IN THE HIGH COURT OF JUSTICE **HP-2014-000037**
CHANCERY DIVISION
PATENTS COURT

BETWEEN:

HOSPIRA UK LIMITED

Claimant

-v-

CUBIST PHARMACEUTICALS INC

Defendant

AMENDED CLAIMS AS REFERRED TO IN STATEMENT OF REASONS IN SUPPORT OF APPLICATION TO CONDITIONALLY AMEND EUROPEAN PATENT (UK) NO. 1 586 580

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1. A method to purify daptomycin, comprising the steps of:

a) fermenting *Streptomyces roseosporus* with a feed of n-decanoic acid to produce daptomycin in a fermentation broth;

b) clarifying the fermentation broth to obtain a clarified solution;

c) subjecting the clarified solution to anion exchange chromatography to obtain an enriched daptomycin preparation;

d) adjusting the enriched daptomycin preparation to a pH of 2.5 to 5.0 using an acid to form micelles;

e) contacting the enriched daptomycin preparation with a HP-20ss resin to obtain a semi-purified daptomycin preparation wherein during the hydrophobic interaction chromatography of step e) daptomycin micelles dissociate at pH 6.0-7.5 into daptomycin monomers by elution with 30-40% isopropyl alcohol at pH 3.5 to 6.5; and

f) subjecting the semi-purified daptomycin preparation to anion exchange chromatography to obtain purified daptomycin;

and wherein said method further comprises the step of separating the enriched daptomycin obtained in step d) from low molecular weight material.

2. The method according to claim 1, wherein the feed of n-decanoic acid in step a) is regulated to achieve a residual concentration of n-decanoic acid of no more than 50 parts per million (ppm) during fermentation.

3. The method according to claim 1, wherein said clarifying in step b) comprises filtration or centrifugation and depth filtration.

4. The method according to claim 1, wherein the anion exchange chromatography in step c) is performed on FP-DA 13 resin.

5. The method according to claim 1, wherein the anion exchange chromatography in step f) is performed on FP-DA 13 resin.

6. The method according to claim 1, wherein the anion exchange chromatography in step f) is used to reduce the level of solvent from step b).

7. The method according to claim 1, wherein the method is performed via continuous flow chromatography.

8. The method according to claim 1, further comprising the step of filtering or concentrating daptomycin.

9. The method according to claim 1, further comprising the step of depyrogenating daptomycin using ultrafiltration.

10. The method according to claim 9, wherein said depyrogenating comprises the steps of:

i) providing a daptomycin solution under conditions in which the daptomycin is in a monomeric and nonmicellar state;

ii) filtering the daptomycin solution under conditions in which the daptomycin passes through the filter but pyrogens do not pass through the filter;

iii) subjecting the daptomycin solution to conditions forming a daptomycin aggregate;

iv) filtering the daptomycin aggregate under conditions in which the daptomycin aggregate is retained on the filter; and

v) collecting the daptomycin aggregate.

11. The method according to claim 9, further comprising the step of lyophilizing daptomycin.

12. The method according to claim 1, wherein said clarifying in step b) comprises microfiltration or centrifugation.

13. The method according to claim 1, further comprising the step of filtering and concentrating daptomycin.

14. The method according to claim 1, ~~further comprising~~wherein ~~the step of separating~~ the enriched daptomycin obtained in step d) is separated from low molecular weight material by ultrafiltration.

15. The method according to claim 13, further comprising the step of depyrogenating the daptomycin.