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Remarks:
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(54) **METHOD FOR PURIFICATION OF MICAFUNGIN**

(57) The present invention relates to a method for the purification of Micafungin.

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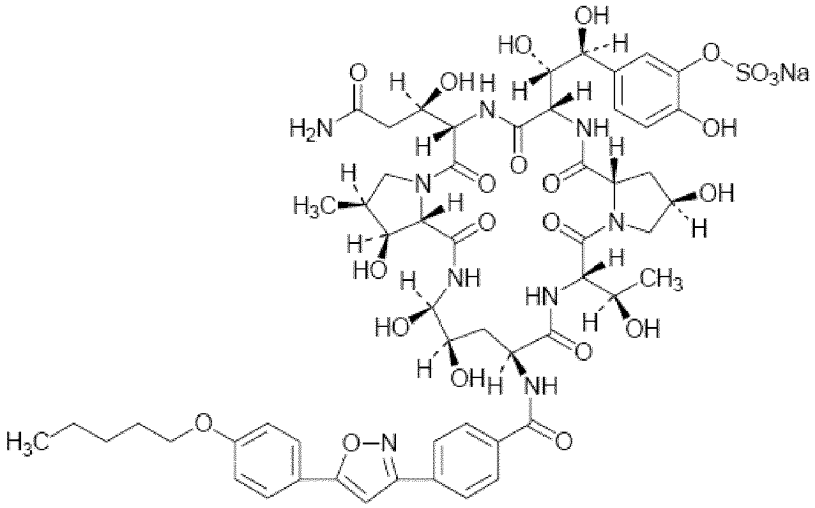
## Description

### Technical filed

**[0001]** The present invention relates to an improved method for purification of a Micafungin salt, in particular Micafungin sodium.

### Background

**[0002]** Micafungin is the active pharmaceutical ingredient in Mycamine®. According to the FDA label, the chemical structure of Micafungin sodium is represented by formula (I):



**[0003]** Micafungin is also known as Pneumocandin A0, 1-[(4*R*,5*R*)-4,5-dihydroxy-*N*2-[4-[5-[4-(pentyloxy)phenyl]-3-isoxazolyl]benzoyl]-L-ornithine]-4-[(4*S*)-4-hydroxy-4-[4-hydroxy-3-(sulfooxy)phenyl]-L-threonine]. Micafungin sodium is furthermore known as FK-463. The assigned Registry No's by Chemical Abstracts are 235114-32-6 for Micafungin and 208538-73-2 for Micafungin sodium.

**[0004]** Micafungin is an echinocandin which inhibits 1,3-β-D-glucan synthase and thus leads to fungal cell lysis. Micafungin is thus useful as an antifungal agent in the treatment of infections caused by strains of e.g. *Aspergillus, Cryptococcus, Candida, Mucor, Actinomyces, Histoplasma, Dermatophyte*, *Malassezia,* and *Fusarium*. Micafungin is the active ingredient in the approved drugs Mycamine® and Funguard® which are used in the treatment and prophylaxis of infections caused by Candida.

**[0005]** Various methods for the preparation and purification of Micafungin are known to the skilled person, see e.g. US 6,107,458 and US 7,199,248. More particularly, US 7,199,248 discloses a method wherein a crude DIPEA salt of Micafungin is purified by filtration and chromatographic separation using a regenerated y Alumina in a 1350-L column and eluting Micafungin DIPEA with Methanol. The Micafungin containing fraction is further purified and transferred to a sodium salt of Micafungin, inter alia by ion exchange chromatography using a regenerated ion exchange resin UBK510L. Micafungin sodium is eluted with aqueous Methanol. Acetone and ethyl acetate is finally used for precipitation of Micafungin sodium.

**[0006]** It is well known in the art that the diisopropylethylamine (DIPEA) salt of Micafungin is more stable than the sodium salt of Micafungin. Therefore, the prior art methods as the method disclosed above often make use of the DIPEA salt as an intermediate when preparing Micafungin sodium. This is e.g. described in the General Thesis, "Process Development of Micafungin, a Novel Lipopeptide Antifungal Agent" by Ohigashi et al. in Journal of Synthetic Organic Chemistry, Japan, vol. 64, No. 12, Dec. 2006. In Ohigashi *et al.,* a method is disclosed wherein impurities present in a DIPEA salt of Micafungin are removed by the use of resins. The DIPEA salt purified by the use of an alumina resin column is according to Ohigashi *et al.* then subjected to ion exchange chromatography for converting the DIPEA salt of Micafungin to a sodium salt of Micafungin.

**[0007]** It is clear that the prior art methods for the preparation of Micafungin sodium as mentioned above involves two consecutive chromatography steps, i.e. firstly the purification of Micafungin DIPEA on a Reverse Phase Chromatography (RPC) resin and secondly the transformation of the DIPEA salt to the sodium salt, i.e. a salt swap, on a ion-exchange resin. The use of two subsequent chromatography steps for the obtainment of the desired sodium salt of Micafungin is

labour-intensive and the providing of an improved method involving fewer processing steps, less chemicals and equipment would be beneficial both from an economical aspect as well as from an environmental and labour-saving point of view.

**[0008]** It is thus still a need for more efficient processes for the preparation of a purified Micafungin and derivates thereof, such as the sodium salt of Micafungin.

## Summary of the invention

**[0009]** The object of the present invention is to provide an improved process for the purification of a salt of Micafungin, such as Micafungin sodium.

**[0010]** As mentioned above, the prior art methods is inter alia attended with the disadvantage of two consecutive chromatography steps. The present invention is based on the surprising findings that the two steps (salt swap and purification) can be performed in the one and same operation. The process of the present invention thus involves several self-evident advantages, both of environmental and economical character, such as reduced consumption of chemicals, shorter process times and the use of less hardware requirements.

**[0011]** According to one embodiment of the invention, a method for producing a pharmaceutically acceptable Micafungin salt is provided wherein said process comprises the steps of:

a) applying a Micafungin starting material to a hydrophobic adsorbent resin support;

b) exposing the bound Micafungin to an aqueous solution of a dissolved pharmaceutically acceptable salt;

c) eluting the dissolved pharmaceutically acceptable salt of Micafungin with a solution comprising a water miscible organic solvent;

provided that at least one of

i) the starting material in step a)
ii) or the aqueous solution in step b)

comprises a water miscible organic solvent.

**[0012]** According to one aspect of the above embodiment, a method is provided which further comprises a step wherein the bound Micafungin of step b) is further exposed to an aqueous solution comprising a water miscible organic solvent one or more times for the removal of impurities.

**[0013]** According to another aspect of the above embodiment, a method is provided wherein the starting material in step a) comprises a water miscible organic solvent in the range of 0 - 30 % v/v.

**[0014]** According to another aspect of the above embodiment, a method is provided wherein the starting material in step a) comprises a water miscible organic solvent in the range of 5-15 % v/v.

**[0015]** According to another aspect of the above embodiment, a method is provided wherein the starting material in step a) comprises a water miscible organic solvent in the range of 10 % v/v.

**[0016]** According to another aspect of the above embodiment, a method is provided wherein the aqueous solution in step b) comprises a water miscible organic solvent in the range of 0 - 40 % v/v.

**[0017]** According to another aspect of the above embodiment, a method is provided wherein the aqueous solution in step b) comprises a water miscible organic solvent in the range of 20 % v/v.

**[0018]** According to another aspect of the above embodiment, a method is provided wherein the water miscible organic solvent is selected from the group consisting of branched or non-branched C1-C3 alcohols, C3-C6 ketones and polar aprotic organic solvents.

**[0019]** According to another aspect of the above embodiment, a method is provided wherein the water miscible organic solvent is selected from the group consisting of methanol, ethanol, propanol, isopropanol, acetone and acetonitrile.

**[0020]** According to another aspect of the above embodiment, a method is provided wherein the aqueous solution in step c) comprises at least 30% v/v acetonitrile as a water miscible organic solvent.

**[0021]** According to another aspect of the above embodiment, a method is provided wherein the water miscible organic solvent is methanol.

**[0022]** According to another aspect of the above embodiment, a method is provided wherein the aqueous solution in step c) comprises at least 70 % v/v methanol, such as at least 90 %v/v methanol, such as at least 95 % v/v methanol as a water miscible organic solvent.

**[0023]** According to another aspect of the above embodiment, a method is provided wherein the starting material in step a), the aqueous solution in step b) and the washing solution in step c) comprises a water miscible organic solvent.

**[0024]** According to another embodiment of the invention, a method for producing a pharmaceutically acceptable Micafungin salt is provided wherein said process comprises the steps of:

a) applying a Micafungin starting material to a hydrophobic adsorbent resin support;

b) exposing the bound Micafungin to an aqueous solution of a dissolved pharmaceutically acceptable salt;

c) optionally exposing the bound salt of Micafungin obtained in b) to an aqueous solution comprising a water miscible organic solvent one or more times for the removal of impurities;

d) eluting the dissolved pharmaceutically acceptable salt of Micafungin with a solution comprising a water miscible organic solvent;

provided that when step c) is not performed, then the Micafungin starting material and/or the aqueous solution in step b) comprises a water miscible organic solvent.

**[0025]** According to one embodiment, the Micafungin starting material or the aqueous solution in step b) of the present method comprises 2-30% v/v of a water miscible organic solvent.

**[0026]** According to one embodiment, the Micafungin starting material and the aqueous solution in step b) of the present method comprises 2-30% v/v of a water miscible organic solvent.

**[0027]** According to one embodiment, the aqueous solution in step c) of the present method comprises 30-50% v/v of a water miscible organic solvent.

**[0028]** According to another embodiment, the cation present in the Micafungin starting material of the present invention is DIPEA.

**[0029]** According to yet another embodiment, the resin used according to the present method is HP20SS.

**[0030]** According to another embodiment, the Micafungin starting material and the aqueous solution in step b) of the present method comprises a water miscible organic solvent.

**[0031]** According to yet another embodiment, the Micafungin starting material and the aqueous solution in step b) of the present method comprises Methanol.

**[0032]** According to yet another embodiment of the present method, the water miscible organic solvent present in the Micafungin starting material and/or the aqueous solution in step b) is Methanol.

**[0033]** According to yet another embodiment of the present method, the aqueous solution in step b) comprises Methanol.

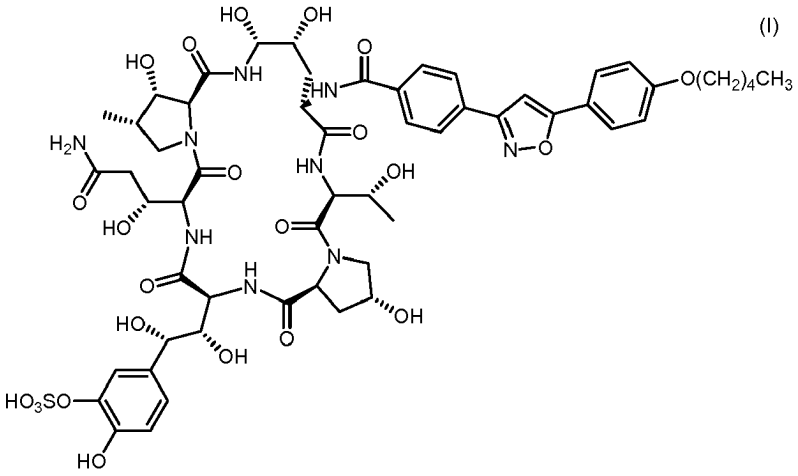
**[0034]** According to a final embodiment of the present invention, a method is provided wherein the pharmaceutically acceptable salt of Micafungin is the sodium salt, and the resin is HP20SS, and the Micafungin starting material comprises Micafungin DIPEA and 10% v/v Methanol, and the solution in step b) comprises 20% v/v Methanol and 80% v/v 3M NaCl, 0.1M $NaCH_3COO$ and the solution in step c) comprises 40% v/v Methanol and the solution in step d) comprises 90% v/v Methanol.

**Brief description of the figures**

**[0035]** Figure 1-9 illustrate the elution profile of DIPEA and Micafungin sodium from HP20SS according to the corresponding Examples 1-9. The pertaining tables show the level of DIPEA in eluted fractions (CV = column volumes).

**Detailed description**

**[0036]** According to the present invention, Micafungin is any compound comprising the structure



(I)

or salts thereof. The expression "salts thereof" is meant to embrace any salts of Micafungin that may be useful for the purpose of preparation and/or purification of Micafungin or any pharmaceutically acceptable salts of Micafungin useful as an active ingredient in a medicinal formulation. In this respect, a non-limiting list of salts of Micafungin is the sodium salt, the potassium salt, the diisopropylethylamine (DIPEA) salt, etc.

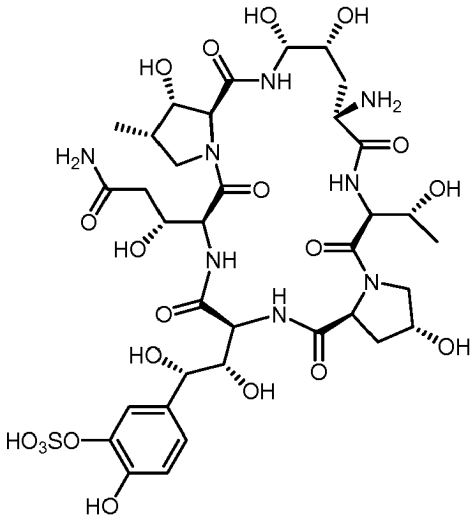
**[0037]** Micafungin is a semisynthetic derivative of FR901379, a fermentation product obtained from *Coleophoma empetri* F-11899 (deposited as FERM BP-2635).

**[0038]** Micafungin may be prepared by deacylation of the fermentation product FR901379, obtaining a compound named the Micafungin peptide core herein, and subsequent coupling of the Micafungin side chain, 4-[5-(4-pentyloxyphenyl)isoxazole-3-yl]benzoic acid, to said peptide core. The Registry Number of the said side chain assigned by Chemical abstracts is 179162-55-1. It is also known by the name FR195752.

**[0039]** Deacylation of FR901379 can be performed by enzymes produced by certain microorganisms of the Actinoplanaceae, for example, *Actinoplanes utahensis* IF0-13244, *Actinoplanes utahensis* ATCC 12301, Actinoplanes missourienses NRRL 12053. The deacylated peptide core is represented here as formula II. The sodium salt of the deacylated peptide core was named FR133303 (see EP462531).

**[0040]** Reacylation of the peptide core represented by formula II can be performed as disclosed in US 7,199,248.

**[0041]** The Micafungin peptide core is represented by the formula II.



II

**[0042]** According to the present invention, a method for the preparation of a purified salt of Micafungin is provided. According to one embodiment of the invention, the salt of Micafungin obtained according to the present invention is the sodium salt of Micafungin. It is to be understood that also other pharmaceutically acceptable salts of Micafungin may

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