



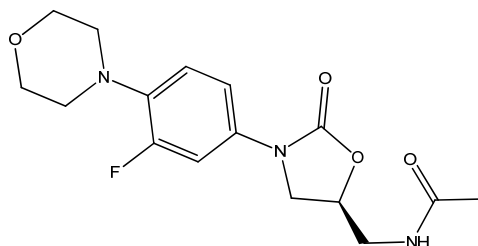
# Mikromol<sup>TM</sup>

## Linezolid impurities: An overview

QUALITY | ISO 9001 | ISO/IEC 17025  
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## Linezolid Impurities – An overview



Linezolid (MM3300.00)

### Introduction:

Linezolid, also known as Zyvox or Zyvoxid, is a synthetic antibiotic which belongs to the relatively new class of oxazolidinones. Linezolid is effective for the treatment of nosocomial infections with gram positive bacteria. Linezolid acts by inhibiting the initiation of bacterial protein synthesis, a mechanism of action which is distinct from that of any other commercially available<sup>1</sup> antibiotic. Linezolid appeared to be an effective option when compared to Vancomycin. Linezolid, administered orally or as an injection,<sup>2</sup> was launched in May 2000<sup>3</sup>.

A representative synthesis route of Linezolid is shown in **scheme 1**<sup>4</sup>.

Starting materials are Morpholine [1] and 1,2-Difluoro-4-nitrobenzene [2] from which carbamate MM3300.18 is formed in three steps. The carbamate is an important **process related impurity** as it is an intermediate in all synthetic routes.

Oxazolidinone MM3300.05 is also an important intermediate of Linezolid, and therefore likely present in the drug substance, which is formed by reaction of MM3300.18 with (R)-glycidyl butyrate<sup>5</sup>. Residuals of (S)-glycidyl butyrate will lead to formation of the enantiomeric impurity (R)-Linezolid MM3300.07.

The reaction of oxazolidinone MM3300.05 with mesyl chloride provides the mesyl derivative MM3300.06 (USP related compound D). During this reaction, the hydroxy group might be substituted by chlorine, leading to the formation of impurity MM3300.08 at trace levels. On the other hand, a remainder of the key intermediate MM3300.05 will not react with mesyl chloride. These remaining traces can subsequently also undergo acetylation towards the end of the synthesis route and might form impurity MM3300.13<sup>3</sup>.

The azide intermediate MM3300.02 (USP related compound A) is formed in the reaction of the mesyl derivative with sodium azide with the aim of introducing a nitrogen moiety. The azide is subsequently reduced to the free amine MM3300.01 (USP related compound C). However, in the course of this reduction the Desfluorolinezolid MM3300.10 can be formed.

<sup>1</sup> Raju T.S., Kuttly O.V., Ganesh V., Swami P.Y., *Journal of Pharmaceutical Analysis*, **2012**; 2(4): 272-278

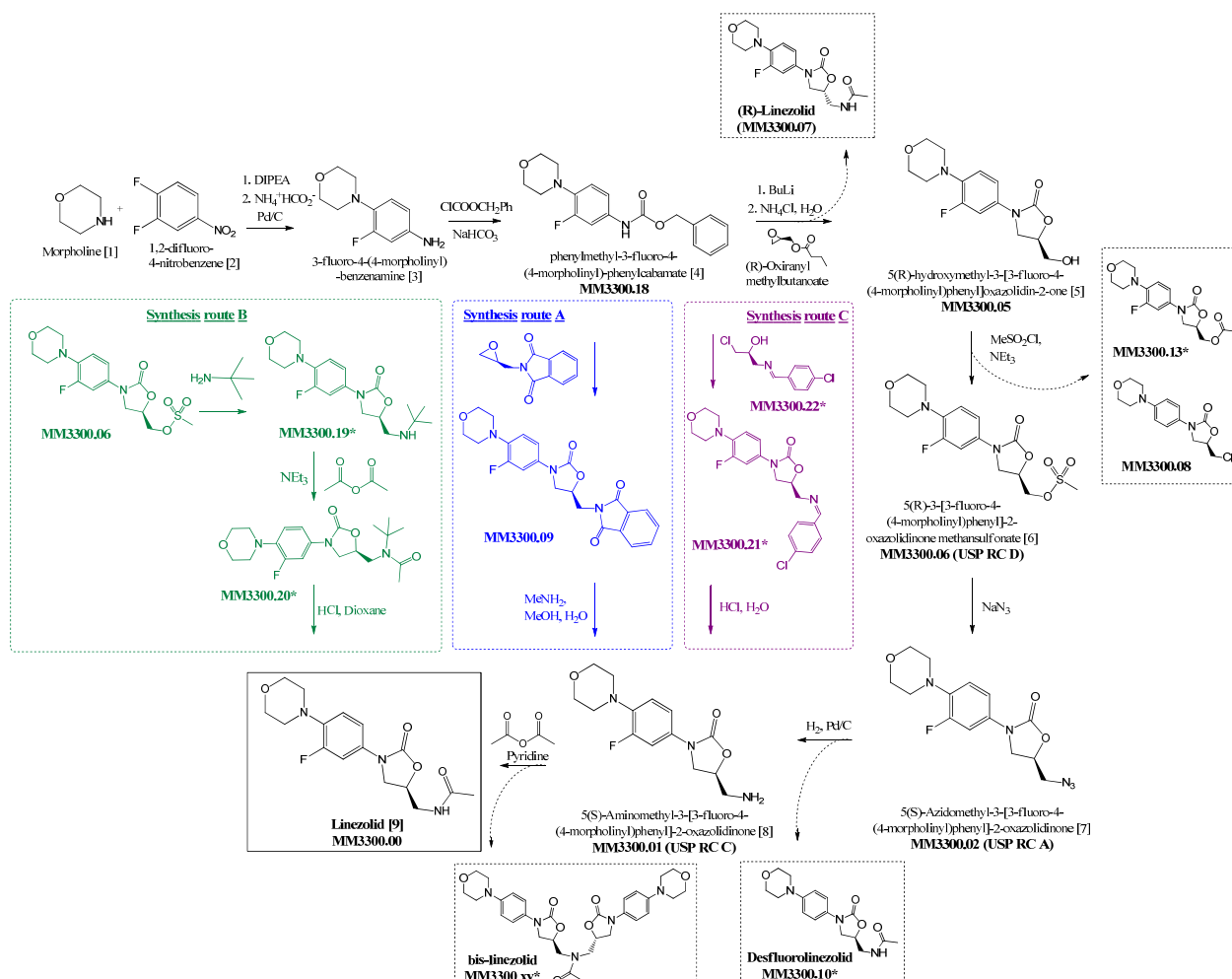
<sup>2</sup> Koltai T. et al, *Crystalline Forms of Linezolid Intermediate*, Nov.16, **2006**, US2006/0258655 A1

<sup>3</sup> Krishna Reddy K.V.S.R., Mahender Rao S. et al., *Journal of Pharmaceutical and Biomedical Analysis*, 30 (**2002**): 635-642

<sup>4</sup> Kleemann et al. *Pharmaceutical Substances Online Database 4.4*, **2018**

<sup>5</sup> Kashyap R. Wadekar, P.Ravi, et al., *Pharmaceutical Technology*, **2012**, Volume 36 (3): 58-72

The final acetylation of the amine yields the API Linezolid MM3300.00. During this step amounts of free amine still present can react in situ with the newly formed API MM3300.00 by dimerization which leads, upon acetylation, to the dimeric structure of bis-linezolid (MM3300.xy)<sup>2</sup>.



Scheme 1 Synthesis Route of Linezolid (\* = available on request)

Impurity MM3300.09 is an intermediate of the alternative **synthesis route A** and derived from the reaction of carbamate MM3300.18 and (S)-glycidyl phthalimide<sup>6</sup>. This synthesis route bypasses the formation of the azide MM3300.02 (USP related compound A), which is difficult to handle within industrial processes.

MM3300.19 is an important intermediate in the alternative **synthesis route B** and is formed by substitution of the mesylate group of derivative MM3300.06 (USP related

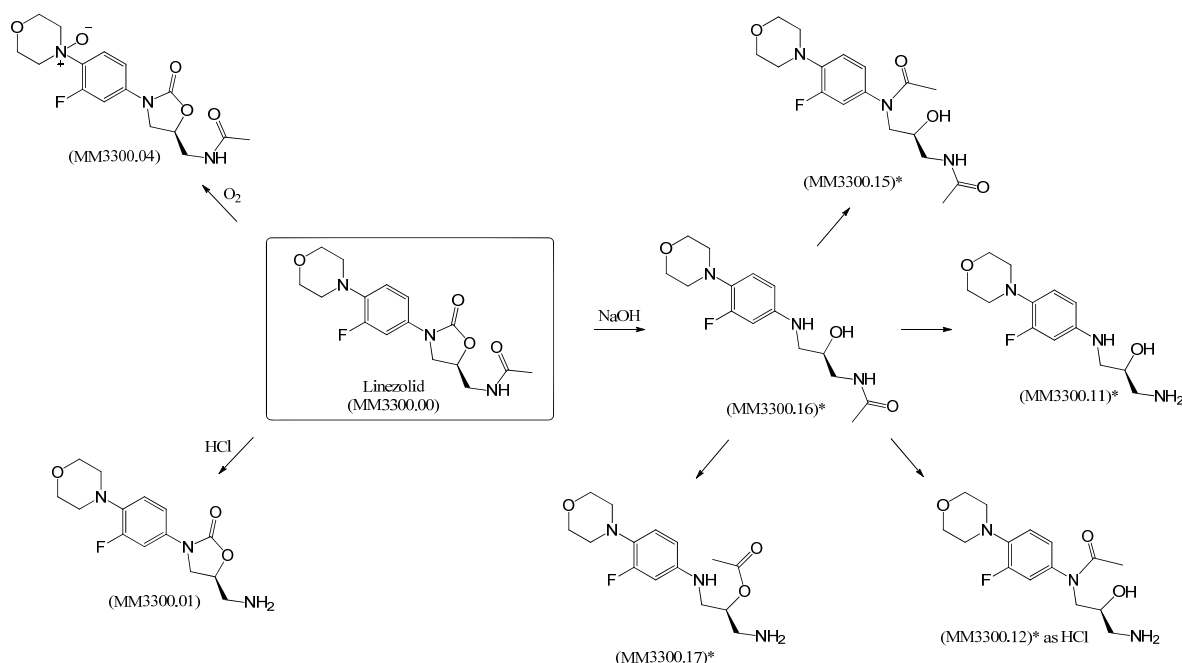
<sup>6</sup> Desy Reddy et al., *Process for the Preparation of Linezolid*, May 9, 2017, US009643939 B1

compound D) with tert-butylamine. Subsequent acetylation forms MM3300.20 and desalkylation yields the desired Linezolid<sup>7</sup>.

It is also possible to build Linezolid from imine MM3300.22, **synthesis route C**, which is coupled with carbamate MM3300.18 to give the (S)-oxazolidinone imine MM3300.21. This is then hydrolyzed to amine MM3300.01 (USP related compound C)<sup>8</sup>. As imines need to be built up carefully because they are sensitive to water, this process might lead to further impurities and by products<sup>6</sup>.

In contrast to **process impurities**, which usually remain at constant levels within the drug substance, **degradation products** can be formed in the final drug product and are therefore likely to enrich. The most important degradation products of linezolid are given in the **scheme 2**.

Stress conditions which need to be taken into account for validations are given in ICH Q3B(R2) and comprise light, heat, humidity, acid/base hydrolysis and oxidation<sup>9</sup>.



*Scheme 2 Impurities of degradation of Linezolid*

Degradation studies for Linezolid show lability to acids, alkalis and oxidation, while the API seems to be relatively stable to water, thermal and photo degradation<sup>1</sup>. Oxidative stress leads to the amine oxide MM3300.04, which is therefore an impurity very likely present in drug products.

<sup>7</sup> Union Quimico Farmaceutica, S.A. (UQUIFA) - EP2163547, **2010**, A1

<sup>8</sup> Process for Preparing Linezolid, October 18, **2007**, WO2007116284 A

<sup>9</sup> ICH (2006) , *Impurities in New Drug Products Q3B(R2)*, 4th version, 02 June, **2006**

Alkaline hydrolysis products of linezolid differ from the acid hydrolysis product. While alkaline hydrolysis leads to ring-opening and then to hydrolysate MM3300.16/17/11/12, acid hydrolysis primarily leads to cleavage of the acetyl moiety and thus to MM3300.01<sup>10</sup>.

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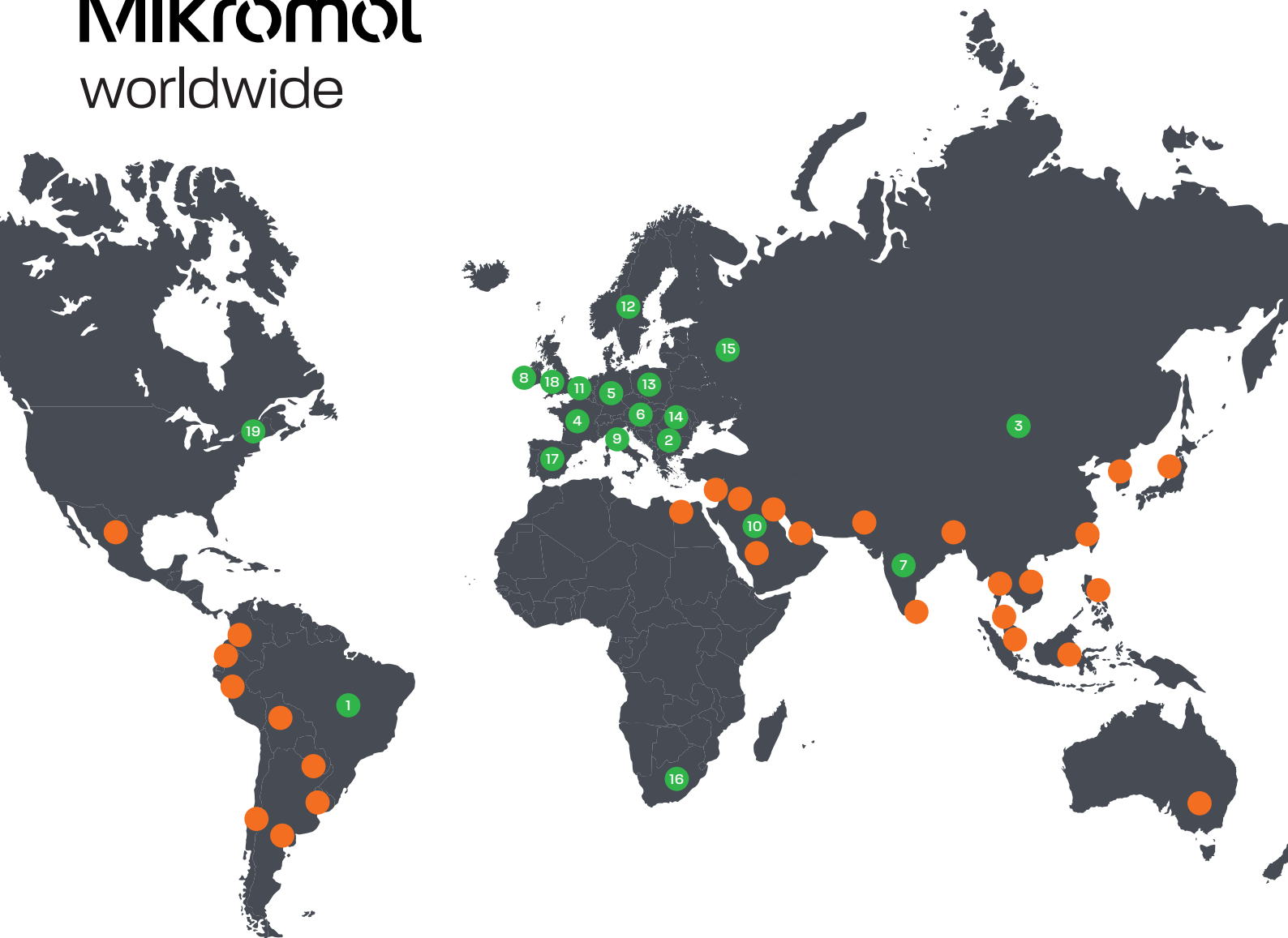
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<sup>10</sup> CAO, Hai-yan; ZHU, Yi-juan; SANG, Zhi-pei; CHAO, Ruo-bing, *Chinese Journal of Pharmaceutical Analysis*, Volume 33, Number 8, **2013**, pp. 1342-1347(6)



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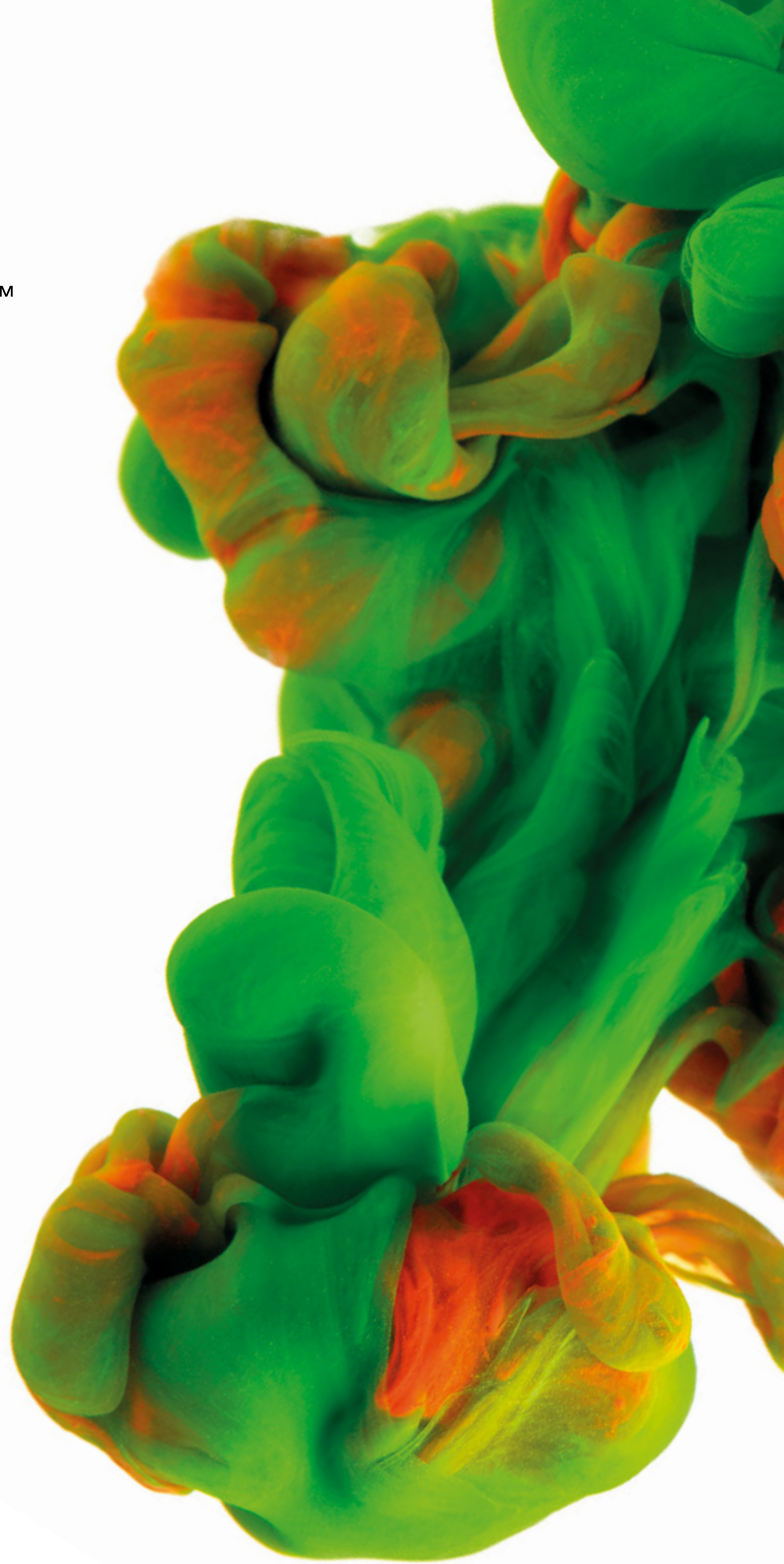
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# Mikromol™



Acetylation of 2,6-Dimethylaniline during the synthesis of Lidocaine.  
(Impurity N-(2,6-Dimethylphenyl) acetamide, MM0102.08)

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