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(54) **Title:** ISOLATED BIS-LINEZOLID, PREPARATION THEREOF, AND ITS USE AS A REFERENCE STANDARD

(57) **Abstract:** The present invention provides an isolated linezolid (1) impurity, bis-linezolid (4), preparation thereof and its use as a reference standard.

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ISOLATED BIS-LINEZOLID, PREPARATION THEREOF, AND ITS USE AS A REFERENCE STANDARD

CROSS-REFERENCE TO RELATED APPLICATIONS

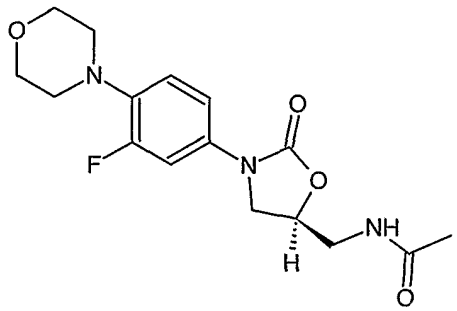
This application claims the benefit of provisional applications Serial Numbers 60/656,778, filed February 24, 2005, 60/656,646, filed February 24, 2005, as well as 60/690,822, filed June 14, 2005 which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to isolated bis-linezolid, methods of preparing and detecting bis-linezolid, and methods of using bis-linezolid as a reference standard.

BACKGROUND OF THE INVENTION

Linezolid [(S)-N-[[3-(3-Fluoro-4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl] acetamide] is an antimicrobial agent. Linezolid is an oxazolidinone, having the empirical formula $C_{16}H_{20}FN_3O_4$ and the following structure (1):



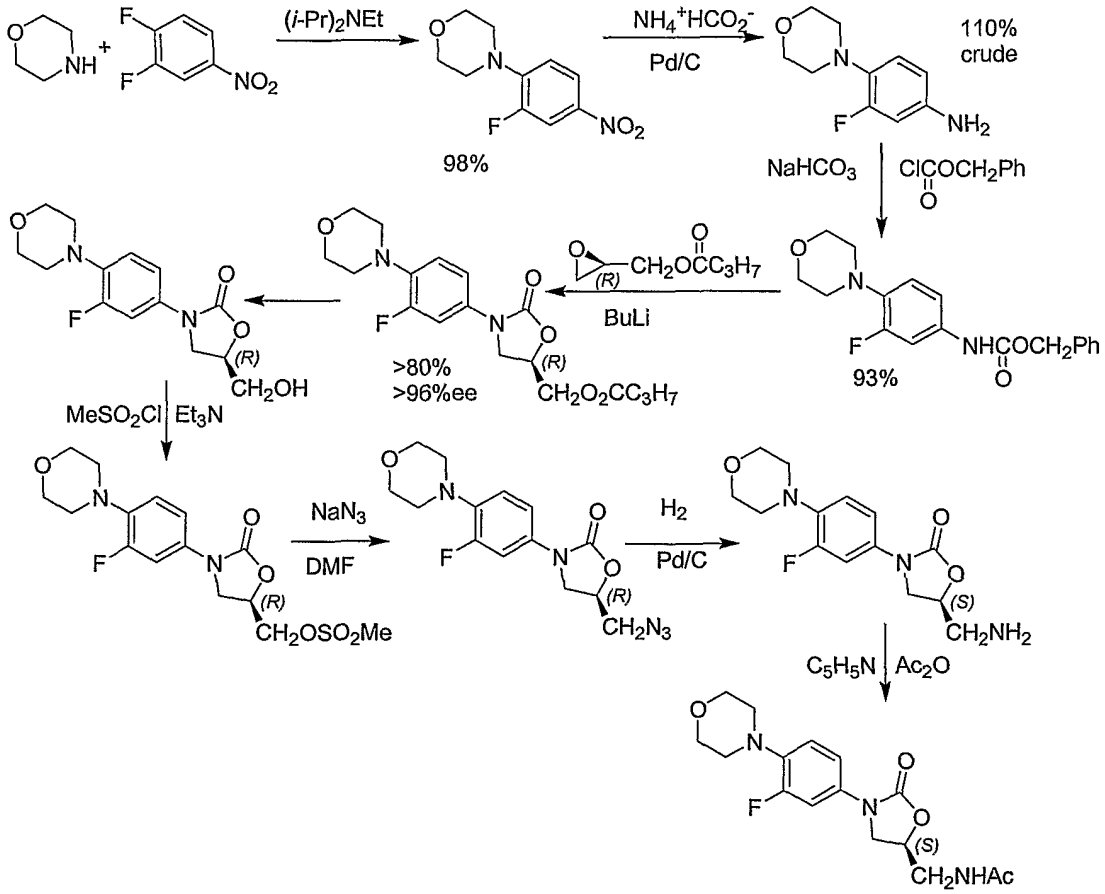
(1)

Linezolid (1) is described in The Merck Index (13th edition, Monograph number: 05526, CAS Registry Number: 165800-03-3) as white crystals, with a melting point of 181.5-182.5°. Linezolid (1), as well as a process for its preparation, is disclosed in U.S. Patent No. 5,688,792 (Example 5), European Patent No. 717738, Israeli Patent No. 110,802, Canadian Patent No. 2,168,560, and International Patent Publication WO 95/07271.

Linezolid (1) is marketed in the United States by Pfizer, Inc. as an injection, tablets, and oral suspension under the name ZYVOX®. Its main indications are

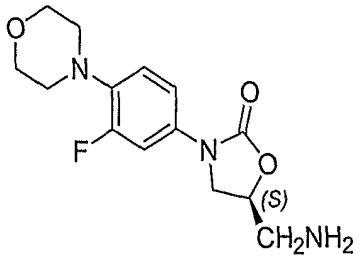
nosocomial pneumonia, skin and skin-structure infections, and vancomycin-resistant *Enterococcus faecium* infections.

U.S. Patent No. 5,688,792 claims linezolid (1) and its use for the treatment of microbial infections. This patent also discloses, but does not claim, the following method of preparation:



This method of preparation was also disclosed in Bricker, et al., J. Med. Chem., 39 673 – 679 (1996), where it was stated that the above route avoids the use of phosgene to make the carbamate precursor of the oxazolidinone ring. The authors also disclose that the use of NaN_3 can be avoided by using potassium phthalimide, followed by deblocking of the phthalimide with aqueous methyl amine.

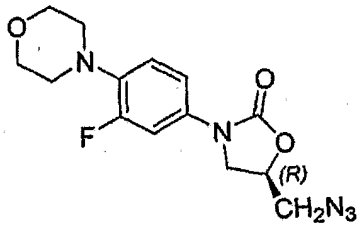
In the above-described synthesis, the intermediate amine, S-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2)



(2)

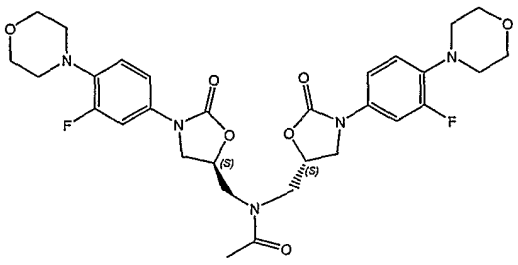
is reacted without isolation with acetic anhydride as an oily product, or in solution, to produce the acetamide, linezolid (1). This is followed by procedures for isolating the linezolid (1) such as those described in U.S. Patent No. 5,688,792, at col. 15, ll. 22-28 (chromatography and separation of the desired fraction, followed by evaporation and trituration of the product to obtain pure linezolid (1)).

In the above-described syntheses, the intermediate azide R-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl azide (3)



(3)

is reduced to its corresponding amine, S-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2) in the solvent ethyl acetate by hydrogenation using hydrogen gas and a palladium/carbon catalyst. These reaction conditions lead to the production of an undesirable level of reaction by-products, and, following the acetylation of the intermediate amine (2) to linezolid (1), to undesirably high levels of bis-linezolid (4)



(4)

It is well known in the art that, for human administration, safety considerations require the establishment, by national and international regulatory authorities, of very low limits for identified, but toxicologically uncharacterized impurities, before an active pharmaceutical ingredient (API) product is commercialized. Typically, these limits are less than about 0.15 percent by weight of each impurity. Limits for unidentified and/or uncharacterized impurities are obviously lower, typically, less than 0.1 percent by weight. Therefore, in the manufacture of APIs, the purity of the products, such as linezolid (1), is required before commercialization, as is the purity of the active agent in the manufacture of formulated pharmaceuticals.

It is also known in the art that impurities in an API may arise from degradation of the API itself, which is related to the stability of the pure API during storage, and from the manufacturing process, including the chemical synthesis. Process impurities include unreacted starting materials, chemical derivatives of impurities contained in starting materials, synthetic by-products, and degradation products.

In addition to stability, which is a factor in the shelf life of the API, the purity of the API produced in the commercial manufacturing process is clearly a necessary condition for commercialization. Impurities introduced during commercial manufacturing processes must be limited to very small amounts, and are preferably substantially absent. For example, the ICH Q7A guidance for API manufacturers requires that process impurities be maintained below set limits by specifying the quality of raw materials, controlling process parameters, such as temperature, pressure, time, and stoichiometric ratios, and including purification steps, such as crystallization, distillation, and liquid-liquid extraction, in the manufacturing process.

The product mixture of a reaction is rarely a single compound with sufficient purity to comply with pharmaceutical standards. Side products and by-products of the reaction and adjunct reagents used in the reaction will, in most cases, also be present in the product mixture. At certain stages during processing of an API, such as linezolid (1), the API must be analyzed for purity, typically, by HPLC or GC analysis, to determine if it is suitable for continued processing and, ultimately, for use in a pharmaceutical product. The API need not be absolutely pure, as absolute purity is a theoretical ideal that is typically unattainable. Rather, purity standards are set with the intention of ensuring that an API is as free of impurities as possible, and, thus, is

as safe as possible for clinical use. As discussed above, in the United States, the Food and Drug Administration guidelines recommend that the amounts of some impurities be limited to less than 0.1 percent.

Generally, side products, by-products, and adjunct reagents (collectively "impurities") are identified spectroscopically and/or with another physical method, and then associated with a peak position, such as that in a chromatogram, or a spot on a TLC plate. (Strobel, H.A.; Heineman, W.R., Chemical Instrumentation: A Systematic Approach, 3rd dd. (Wiley & Sons: New York 1989) (hereinafter "Strobel"), page 953). Thereafter, the impurity can be identified, e.g., by its relative position in the chromatogram, where the position in a chromatogram is conventionally measured in minutes between injection of the sample on the column and elution of the particular component through the detector. The relative position in the chromatogram is known as the "retention time." The retention time varies daily, or even over the course of a day, based upon the condition of the instrumentation, as well as many other factors. To mitigate the effects such variations have upon accurate identification of an impurity, practitioners use the "relative retention time" ("RRT") to identify impurities. (Strobel, page 922). The RRT of an impurity is its retention time divided by the retention time of a reference marker. In theory, linezolid (1) itself could be used as the reference marker, but as a practical matter it is present in such a large proportion in the mixture that it can saturate the column, leading to irreproducible retention times, as the maximum of the peak can wander (Strobel, Fig. 24.8(b), page 879, illustrates an asymmetric peak observed when a column is overloaded). Thus, it may be advantageous to select a compound other than the API that is added to, or present in, the mixture in an amount sufficiently large to be detectable and sufficiently low as not to saturate the column, and to use that compound as the reference marker.

Those skilled in the art of drug manufacturing research and development understand that a compound in a relatively pure state can be used as a "reference standard." A reference standard is similar to a reference marker, which is used for qualitative analysis only, but is used to quantify the amount of the compound of the reference standard in an unknown mixture as well. A reference standard is an "external standard" when a solution of a known concentration of the reference standard and an unknown mixture are analyzed using the same technique. (Strobel

page 924; Snyder, L.R.; Kirkland, J.J. Introduction to Modern Liquid Chromatography, 2nd ed. (John Wiley & Sons: New York 1979) (hereinafter "Snyder"), page 549). The amount of the compound in the mixture can be determined by comparing the magnitude of the detector response to the reference standard and to the compound in the mixture. See also U.S. Patent No. 6,333,198, incorporated herein by reference.

The reference standard can also be used to quantify the amount of another compound in the mixture if a "response factor," which compensates for differences in the sensitivity of the detector to the two compounds, has been predetermined. (Strobel page 894). For this purpose, the reference standard is added directly to the mixture, and is known as an "internal standard." (Strobel page 925, Snyder page 552).

The reference standard can even be used as an internal standard when, without the addition of the reference standard, an unknown mixture contains a detectable amount of the reference standard compound using a technique known as "standard addition." In a "standard addition," at least two samples are prepared by adding known and differing amounts of the internal standard. (Strobel pp. 391-393, Snyder pp. 571, 572). The proportion of the detector response due to the reference standard present in the mixture without the addition can be determined by plotting the detector response against the amount of the reference standard added to each of the samples, and extrapolating the plot to zero. (See, e.g., Strobel, Fig. 11.4 page 392).

There is a need to isolate the bis-linezolid (4) impurity. This impurity may also be used as a reference marker and/or standard.

SUMMARY OF THE INVENTION

In one embodiment, the invention is directed to isolated bis-linezolid (4), characterized by data selected from: a ^{1}H NMR spectrum (400MHz, D_2O+TFA) δ (ppm):2.08 (s), 3.63 (m), 3.83 (t), 4.02 (s), 4.14 (m), 4.94 (m), 7.23 (d), 7.58 (m); a ^{13}C NMR spectrum (400MHz, $CDCl_3$) δ (ppm):16.6 (q), 43-50 (s), 50.4 (s), 50.9 (s), 68.3 (s), 103.6, 104.0 (d), 111.1 (s) , 118.7 (s), 130-140 (s) , 149-153 (d), 171.4 (s); FAB+m/z (MH^+): 616; and an IR spectrum at 1519, 1572, 1644, 1743, 2825, 2858, 2891, 2965 cm^{-1}

In another embodiment, the invention is directed to the preparation of bis-linezolid (4) by a method comprising converting the azide intermediate to linezolid (1) enriched in bis-linezolid (4), and isolating the bis-linezolid (4) impurity.

In yet another embodiment, the invention is directed to a method of using bis-linezolid (4) as a reference standard to analytically quantify the purity of linezolid (1), and to set specific limits to the amount of bis-linezolid (4) and other impurities during the synthesis of linezolid (1).

In a further embodiment, the invention is directed to analytical methods for testing the impurity profile of linezolid (1). These methods are also suitable for analyzing and assaying linezolid (1) and bis-linezolid (4).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the ^{1}H-NMR spectrum of bis-linezolid (4)

Figure 2 shows the ^{13}C-NMR spectrum of bis-linezolid (4)

Figure 3 shows the IR spectrum of bis-linezolid (4)

Detailed Description of the Invention

As used herein, the term "reference standard" refers to a compound that may be used both for quantitative and qualitative analysis of an active pharmaceutical ingredient. For example, the HPLC retention time of the reference standard compound allows a relative retention time with respect to the active pharmaceutical ingredient to be determined, thus making qualitative analysis possible. Furthermore, the concentration of the compound in solution before injection into an HPLC column allows the areas under the HPLC peaks to be compared, thus making quantitative analysis possible.

A "reference marker" is used in qualitative analysis to identify components of a mixture based upon their position, e.g., in a chromatogram or on a Thin Layer Chromatography (TLC) plate (Strobel pages 921, 922, 953). For this purpose, the compound does not necessarily have to be added to the mixture if it is present in the mixture. A "reference marker" is used only for qualitative analysis, while a reference standard may be used for quantitative or qualitative analysis, or both. Hence, a reference marker is a subset of a reference standard, and is included within the definition of a reference standard.

Prior art methods of preparing linezolid (1) that utilize the intermediate amine (2) have relied on purification steps applied to the linezolid (1) final product. This was necessary because the reduction of the intermediate azide (3) to the intermediate amine (2) in those methods did not result in complete conversion of the intermediate azide (3) to the intermediate amine (2) but instead yielded significant amounts of by-products. Those methods yielded undesirably high levels of contaminating by-products, e.g., bis-linezolid (4), following acetylation of the intermediate amine (2) to linezolid (1).

This can be seen in Example 2. Example 2 is a comparative example in which the intermediate azide (3) is converted to the intermediate amine (2) by hydrogenation in the solvent ethyl acetate, as in U.S. Patent No. 5,688,792. Following acetylation of the intermediate amine (2), Example 2 produced a final product that contained 3.2% bis-linezolid (4). Similarly, when linezolid (1) was prepared directly from the intermediate amine (2) that had been prepared as in Example 4, a high molecular weight compound was obtained as main impurity (~10%). It was isolated and identified by MS and NMR as bis-linezolid (4).

The present invention provides isolated bis-linezolid (4).

The bis-linezolid (4) is isolated in at least 98% purity by weight with respect to other compounds, including linezolid (1). Thus, the isolated bis-linezolid (4) contains less than about 5%, preferably less than about 2%, and even more preferably less than about 1%, by weight of linezolid (1).

The isolated bis-linezolid (4) of the present invention is characterized by data selected from: a ^{1}H NMR spectrum (400MHz, D_2O+TFA) δ (ppm):2.08 (s), 3.63 (m), 3.83 (t), 4.02 (s), 4.14 (m), 4.94 (m), 7.23 (d), 7.58 (m); a ^{13}C NMR spectrum (400MHz, $CDCl_3$) δ (ppm):16.6 (q), 43-50 (s), 50.4 (s), 50.9 (s), 68.3 (s), 103.6, 104.0 (d), 111.1 (s) , 118.7 (s), 130-140 (s) , 149-153 (d), 171.4 (s); FAB+m/z (MH^+): 616; and an IR spectrum at 1519, 1572, 1644, 1743, 2825, 2858, 2891, 2965 cm^{-1}.

The isolated bis-linezolid (4) is characterized by ^{1}H NMR, substantially as depicted in Figure 1. The isolated bis-linezolid (4) is characterized by ^{13}C NMR, substantially as depicted in Figure 2. The isolated bis-linezolid (4) is characterized by an IR spectrum, substantially as depicted in Figure 3.

The present invention also provides a method for the preparation and isolation of bis-linezolid (4). This method comprises:

a) combining R-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl methyl azide (3) with an organic solvent and hydrogen gas in the presence of a catalyst to obtain crude (S)-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2);

b) combining the crude product of step a) with an organic solvent to obtain a solution;

c) adding acetic anhydride to the solution and maintaining the reaction mixture for at least 12 hours to obtain crude linezolid (1);

d) combining the crude linezolid (1) of step c) with an organic solvent to obtain a mixture;

e) heating the mixture to reflux; and

f) recovering bis-linezolid (4) from the mixture of step e).

Preferably, the organic solvent in steps a), b) and d) is a C_{1-4} alkyl ester or an aromatic hydrocarbon. Preferably, the C_{1-4} alkyl ester is selected from the group consisting of methyl acetate, ethyl acetate, butyl acetate and isobutyl acetate. Preferably, the aromatic hydrocarbon is toluene. Most preferably, the organic solvent in steps a), b) and d) is ethyl acetate. Preferably the catalyst in step a) is Pd/C.

Optionally, an amine, such as pyridine or a tertiary amine, may be added to the reaction mixture prior to step c). The most preferred amine is triethyl amine.

The isolated bis-linezolid (4) of the present invention is useful as a reference standard for linezolid (1). Isolated bis-linezolid (4) may be used to quantify impurities in a linezolid (1) sample. A sample of linezolid (1) may be spiked with a known amount of purified bis-linezolid (4) and analyzed by HPLC to identify peaks associated with the impurities. Impurity levels can be determined by comparing the area percent by HPLC of the impurities with the area percent of the bis-linezolid (4) injected in a known amount within linearity ranges. A control sample without added bis-linezolid (4) can be run to determine the amount of the bis-linezolid (4) peak associated with the added amount of bis-linezolid (4). Alternatively, at least two samples can be prepared by adding known and differing amounts of bis-linezolid (4) to the samples. The proportion of the HPLC peak due to the bis-linezolid (4) present in the mixture without the addition of bis-linezolid (4) can be determined by plotting

the HPLC peak area against the amount of bis-linezolid (4) added to each of the samples, and extrapolating the plot to zero.

The present invention comprises the use of bis-linezolid (4) as a reference standard.

The present invention provides a method of determining the amount of an impurity in a sample of linezolid (1) comprising:

a) measuring by HPLC the area under a peak corresponding to bis-linezolid (4) in a reference standard comprising a known amount of bis-linezolid (4);
b) measuring by HPLC the area under a peak corresponding to bis-linezolid (4) in a sample comprising linezolid (1) and bis-linezolid (4); and
c) determining the amount of bis-linezolid (4) in the sample by comparing the area of step (a) to the area of step (b).

In another embodiment, the present invention provides a method of determining the amount of bis-linezolid (4) in a sample of linezolid (1) comprising:

a) subjecting a reference standard solution of bis-linezolid (4) comprising a known amount of bis-linezolid (4) to HPLC for at least 45 minutes and measuring by HPLC the area under a peak corresponding to bis-linezolid (4);
b) subjecting a sample solution comprising linezolid (1) and bis-linezolid (4) to HPLC for at least 45 minutes and measuring by HPLC the area under a peak corresponding to bis-linezolid (4); and
c) determining the amount of bis-linezolid (4) in the sample by comparing the area of step (a) to the area of step (b).

The present invention discloses that bis-linezolid (4) has an HPLC retention time that is unusually long with respect to linezolid (1). Bis-linezolid (4) is detected at an RRT (relative retention time) of about 3.8 relative to linezolid (1).

The present invention also provides a method for detecting bis-linezolid (4) comprising:

a) providing a preparation of crude linezolid (1) known to contain or suspected of containing bis-linezolid (4);
b) subjecting the preparation of step a) to HPLC;

c) determining whether any material has an RRT of about 3.8 with respect to linezolid (1);

wherein the presence of material with an RRT of about 3.8 with respect to linezolid (1) indicates that the preparation contained bis-linezolid (4).

Under certain conditions, step b) includes carrying out the HPLC for at least about 48 minutes.

The present invention further provides an HPLC method for assaying linezolid (1) comprising the steps:

a) combining a linezolid (1) sample with a mixture of acetonitrile/water diluent having a ratio of about 1:1 to obtain a solution;

b) injecting the solution of step a) into a Hypersil Gold 150x4.6 (or similar) column;

c) eluting the sample from the column at about 5 times the elution time of linezolid (1) using a mixture of about 0.01M K_2HPO_4 : MeOH as an eluent at a gradient of about 80:20 to about 50:50; and

d) measuring the bis-linezolid (4) content in the relevant sample with a UV detector.

Preferably, the bis-linezolid (4) at step d) is measured at a wavelength of 254nm. Preferably, the eluting time in step c) is about 45 minutes.

Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the composition and methods of use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

EXAMPLES

HPLC method

Column Hypersil Gold 150x4.6, 5μ

Detection limit: 0.1%

Eluents: 0.01M K_2HPO_4: MeOH A: 80:20 B: 50:50

Table 1

Time	A	B	Flow
0	100	0	1.5
15	57	43	2
25	35	65	2

Example 1 - isolation of bis-linezolid (4)

Step A: preparation of (S)-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2) enriched with impurities

Into a 1L stainless steel pressure reactor, 10 g R-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl methyl azide (3) and 455 ml ethyl acetate were charged, followed by 2.5 g of 10% Pd/C. After the addition, the system was blanketed twice with nitrogen. Hydrogen was bubbled into the reaction until a pressure of 1.5 atm. was reached. The reaction was completed after 6 hrs. The reaction mixture was filtered. The filtrate was evaporated to dryness to obtain 9.2 g of crude (S)-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2)

Step B: preparation of crude linezolid (1), enriched with bis-linezolid (4)

Into a 500 ml three-necked reactor equipped, 7.7 g crude (S)-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2) from step A was charged followed by 150 ml ethyl acetate and 7.4 ml triethyl amine. The solution was stirred at RT and then 6.2 ml of acetic anhydride was added. The reaction mixture was stirred overnight and then filtered. 5.8 g of dry, crude linezolid (1) was obtained. (yield: 65.8%, bis-linezolid (4) content as per A % in HPLC was 4.1%).

Step C: Isolation of bis-linezolid (4)

Crude linezolid (1) (2.5 g, as prepared in Step B described above), was mixed with 150 ml ethyl acetate. The mixture was heated to reflux; but it remained turbid. The reaction mixture was filtrated while hot. The cake contained 0.07 g bis-linezolid (4) (A % (HPLC) 98.1).

Example 2 – comparative example

In a 1L reactor, 6 g R-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl azide (3) was charged with 150 ml ethyl acetate, followed by 0.6 g Pd/C. The system was flushed 3 times with nitrogen and 3 times with hydrogen. The pressure of hydrogen was set to 1.5 atm. The reaction mixture was stirred at RT and the reaction followed by TLC or HPLC until completion. The reaction mixture was filtered through celite and the solution was treated with acetic anhydride in the presence of triethyl amine at RT. The precipitate was filtered and dried to obtain linezolid (1) crystalline Form IV with a 3.2% content of bis-linezolid (4).

Example 3 – use of bis-linezolid (4) as a reference marker

Two different injections were compared according to the area under the peak:

a) 10 microliters of a solution containing at least 0.2mg/ml bis linezolid (1) in acetonitrile : water 1:1 were injected to an HPLC equipped with an Hypersil Gold type column. The retention time of the sample was recorded.

b) 10 microliters of a solution containing at least 0.8mg/ml linezolid (1) (that might contain impurities such as bis-linezolid (4)) in acetonitrile : water 1:1 were injected to an HPLC equipped with an Hypersil Gold type column. The retention time of all sample components was recorded.

c) The retention time and the area under the peaks of the impurities in the linezolid (1) sample were compared. Bis-linezolid (4) eluted at about 3.8 times longer than linezolid (1).

The relative area percentage of the bis-linezolid (4) peak represents its content in the sample.

We claim:

1. Isolated bis-linezolid (4).

2. The isolated bis-linezolid (4) of claim 1, characterized by data selected from: ^{1}H NMR (400MHz, D_2O+TFA) δ (ppm): 2.08 (s), 3.63 (m), 3.83 (t), 4.02 (s), 4.14 (m), 4.94 (m), 7.23 (d), 7.58 (m); ^{13}C NMR (400MHz, $CDCl_3$) δ (ppm):16.6 (q), 43-50 (s), 50.4 (s), 50.9 (s), 68.3 (s), 103.6, 104.0 (d), 111.1 (s) , 118.7 (s), 130-140 (s) , 149-153 (d), 171.4 (s); FAB+m/z (MH^+): 616; and an IR spectrum at 1519, 1572, 1644, 1743, 2825, 2858, 2891, 2965 cm^{-1}.

3. The isolated bis-linezolid (4) of claim 2, characterized by ^{1}H NMR (400MHz, D_2O+TFA) δ (ppm): 2.08 (s), 3.63 (m), 3.83 (t), 4.02 (s), 4.14 (m), 4.94 (m), 7.23 (d), 7.58 (m).

4. The isolated bis-linezolid (4) of claim 3, characterized by ^{1}H NMR substantially as depicted in Figure 1.

5. The isolated bis-linezolid (4) of claim 2, characterized by ^{13}C NMR (400MHz, $CDCl_3$) δ (ppm): 16.6 (q), 43-50 (s), 50.4 (s), 50.9 (s), 68.3 (s), 103.6, 104.0 (d), 111.1 (s), 118.7 (s), 130-140 (s), 149-153 (d), 171.4 (s).

6. The isolated bis-linezolid (4) of claim 5, characterized by ^{13}C NMR substantially as depicted in Figure 2.

7. The isolated bis-linezolid (4) of claim 2, characterized by FAB+m/z (MH^+): 616.

8. The isolated bis-linezolid (4) of claim 2, characterized by an IR spectrum at 1519, 1572, 1644, 1743, 2825, 2858, 2891, 2965 cm^{-1}.

9. The isolated bis-linezolid (4) of claim 8, characterized by an IR spectrum substantially as depicted in Figure 3.

10. The isolated bis-linezolid (4) of claims 1-8 or 9, containing less than about 5% by weight of linezolid (1).

11. The isolated bis-linezolid (4) of claim 10, containing less than about 2% by weight of linezolid (1).

12. The isolated bis-linezolid (4) of claim 11, containing less than about 1% by weight of linezolid (1).

13. A process for preparing the isolated bis-linezolid (4) of claim 1, comprising:
    a) combining R-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl methyl azide (3) with an organic solvent and hydrogen gas in the presence of a catalyst to obtain crude (S)-N-(4-morpholinyl-3-fluorophenyl)-2-oxo-5-oxazolidinyl-methyl amine (2);
    b) combining the crude product of step a) with an organic solvent to obtain a solution;
    c) adding acetic anhydride to the solution and maintaining the reaction mixture for at least 12 hours to obtain crude linezolid (1);
    d) combining the crude linezolid (1) of step c) with an organic solvent to obtain a mixture;
    e) heating the mixture to reflux; and
    f) recovering bis-linezolid (4).

14. The process of claim 13, wherein the organic solvent in steps a), b) and d) is a C_{1-4} alkyl ester or an aromatic hydrocarbon.

15. The process of claim 14, wherein said organic solvent is selected from the group consisting of methyl acetate, ethyl acetate, butyl acetate, isobutyl acetate and toluene.

16. The process of claim 15, wherein said organic solvent is ethyl acetate.

17. The process of claims 13, 14, or 15 wherein the catalyst in step a) is Pd/C.

18. The process of claims 13, 14, or 15, further comprising adding an amine prior to step c).

19. The process of claim 18, wherein said amine is pyridine or a tertiary amine.

20. The process of claim 19, wherein said amine is triethyl amine.

21. Use of the isolated bis-linezolid (4) of claim 1 as a reference standard.

22. A method of determining the amount of bis-linezolid (4) in a sample of linezolid (1) comprising:
    a) measuring by HPLC the area under a peak corresponding to bis-linezolid (4) in a reference standard comprising a known amount of bis-linezolid (4);
    b) measuring by HPLC the area under a peak corresponding to bis-linezolid (4) in a sample comprising linezolid (1) and bis-linezolid (4); and

c) determining the amount of bis-linezolid (4) in the sample by comparing the area of step (a) to the area of step (b).

23. A method of determining the amount of bis-linezolid (4) in a sample of linezolid (1) comprising:

a) subjecting a reference standard solution of bis-linezolid (4) comprising a known amount of bis-linezolid (4) to HPLC for at least 45 minutes and measuring by HPLC the area under a peak corresponding to bis-linezolid (4);

b) subjecting a sample solution comprising linezolid (1) and bis-linezolid (4) to HPLC for at least 45 minutes and measuring by HPLC the area under a peak corresponding to bis-linezolid (4); and

c) determining the amount of bis-linezolid (4) in the sample by comparing the area of step (a) to the area of step (b).

24. A method for detecting bis-linezolid (4) comprising:

a) providing a preparation of crude linezolid (1) containing bis-linezolid (4);

b) subjecting the preparation of step a) to HPLC;

c) determining whether any material has an RRT of about 3.4 with respect to linezolid (1);

wherein the presence of material with an RRT of about 3.4 with respect to linezolid (1) indicates that the preparation contained bis-linezolid (4).

25. The process of claim 24, wherein the HPLC in step b) is carried out for at least about 34 minutes.

26. An HPLC method for assaying linezolid (1) comprising the steps:

a) combining a linezolid (1) sample with a mixture of acetonitrile/water diluent to obtain a solution;

b) injecting the solution of step a) into a Hypersil Gold 150x4.6 (or similar) column;

c) eluting the sample from the column at about 25 min using a mixture of about 0.01M K_2HPO_4 : MeOH as an eluent at a gradient of about 80:20 to about 50:50; and

d) measuring the bis-linezolid (4) content in the relevant sample with a UV detector.

27. The process of claim 26, wherein the bis-linezolid (4) at step d) is measured at a wavelength of 254nm.

Figure 1: ^{1}H-NMR spectrum of bis-linezolid (4)

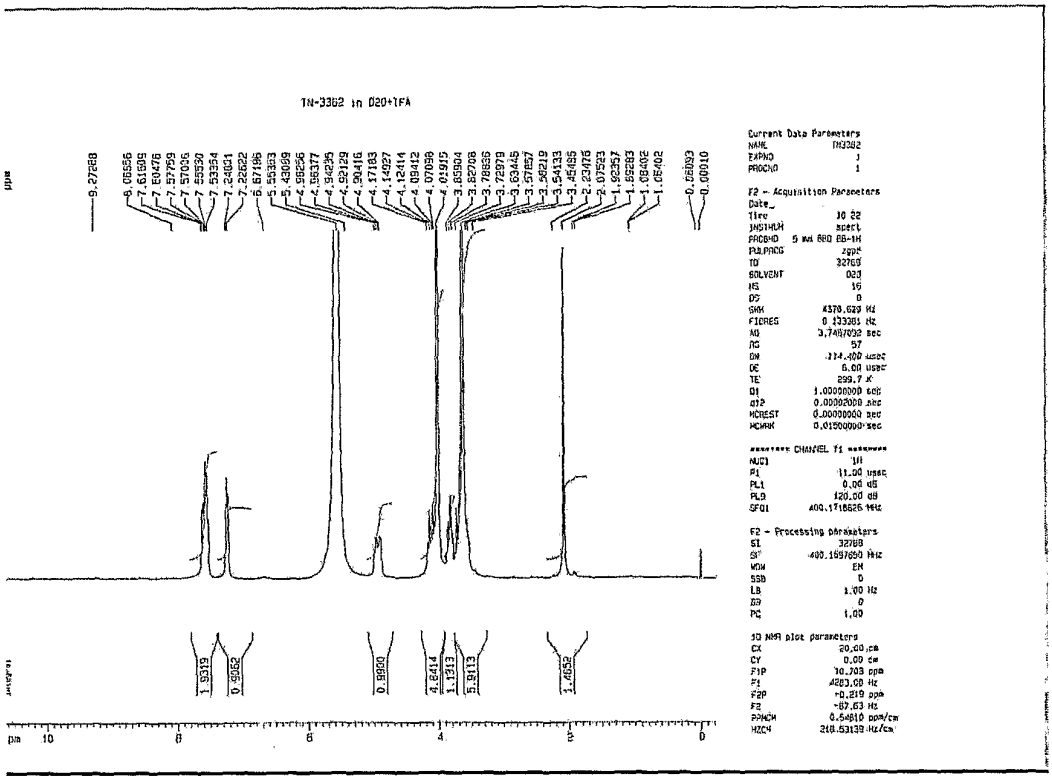


Figure 2: ^{13}C-NMR spectrum of bis-linezolid (4)

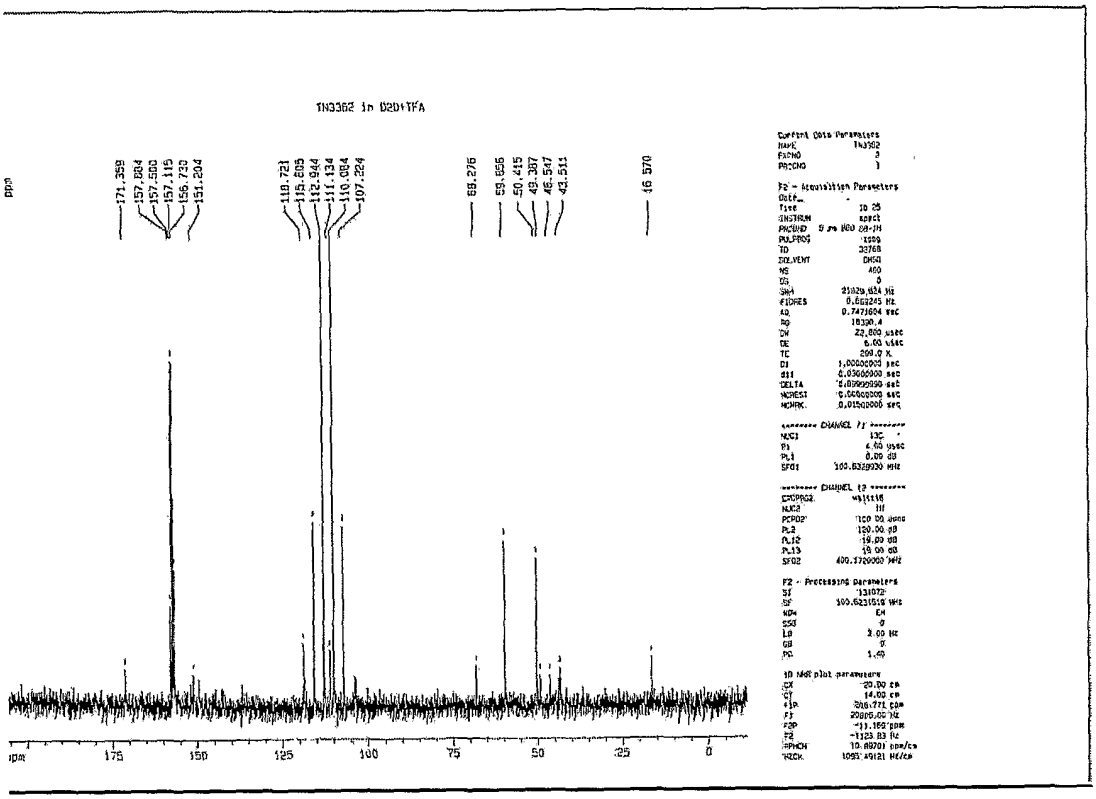


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Figure 3: IR spectrum of bis-linezolid (4)