, e.g., a mixture of hydrophilic and/or hydrophobic materials.

Other suitable oils or oily-like materials may be solvents or semi-solid excipients like, e.g. propylene glycol, polyglycolised glycerides including Gelucire 44/14, complex fatty materials of plant origin including theobroma oil, carnauba wax, vegetable oils like e.g. almond oil, coconut oil, corn oil, cottonseed oil, sesame oil, soya oil, olive oil, castor oil, palm kernels oil, peanut oil, rape oil, grape seed oil etc., hydrogenated vegetable oils such as, e.g. hydrogenated peanut oil, hydrogenated palm kernels oil, hydrogenated cottonseed oil, hydrogenated soya oil, hydrogenated castor oil, hydrogenated coconut oil; natural fatty materials of animal origin including beeswax, lanolin, fatty alcohols including cetyl, stearyl, lauric, myristic, palmitic, stearic fatty alcohols; esters including glycerol stearate, glycol stearate, ethyl oleate, isopropyl myristate; liquid interesterified semi-synthetic glycerides including Miglycol 810/812; amide or fatty acid alcolamides including stearamide ethanol, diethanolamide of fatty coconut acids, acetic acid esters of mono and di-glycerides, citric acid esters of mono and di-glycerides, lactic acid esters of mono and diglycerides, mono and diglycerides, poly-glycerol esters of fatty acids, poly-glycerol poly-ricinoleate, propylene glycol esters of fatty acids, sorbitan monostearates, sorbitan tristearates, sodium stearoyl lactylates, calcium stearoyl lactylates, diacetyl tartaric acid esters of mono and di-glycerides etc.

A delayed release of active ingredient is desired in order to increase the bioavailability of active ingredient by delivering the ingredient in the gastrointestinal tract, i.e.
the release predominantly takes place after passage of the stomach. For example, the
dosage form of the present invention may be designed in order to release, after oral
administration to a mammal in need thereof, at the most about 10 w/w%, preferably at the
most about 7.5 w/w%, more preferably at the most about 5 w/w%, especially at the most
about 2 w/w% of the total amount of active ingredient within the first 3 hours, preferably
within 2 hours, more preferably within 1 hour, in particular within about 30 minutes after
administration.

Further, the solid dosage form of the invention may, upon oral administration to a
mammal in need thereof, release at least about 50 w/w% of the active ingredient within 24
hours, preferably within about 20 hours, more preferably within about 18 hours, especially
within about 15 hours, in particular within about 12 hours.

Delayed release is mainly brought about by some kind of enteric coating. Whereas
semipermeable coating will show some kind of delayed release, it may not precisely enough
"delay" release. Additionally it requires a certain amount of time to release the content. The
coating sought for this invention, is a pH dependant coating. This type of coating is very
resistant to release of drug until a certain pH is reached. Within a small increment in the pH
value, i.e. within an increase in pH of about 0.2 to 0.4, the film alters properties and becomes
permeable.

Accordingly, the solid dosage forms of the invention may exhibit a delayed release of
active ingredient by means of an enteric coating using a water-miscible polymer having a pH-
dependant solubility in water. Examples of pH-sensitive polymers, which are relatively
insoluble and impermeable at the pH of the stomach, but which are more soluble and
permeable at the pH of the small intestine and colon include, but are not limited to,
polyacrylamides; phthalate derivatives such as acid phthalates of carbohydrates including
amylose acetate phthalate, cellulose acetate phthalate, cellulose acetate terephthalate,
cellulose acetate isophthalate, other cellulose ester phthalates, cellulose ether phthalates,
hydroxypropyl cellulose phthalate, hydroxypropylcellulose acetate phthalate, hydroxypropyl
ethylcellulose phthalate, hydroxypropyl methylcellulose phthalate (HPMCP), methylcellulose
phthalate, methyl cellulose acetate phthalate, polyvinyl acetate phthalate, polyvinyl acetate
hydrogen phthalate, sodium cellulose acetate phthalate, starch acid phthalate; phthalates of
other compounds including polyvinyl acetate phthalate (PVAP); other cellulose derivatives
including hydroxypropyl methylcellulose acetate succinate (HPMCAS),
carboxymethylcellulose, cellulose acetate trimellitate; alginates; caromers; polyacrylic acid
derivatives such as acrylic acid and acrylic ester copolymers, polymethacrylic acid and esters
thereof, poly acrylic methacrylic acid copolymers, methacrylic acid copolymers (for example
Eudragit® L and Eudragit® S); styrene-maleic acid dibutyl phthalate copolymer, styrene-
maleic acid polyvinylacetate phthalate copolymer, styrene and maleic acid copolymers;
shellac, starch glycolate; polacrylin; vinyl acetate and crotonic acid copolymers and mixtures thereof. pH-sensitive polymers of specific interest include shellac; phthalate derivatives, particularly cellulose acetate phthalate, polyvinylacetate phthalate, and hydroxypropylmethylcellulose phthalate; polyacrylic acid derivatives, particularly polymethyl methacrylate blended with acrylic acid and acrylic ester copolymers; and vinyl acetate and crotonic acid copolymers.

A first delayed release embodiment according to the invention is a "pH-dependent coated dosage form" such as, e.g., a tablet or a capsule. In the case of a tablet it comprises a tablet core comprising tacrolimus e.g. in a solid solution/ dispersion as a multiparticulate product, a controlled release matrix of e.g. HPMC (hypromellose), a disintegrant, a lubricant, and one or more pharmaceutical carriers, such core being coated with a material, preferably a polymer, which is substantially insoluble and impermeable at the pH of the stomach, and which is more soluble and permeable at the pH of the small intestine. Preferably, the coating polymer is substantially insoluble and impermeable at pH <5.0, and water-soluble at pH>5.0.

The tablet core may be coated with an amount of polymer sufficient to assure that substantially no release of tacrolimus from the dosage form occurs until the dosage form has exited the stomach and has resided in the small intestine for about 15 minutes or greater, preferably about 30 minutes or greater, thus assuring that minimal tacrolimus is released in the duodenum. Mixtures of a pH-sensitive polymer with a water-insoluble polymer may also be employed. Tablets are coated with an amount of polymer comprising from about 10% to about 80% of the weight of the tacrolimus-containing tablet core. Preferred tablets are coated with an amount of polymer comprising about 15% to about 50% of the weight of the tacrolimus tablet core.

pH-sensitive polymers which are very insoluble and impermeable at the pH of the stomach, but which are more soluble and permeable at the pH of the small intestine and colon include polyacrylamides, phthalate derivatives such as acid phthalates of carbohydrates, amylose acetate phthalate, cellulose acetate phthalate, other cellulose ester phthalates, cellulose ether phthalates, hydroxypropylcellulose phthalate, hydroxypropylethylcellulose phthalate, hydroxypropylmethylcellulose phthalate, methylcellulose phthalate, polyvinyl acetate phthalate, polyvinyl acetate hydrogen phthalate, sodium cellulose acetate phthalate, starch acid phthalate, styrene-maleic acid dibutyl phthalate copolymer, styrene-maleic acid polyvinylacetate phthalate copolymer, styrene and maleic acid copolymers, polyacrylic acid derivatives such as acrylic acid and acrylic ester copolymers, polymethacrylic acid and esters thereof, poly acrylic methacrylic acid copolymers, shellac, and vinyl acetate and crotonic acid copolymers.

Preferred pH-sensitive polymers include shellac; phthalate derivatives, particularly cellulose acetate phthalate, polyvinylacetate phthalate, and hydroxypropylmethylcellulose
phthalate; polyacrylic acid derivatives, particularly polymethyl methacrylate blended with acrylic acid and acrylic ester copolymers; and vinyl acetate and crotonic acid copolymers.

The delay time before release of tacrolimus, after the "pH-dependent coated tablet" dosage form has exited the stomach, may be controlled by choice of the relative amounts of Eudragit-L® and Eudragit-S® in the coating, and by choice of the coating thickness. Eudragit-L® films dissolve above pH 6.0, and Eudragit-S® films dissolve above 7.0, and mixtures dissolve at an intermediate pH. Since the pH of the duodenum is approximately 6.0 and the pH of the colon is approximately 7.0, coatings composed of mixtures of Eudragit-L® and Eudragit-S® provide protection of the duodenum from tacrolimus. If it is desired to delay release of tacrolimus until the tacrolimus-containing "pH-dependent coated tablet" has reached the colon, Eudragit-S® may be used as the coating material, as described by Dew et al. (Br. J. Clin. Pharmac. 14 (1982) 405-408). In order to delay the release of tacrolimus for about 15 minutes or more, preferably 30 minutes or more, after the dosage form has exited the stomach, preferred coatings comprise from about 9:1 to about 1:9 Eudragit-L®/Eudragit-S®, more preferably from about 9:1 to about 1:4 Eudragit-L®/Eudragit-S®. The coating may comprise from about 3% to about 70% of the weight of the uncoated tablet core. Preferably, the coating comprises from about 5% to about 50% of the weight of the tablet core.

The release of the active substance from a composition having a delayed release coating could also be an enzymatic reaction, if for example Zein or mono/di-glyceride mixtures are employed as coating material.

In a further embodiment, a pharmaceutical composition can be prepared in the form of an emulsion or a suspension for preparation of a liquid or semisolid dosage form. Such oral formulations may be prepared by well known pharmaceutical technologies in the art, for instance as described in Remington: The Science and Practice of Pharmacy, (formerly called Remington's Pharmaceutical Sciences), Mack Publishing Co, and Martindale The Complete Drug Reference, (formerly called Martindale The Extra Pharmacopoeia), 34th edition, The Pharmaceutical Press

Materials and methods

Materials
Tacrolimus (supplied by Eurotrade); batch no RD 03-111
Lactose monohydrate 200 mesh or 125 mesh (from DMV)
Polyethylene glycol 6000, Pluracol® E6000 (from BASF)
Poloxamer 188, Pluronic® F-68 (from BASF)
Glyceryl monostearate, Rylo® MD50, (from Danisco Cultor), Ph.Eur.; batch no. 4010056276
Magnesium stearate
Croscarmellose sodium, Ac-Di-Sol® (from FMC)
Eudragit® L30D.55 (from Degussa)
Triethyl citrate (from Merck)
Anti-foam emulsion (from Unikem)
Micro talc

5
Ketonazole
Limonin
Spiro ortho esters prepared as disclosed in WO99/09976 (compounds V-XIV) and WO2004/037827

10 HPMC/hypromellose refers to Metolose 90SH (type 2910, 2208 – for example 15,000cP) or Metolose 60SH (type 2910) from ShinEtsu available in various degrees of polymerization (viscosity 3-100,000cP).

15 Tablets, capsules or granules might be enteric coated with different types of polymers such as hydroxypropylmethylcellulose acetate succinate (Aqoat), cellulose acetate phthalate CAP, hydroxypropylmethylcellulose phtalate HPMCP or methacrylic acid copolymers such as Eudragit L30D, Eudragit 100/S, Eudragit 100/L.

Methods

Determination of weight variation

The tablets prepared in the Examples herein were subjected to a test for weight variation performed in accordance with Ph. Eur.

Determination of average tablet hardness

The tablets prepared in the Examples herein were subjected to a test for tablet hardness employing Schleuniger Model 6D apparatus and performed in accordance with the general instructions for the apparatus.

Determination of disintegration time

The time for a tablet to disintegrate, i.e. to decompose into particles or agglomerates, was determined in accordance with Ph. Eur.

Determination of geometric weight mean diameter $d_{gw}$

The geometric weight mean diameter was determined by employment of a method of laser diffraction dispersing the particulate material obtained (or the starting material) in air. The measurements were performed at 1 bar dispersive pressure in Sympatec Helos equipment, which records the distribution of the equivalent spherical diameter. This distribution is fitted to a log normal volume-size distribution.
When used herein, “geometric weight mean diameter” means the mean diameter of the log normal volume-size distribution.

In vitro dissolution tests

The following test methods were applies to the compositions and dosage forms of the present invention.

Test 1:
In vitro dissolution test according to USP Method A, delayed release articles (USP paddle method; rotation speed: 50 rpm; 37°C; after 2 hours in acidic medium, the medium is changed to phosphate buffer pH 6.8.).

Test 2:
In vitro dissolution test in aqueous dissolution medium adjusted to pH 4.5 (900 ml water with 0.005% HPC (hydroxypropylcellulose) adjusted to pH 4.5; 37°C; USP Paddle method; rotation speed: 50 rpm).

The following non-limiting examples illustrate the invention.

Example 1
Immediate release composition of tacrolimus and a CYP3A4 inhibitor compound

The following tablet compositions were prepared:

Composition 1A:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Active ingredient</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Spiro ortho ester</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Lactose 200 mesh</td>
<td>Carrier</td>
<td>41.7</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>Vehicle</td>
<td>34.3</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>Vehicle</td>
<td>14.7</td>
</tr>
<tr>
<td>Croscarmellose sodium (Ac-di-sol)</td>
<td>Filler</td>
<td>4.9</td>
</tr>
<tr>
<td>Dimeticone 350</td>
<td>Antioxidant</td>
<td>0.25microgram</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>Antioxidant</td>
<td>25microgram</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Antioxidant</td>
<td>0.5 microL</td>
</tr>
<tr>
<td>Butyl hydroxy toluene (BHT)</td>
<td>Antioxidant</td>
<td>5 microgram</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
Composition 1B:

<table>
<thead>
<tr>
<th>Substance</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus Active ingredient</td>
<td>1.0</td>
</tr>
<tr>
<td>Limonin CYP3A4 inhibitor</td>
<td>5.0</td>
</tr>
<tr>
<td>Lactose 200 mesh Carrier</td>
<td>41.7</td>
</tr>
<tr>
<td>PEG 6000 Vehicle</td>
<td>34.3</td>
</tr>
<tr>
<td>Poloxamer 188 Vehicle</td>
<td>14.7</td>
</tr>
<tr>
<td>Croscarmellose sodium (Ac-di-sol) Filler</td>
<td>4.9</td>
</tr>
<tr>
<td>Dimeticone 350 Antioxidant 0.25 microgram</td>
<td></td>
</tr>
<tr>
<td>Citric acid monohydrate Antioxidant 25 microgram</td>
<td></td>
</tr>
<tr>
<td>Isopropyl alcohol Antioxidant 0.5 microL</td>
<td></td>
</tr>
<tr>
<td>Butyl hydroxy toluene (BHT) Antioxidant 5 microgram</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>103.0</td>
</tr>
</tbody>
</table>

Composition 1C:

<table>
<thead>
<tr>
<th>Substance</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus Active ingredient</td>
<td>1.0</td>
</tr>
<tr>
<td>Ketoconazole CYP3A4 inhibitor</td>
<td>200.0</td>
</tr>
<tr>
<td>Lactose 200 mesh Carrier</td>
<td>41.7</td>
</tr>
<tr>
<td>PEG 6000 Vehicle</td>
<td>34.3</td>
</tr>
<tr>
<td>Poloxamer 188 Vehicle</td>
<td>14.7</td>
</tr>
<tr>
<td>Croscarmellose sodium (Ac-di-sol) Filler</td>
<td>4.9</td>
</tr>
<tr>
<td>Dimeticone 350 Antioxidant 0.25 microgram</td>
<td></td>
</tr>
<tr>
<td>Citric acid monohydrate Antioxidant 25 microgram</td>
<td></td>
</tr>
<tr>
<td>Isopropyl alcohol Antioxidant 0.5 microL</td>
<td></td>
</tr>
<tr>
<td>Butyl hydroxy toluene (BHT) Antioxidant 5 microgram</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>298.0</td>
</tr>
</tbody>
</table>

Film coating:

The tablets of examples 1A, 1B, 1C and 1D were subsequently coated with the following film coating:
5

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>mg/tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit® L30D</td>
<td>42.4</td>
</tr>
<tr>
<td>Purified water</td>
<td>58.2</td>
</tr>
<tr>
<td>Triethyl acetylcitrate</td>
<td>1.9</td>
</tr>
<tr>
<td>Dow Corning 1510</td>
<td>0.2</td>
</tr>
<tr>
<td>Talc</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>105.9</strong></td>
</tr>
</tbody>
</table>

The coating suspension was prepared by mixing triethyl acetylcitrate, anti-foam emulsion and purified water in Ultra Turrax apparatus at 9500 rpm for 30 min. After 1 minute, talc was added. The mixture was passed through sieve no. 300 and stirred by a magnet stirrer. Eudragit was passed through sieve no. 300 and added the mixture, which was stirred for 5 minutes.

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Example 2
Tacrolimus tablet film-coated with a CP3A4 inhibitor compound

The following tablet composition was prepared:

<table>
<thead>
<tr>
<th>Substance</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>Active ingredient</td>
</tr>
<tr>
<td>Lactose 200 mesh</td>
<td>Carrier</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Croscarmellose sodium (Ac-di-sol)</td>
<td>Filler</td>
</tr>
<tr>
<td>Dimeticone 350</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Butyl hydroxy toluene (BHT)</td>
<td>Antioxidant</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

Film coating:

The tablet was subsequently coated with the following film coating:
The coating suspension was prepared by mixing triethyl acetylcitrate, spiro ortho ester, antifoam emulsion and purified water in Ultra Turrax apparatus at 9500 rpm for 30 min. After 1 minute talc was added. The mixture was passed through sieve no. 300 and stirred by a magnet stirrer. Eudragit was passed through sieve no. 300 and added the mixture, which was stirred for 5 minutes.

Example 3
Tacrolimus and a CYP3A4 inhibitor compound

The following tablet compositions were prepared:

Composition 3A:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Active ingredient</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Spiro ortho ester</td>
<td>CYP3A4 inhibitor</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactose 200 mesh</td>
<td>Carrier</td>
<td>41.7</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>Vehicle</td>
<td>34.3</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>Vehicle</td>
<td>14.7</td>
</tr>
<tr>
<td>Hypermellose USP (Metolose 90SH) Filler</td>
<td></td>
<td>61.8</td>
</tr>
<tr>
<td>Dimeticone 350</td>
<td>Antioxidant</td>
<td>0.25 microgram</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>Antioxidant</td>
<td>25 microgram</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Antioxidant</td>
<td>0.5 microL</td>
</tr>
<tr>
<td>Butyl hydroxy toluene (BHT)</td>
<td>Antioxidant</td>
<td>5 microgram</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
Composition 3B:

<table>
<thead>
<tr>
<th>Substance</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>2.0</td>
</tr>
<tr>
<td>Spiro ortho ester</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactose monohydrate 125 mesh</td>
<td>14.3</td>
</tr>
<tr>
<td>Rylo MD50 (glyceryl monostearate)</td>
<td>64.7</td>
</tr>
<tr>
<td>Pharmacoat 606</td>
<td>14.3</td>
</tr>
<tr>
<td>Pharmatose DCL14</td>
<td>105.8</td>
</tr>
<tr>
<td>Talc: Magnesium stearate</td>
<td>9.52:1.06</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.0</td>
</tr>
</tbody>
</table>

The CYP3A4 inhibitor of compositions 3A and 3B may be substituted with 5 or 10 mg of limonin or 200 mg of ketoconazole.

Example 4

Preparation of a delayed release tacrolimus formulation (granulate)

The following granulate was prepared:

Composition (dosage form)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>2</td>
</tr>
<tr>
<td>Lactose</td>
<td>80</td>
</tr>
<tr>
<td>PEG 6000</td>
<td>15</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>6</td>
</tr>
<tr>
<td>Metolose SH 90</td>
<td>80</td>
</tr>
<tr>
<td>Avicel PH200</td>
<td>60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>243</td>
</tr>
</tbody>
</table>

The formulation was based on melt granulation in a high shear mixer Pellmix 1/8. 16g micronized tacrolimus was mixed with 640 g lactose 125 mesh and 120 g polyethylene glycol 6000, 48g Poloxamer 188 and 640 g hydroxypropylmethylcellulose (hypromellose) Metolose SH 90 15,000 cP in the high shear mixer. The jacket of the mixer bowl was heated to 80°C and the blend was heated at an impeller rotation speed of 1000 rpm until melting point of PEG and Poloxamer. After melting the kneading was continued for 4 minutes at 800 rpm. The granulated was sieved through sieve size of 0.7 mm and cooled on a tray. The granulate was mixed with 480 g Avicel PH200 for 3 minutes. The mixture may be compressed into tablets after addition of magnesium stearate and a granulate comprising a CYP3A4 inhibitor.
CLAIMS

1. A pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the composition upon administration to a human in need thereof results in a relative $\frac{AUC_{\text{inventor}}}{AUC_{\text{separate}}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the AUC$_{\text{separate}}$ value is determined after administration of tacrolimus and CYP3A4 inhibitor in separate dosage forms.

2. The pharmaceutical composition according to claim 1, wherein the relative $\frac{AUC_{\text{inventor}}}{AUC_{\text{separate}}}$ value is at least about 1.2, or at least about 1.3, or at least about 1.4, or at least about 1.5 or at least about 1.6.

3. A pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the composition upon administration to a human in need thereof results in a relative $\frac{AUC_{\text{inventor}}}{AUC_{\text{per se}}}$ value of tacrolimus of at least about 1.1, the AUC values being determined under similar conditions and the AUC$_{\text{per se}}$ value is determined after administration of tacrolimus per se (without CYP3A4 inhibitor).

4. The pharmaceutical composition according to claim 3, wherein the relative $\frac{AUC_{\text{inventor}}}{AUC_{\text{per se}}}$ value is at least about 1.2, or at least about 1.3, or at least about 1.4, or at least about 1.5 or at least about 1.6.

5. A pharmaceutical composition comprising a CYP3A4 inhibitor and tacrolimus or an analogue thereof, wherein the CYP3A4 inhibitor or the tacrolimus or an analogue of tacrolimus is present in the composition in an amount of at least 90%, or at least 95%, or at least 100%, relative to the amount prior to storage, when assayed after 3 months of storage at a temperature of about 25°C and a relative humidity of about 60%.

6. The pharmaceutical composition according to any of the preceding claims, wherein at least 80 w/w% of the tacrolimus is present in the composition as a solid dispersion or a solid solution in a hydrophilic or water-miscible vehicle.

7. The pharmaceutical composition according to claim 6, wherein at least 90 w/w% of the tacrolimus is present in the composition as a solid dispersion or a solid solution in a hydrophilic or water-miscible vehicle.
8. The pharmaceutical composition according to claim 7, wherein at least 99 w/w% of the tacrolimus is present in the composition as a solid dispersion or a solid solution in a hydrophilic or water-miscible vehicle.

9. The pharmaceutical composition according to any of the preceding claims, wherein the CYP3A4 inhibitor is selected from the group consisting of cyclic and spiro ortho esters and cyclic and spiro ortho carbonates capable of inhibiting cytochrome P430.

10. The pharmaceutical composition according to claim any of claims 1-8, wherein the CYP3A4 inhibitor is selected from the group consisting of diethyl dithiocarbamate, ketoconazole, itraconazole, erythromycin, ritonavir and lansoprazol.

11. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is selected from the group consisting of safrole, rutaecarpine, limonin, dipiperamide A (from white pepper), gomisin C (from schisandra fruit), paradisin A and paradisin B (from grape fruit juice).

12. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of Formulae I-IV:

wherein, in each of the above structures, R is, independently, H or an optionally substituted
C₁⁻C₁₅ alkyl group, L is an optionally substituted C₁⁻C₁₅ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen,

HAr is an optionally substituted C₆⁻C₂₄ aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P, and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C₁⁻C₈ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C₃⁻C₈ optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr,

wherein the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C₁⁻C₆ linear, branched or cyclic alkyl group, -OH, halogen, a C₁⁻C₅ alkoxy group, a C₁⁻C₅ alkyl carbonyloxy group and a C₁⁻C₅ alkoxycarbonyl group.

13. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of the formula:

wherein, in each of the above structures, R is, independently, H or an optionally substituted C₁⁻C₁₅ alkyl group, L is an optionally substituted C₁⁻C₁₅ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen,

HAr is an optionally substituted C₆⁻C₂₄ aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P, and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C₁⁻C₈ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C₃⁻C₈ optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr,
wherein the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C<sub>1</sub>-C<sub>6</sub> linear, branched or cyclic alkyl group, -OH, halogen, a C<sub>1</sub>-C<sub>5</sub> alkoxy group, a C<sub>1</sub>-C<sub>5</sub> alkyl carbonyloxy group and a C<sub>1</sub>-C<sub>5</sub> alkoxy carbonyl group.

14. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of the formula:

![Chemical Structure](image)

wherein, in each of the above structures, R is, independently, H or an optionally substituted C<sub>1</sub>-C<sub>15</sub> alkyl group, L is an optionally substituted C<sub>1</sub>-C<sub>15</sub> linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen,

HAr is an optionally substituted C<sub>6</sub>-C<sub>24</sub> aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P,

and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C<sub>1</sub>-C<sub>8</sub> linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C<sub>3</sub>-C<sub>8</sub> optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr,

wherein the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C<sub>1</sub>-C<sub>6</sub> linear, branched or cyclic alkyl group, -OH, halogen, a C<sub>1</sub>-C<sub>5</sub> alkoxy group, a C<sub>1</sub>-C<sub>5</sub> alkyl carbonyloxy group and a C<sub>1</sub>-C<sub>5</sub> alkoxy carbonyl group.

15. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of the formula:
wherein, in each of the above structures, R is, independently, H or an optionally substituted C_1 -C_{15} alkyl group, L is an optionally substituted C_1 -C_{15} linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen,
HAr is an optionally substituted C_6 -C_{24} aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P, and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C_1 -C_8 linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C_3 -C_{8} optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted HAr,
wherein the optional substituents for the groups R, L, HAr and E are selected from the group consisting of a C_1-C_6 linear, branched or cyclic alkyl group, -OH, halogen, a C_1-C_5 alkoxy group, a C_1-C_5 alkyl carboxyloxy group and a C_1-C_5 alkoxy carbonyl group.

16. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of any of the formulae V-X:
17. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of formulae XI-XII:
18. The pharmaceutical composition according to claim 1 or 3 or 5, wherein the CYP3A4 inhibitor is a compound of formulae XIII-XIV:

19. The pharmaceutical composition according to any of claims 1-8, wherein the CYP3A4 inhibitor is a compound of formulae XV-XVI:
20. A pharmaceutical composition according to any of the preceding claims, which further comprises one or more pharmaceutically acceptable excipients.

21. The composition according to claim 20, wherein the pharmaceutically acceptable excipient(s) is/are selected from the group consisting of fillers, disintegrants, binders and lubricants.

22. The composition according to any of the preceding claims comprising a first composition fraction comprising tacrolimus and a second composition fraction comprising the CYP3A4 inhibitor.

23. The composition according to claim 22 wherein the first and second composition fractions are in the form of individual compositions and being selected from solid, semi solid and liquid forms, respectively.

24. The composition according to claim 22 or 23 wherein one first or second composition fraction is in the form of a granulate composition and the other composition fraction is in the form of a coating composition.

25. A method for the preparation of a pharmaceutical composition according to any of claims 1 to 21, the method comprising the steps of dissolving or dispersing tacrolimus in a solid, hydrophilic or water-miscible vehicle to obtain a solid dispersion or a solid solution or a mixture thereof, followed by mixing the CYP3A4 inhibitor with the solid dispersion or solid solution.
26. A method for the preparation of a pharmaceutical composition according to claim 23 comprising the steps of
   a) preparing a first composition fraction in the form of a granulate or a compressible composition comprising tacrolimus,
   b) preparing a second composition fraction in the form of a granulate or a compressible composition comprising the CYP3A4 inhibitor,
   c) mixing the first and second fractions into one single composition.

27. A method for the preparation of a pharmaceutical composition according to claim 23 comprising the steps of
   a) preparing a first composition fraction in the form of a granulate or a compressible composition comprising tacrolimus,
   b) preparing a second composition fraction in the form of a granulate or a compressible composition comprising the CYP3A4 inhibitor,
   c) preparing a single pharmaceutical composition by compressing the two composition fractions into a double layer tablet.

28. A method for the preparation of a pharmaceutical composition according to claim 24 comprising the steps of
   a) preparing one composition fraction in the form of a solid composition comprising either tacrolimus or the CYP3A4 inhibitor,
   b) preparing the other composition fraction in the form of a composition suitable for coating the solid composition obtained in step a) and comprising the CYP3A4 inhibitor or Tacrolimus not present in the first composition fraction,
   c) coating the composition obtained in step a) with the composition obtained in step b) to form a single pharmaceutical composition.

29. A pharmaceutical dosage form comprising the pharmaceutical composition according to any of the preceding claims.

30. A dosage form comprising the pharmaceutical composition according to claim 29 which is a solid oral dosage form.

31. The dosage form according to claim 29 or 30, which is a unit dosage form.

32. The dosage form according to any of claims 29 to 31, which further comprises a pharmaceutically acceptable additive selected from the group consisting of flavoring agents,
coloring agents, taste-masking agents, pH-adjusting agents, buffering agents, preservatives, stabilizing agents, anti-oxidants, wetting agents, humidity-adjusting agents, surface-active agents, suspending agents and absorption enhancing agents.

33. The solid dosage form according to any of claims 29 to 32, wherein the CYP3A4 inhibitor and the tacrolimus or an analogue thereof is stable.

34. The solid dosage form according to any of claims 29 to 33, wherein the CYP3A4 inhibitor and the tacrolimus or an analogue thereof is present in an amount of at least 90%, or at least 95%, or at least 100%, relative to the amount prior to storage, when assayed after 3 months of storage at a temperature of about 40°C and a relative humidity of about 75%.

35. Use of the pharmaceutical composition according to any of claims 1-24 for the preparation of medicament in the form of suspensions, emulsions, granules, pellets, microspheres or nanoparticles.

36. Use of the pharmaceutical composition according to any of claims 1 to 24 in the preparation of a medicament in an oral dosage form or in a sublingual dosage form.

37. Use of the pharmaceutical composition according to claim 35 or 36 for the preparation of a medicament in the form of tablets, capsules or sachets.

38. Use of the medicament prepared according to any of claims 35 to 37 for enhancing the oral bioavailability of tacrolimus.

39. A single solid dosage form for oral administration comprising a first solid pharmaceutical composition containing tacrolimus or an analogue thereof as the active substance and second solid pharmaceutical composition containing a CYP3A4 inhibitor as the active substance, wherein the first and the second pharmaceutical composition are present in separate entities.

40. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is selected from the group consisting of cyclic and spiro ortho esters and cyclic and spiro ortho carbones capable of inhibiting cytochrome P430.

41. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is selected from the group consisting of diethyl dithiocarbamate, ketoconazole, itraconazole, erythromycin,
ritonavir, lansoprazol, safrole, rutaecarpine, limonin, dipiperamide A (from white pepper),
gomisin C (from schisandra fruit), paradisin A and paradisin B (from grape fruit juice).

42. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of

Formulae I-IV:

wherein, in each of the above structures, R is, independently, H or an optionally substituted
C₁₋C₁₅ alkyl group, L is an optionally substituted C₁₋C₁₅ linear or branched, saturated,
monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural
nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by

10 oxygen,

HAr is an optionally substituted C₆₋C₂₄ aromatic group or heteroaromatic group optionally
containing one or plural ring atoms selected from the group consisting of N, O, S, and P,
and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C₁₋C₈
linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally
interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C₃₋C₈ optionally
substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally
interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted
HAr,
wherein the optional substituents for the groups R, L, Har and E are selected from the group consisting of a C₁-C₆ linear, branched or cyclic alkyl group, -OH, halogen, a C₁-C₅ alkoxy group, a C₁-C₅ alkyl carbonyloxy group and a C₁-C₅ alkoxy carbonyl group.

43. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of the formulae:

![Chemical Structures]

wherein, in each of the above structures, R is, independently, H or an optionally substituted C₁-C₁₅ alkyl group, L is an optionally substituted C₁-C₁₅ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent sulfur or oxygen atoms and optionally terminated at one or both ends by oxygen,

Har is an optionally substituted C₆-C₂₄ aromatic group or heteroaromatic group optionally containing one or plural ring atoms selected from the group consisting of N, O, S, and P,

and E is -OH, -COOH, -COOR, where R is defined above, or an optionally substituted C₁-C₈ linear or branched, saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is a C₃-C₅ optionally substituted cyclic saturated, monounsaturated or polyunsaturated alkyl group optionally interrupted by one or plural nonadjacent oxygen or sulfur atoms, or E is optionally substituted Har,

wherein the optional substituents for the groups R, L, Har and E are selected from the group consisting of a C₁-C₆ linear, branched or cyclic alkyl group, -OH, halogen, a C₁-C₅ alkoxy group, a C₁-C₅ alkyl carbonyloxy group and a C₁-C₅ alkoxy carbonyl group.

44. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of any of the formulae V-X:
45. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of formulae XI-XII:
46. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of formulae XIII-XIV:

47. The dosage form according to claim 39, wherein the CYP3A4 inhibitor is a compound of formulae XV-XVI:
48. The dosage form according to any of claims 39-47, wherein the first solid pharmaceutical composition is in the form of granulate, granules, grains, beads or pellets.

49. The dosage form according to any of claims 39-48, wherein the second solid pharmaceutical composition is in the form of granulate, granules, grains, beads or pellets.

50. The dosage form according to any of claims 39 to 49, wherein the granulate, granules, grains, beads or pellets are entero-coated.

51. The dosage form according to any of claims 48 to 49, wherein the granules, granulate, grains, beads or pellets are coated with a protective coating.

52. The dosage form according to any of claims 39-51, which is a capsule or a sachet.

53. The dosage form according to claim 39-51, which is a tablet.

54. The dosage form according to claim 53, in which the first and second pharmaceutical compositions are present in at least two separate layers.

55. The dosage form according to claim 54, wherein the layers comprising the first and second pharmaceutical compositions are separated by an intermediate, inactive layer.
56. The dosage form according to claim 53, which is a tablet prepared by compressing the first pharmaceutical composition in the form of granulate together with the second pharmaceutical composition in the form of granulate having a protective coating.

57. The dosage form according to claim 53, which is a tablet prepared by compressing the first pharmaceutical composition in the form of granulate together with the second pharmaceutical composition in the form of entero-coated granulate.

58. The dosage form according to any of claims 39-57, wherein the CYP3A4 inhibitor and the tacrolimus or an analogue thereof is stable.

59. The solid dosage form according to claim 58, wherein the CYP3A4 inhibitor and the tacrolimus or an analogue thereof is present in an amount of at least 90%, or at least 95%, or at least 100%, relative to the amount prior to storage, when assayed after 3 months of storage at a temperature of about 40°C and a relative humidity of about 75%.

60. A single solid dosage form suitable for oral administration comprising tacrolimus or an analogue thereof as one active substance and a CYP3A4 inhibitor as a second active substance, wherein the tacrolimus is present as an active ingredient either of an immediate release pharmaceutical formulation, an entero-coated immediate release pharmaceutical formulation or of a delayed release pharmaceutical formulation; and the CYP3A4 inhibitor is present as an active ingredient of an immediate release pharmaceutical formulation or of a delayed release pharmaceutical formulation.

61. A method for preparing a single solid dosage form comprising a first solid pharmaceutical composition containing tacrolimus as the active substance and second solid pharmaceutical composition containing a CYP3A4 inhibitor as the active substance, the first and the second pharmaceutical composition being present in separate entities, which method comprising the steps of:

   i) preparing the first solid pharmaceutical composition,

   ii) preparing the second solid pharmaceutical composition, and

   iii) compressing the first and second compositions into a multilayer tablet, the first and second compositions being present in separate layers.

62. A method for treating a patient with tacrolimus comprising administering to said patient in need thereof a pharmaceutically effective amount of tacrolimus in a dosage form according to any of claims 29 to 34 and 39 to 60.