Pharmaceutical compositions including a cephalosporin disclosed herein as having the structure of formula (III).
Figure 2

Atmospheric pressure

100 to 150 mTorr

30°C → Shelf temperature

-40°C

-20°C

NMT 10 mTorr → Chamber pressure
**Table 1**

<table>
<thead>
<tr>
<th>Impurity</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1 (P1)</td>
<td>~0.1</td>
</tr>
<tr>
<td>Peak 2 (P2)</td>
<td>~0.2</td>
</tr>
<tr>
<td>Peak 3 (P3)</td>
<td>~0.4</td>
</tr>
<tr>
<td>Peak 4 (P4)</td>
<td>~0.6</td>
</tr>
<tr>
<td>Peak 5 (P5)</td>
<td>~0.9</td>
</tr>
<tr>
<td>CXA-101</td>
<td>1.0</td>
</tr>
<tr>
<td>Peak 6 (P6)</td>
<td>~1.1</td>
</tr>
<tr>
<td>Peak 7 (P7)</td>
<td>~1.3</td>
</tr>
<tr>
<td>Peak 8 (P8)</td>
<td>~1.4</td>
</tr>
<tr>
<td>Peak 9 (P9)</td>
<td>~1.7</td>
</tr>
<tr>
<td>Peaks 10, 11 (P10, 11)</td>
<td>~2.3</td>
</tr>
</tbody>
</table>

1. The absolute retention time for Peak 1 is 3.5 minutes.
2. The absolute retention time for CXA-101 (ceftolozane) is 24 minutes.
Figure 4B

Table 2: Compositions of Co-lyophilization Drug Product.

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
<th>Amount (mg/vial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXA-101</td>
<td>Active pharmaceutical ingredient</td>
<td>1000 (potency)</td>
</tr>
<tr>
<td>L-arginine</td>
<td>Alkalization reagent</td>
<td>587</td>
</tr>
<tr>
<td>Citric acid (anhydrous)</td>
<td>Buffer</td>
<td>21</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Stabilizer</td>
<td>476</td>
</tr>
<tr>
<td>Tazobactam (free acid)</td>
<td>Active pharmaceutical ingredient</td>
<td>500</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Alkalization reagent</td>
<td>Quantity sufficient(^1) for pH 4.8 to 7.0</td>
</tr>
<tr>
<td>water</td>
<td>Dissolution solvent</td>
<td>Not more than 4% by weight(^2)</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Inert gas</td>
<td>Sufficient quantity</td>
</tr>
</tbody>
</table>

1. Sodium content is approximately 78 mg/g of tazobactam in drug product after lyophilization.
2. Water is removed during the lyophilization process and is controlled at no more than 4% by weight.
**Figure 5**

Table 3: Formulation composition of the Co-Lyo Combo Drug Product.

<table>
<thead>
<tr>
<th>CXA-201 Comp.</th>
<th>16.3 g active</th>
<th>ceftolozane</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1 g active</td>
<td>Tazobactam free ac.</td>
<td></td>
</tr>
<tr>
<td>15.5 g</td>
<td>L-Arginine</td>
<td></td>
</tr>
<tr>
<td>350 mg</td>
<td>Citric acid</td>
<td></td>
</tr>
<tr>
<td>7.9 g</td>
<td>NaCl</td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>pH compounded solution</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6A

Table 4: Stability Data of Co-Lyo Combo Drug Product at 25 °C.

<table>
<thead>
<tr>
<th>Test items</th>
<th>Spec. D.P.</th>
<th>T0</th>
<th>T1 25°C</th>
<th>T2 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related Substances</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Peak1</td>
<td>≤ 1.50%</td>
<td>0.31%</td>
<td>0.54%</td>
<td>0.71%</td>
</tr>
<tr>
<td>-Peak2</td>
<td>≤ 0.40%</td>
<td>0.07%</td>
<td>0.07%</td>
<td>0.09%</td>
</tr>
<tr>
<td>-Peak3</td>
<td>≤ 0.30%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>-Peak4</td>
<td>≤ 0.80%</td>
<td>0.08%</td>
<td>0.08%</td>
<td>0.09%</td>
</tr>
<tr>
<td>-Peak5</td>
<td>≤ 1.00%</td>
<td>0.27%</td>
<td>0.26%</td>
<td>0.29%</td>
</tr>
<tr>
<td>-Peak6</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>-Peak7</td>
<td>≤ 2.00%</td>
<td>0.64%</td>
<td>0.65%</td>
<td>0.66%</td>
</tr>
<tr>
<td>-Peak8</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>-Peak9</td>
<td>≤ 0.60%</td>
<td>0.05%</td>
<td>0.11%</td>
<td>0.10%</td>
</tr>
<tr>
<td>-Peak10,11</td>
<td>≤ 0.15% each</td>
<td>0.04%</td>
<td>0.04%</td>
<td>0.04%</td>
</tr>
<tr>
<td>-Peak12</td>
<td>≤ 2.00%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>Others (RRT 0.43)</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Others (RRT 1.22)</td>
<td>≤ 0.15%</td>
<td>0.13%</td>
<td>0.30%</td>
<td>0.38%</td>
</tr>
<tr>
<td>Others (RRT 2.18)</td>
<td>≤ 0.15%</td>
<td>0.03%</td>
<td>&lt;0.03%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Others (RRT 2.77)</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>0.03%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Sing. Unk.</td>
<td>≤ 0.15%</td>
<td>0.05%</td>
<td>0.07%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Total</td>
<td>≤ 5.00%</td>
<td>1.67%</td>
<td>2.19%</td>
<td>2.77%</td>
</tr>
<tr>
<td>pH</td>
<td>report value</td>
<td>5.5</td>
<td></td>
<td>4.83</td>
</tr>
</tbody>
</table>
Table 5: Stability Data of Co-Lyo Combo Drug Product at 40 °C.

<table>
<thead>
<tr>
<th>Test items</th>
<th>Spec. D.P.</th>
<th>T0</th>
<th>T1 40°C</th>
<th>T2 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related Substances</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Peak1</td>
<td>≤ 1.50%</td>
<td>0.31%</td>
<td>1.77%</td>
<td>2.22%</td>
</tr>
<tr>
<td>-Peak2</td>
<td>≤ 0.40%</td>
<td>0.07%</td>
<td>0.10%</td>
<td>0.16%</td>
</tr>
<tr>
<td>-Peak3</td>
<td>≤ 0.30%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>0.06%</td>
</tr>
<tr>
<td>-Peak4</td>
<td>≤ 0.80%</td>
<td>0.08%</td>
<td>0.09%</td>
<td>0.09%</td>
</tr>
<tr>
<td>-Peak5</td>
<td>≤ 1.00%</td>
<td>0.27%</td>
<td>0.27%</td>
<td>0.30%</td>
</tr>
<tr>
<td>-Peak6</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>-Peak7</td>
<td>≤ 2.00%</td>
<td>0.64%</td>
<td>0.69%</td>
<td>0.78%</td>
</tr>
<tr>
<td>-Peak8</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>0.10%</td>
</tr>
<tr>
<td>-Peak9</td>
<td>≤ 0.60%</td>
<td>0.05%</td>
<td>0.09%</td>
<td>0.09%</td>
</tr>
<tr>
<td>-Peak10,11</td>
<td>≤ 0.15% each</td>
<td>0.04%</td>
<td>0.04%</td>
<td>0.05%</td>
</tr>
<tr>
<td>-Peak12</td>
<td>≤ 2.00%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
</tr>
<tr>
<td>Others (RRT 0.43)</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>0.09%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Others (RRT 1.22)</td>
<td>≤ 0.15%</td>
<td>0.13%</td>
<td>0.74%</td>
<td>0.97%</td>
</tr>
<tr>
<td>Others (RRT 2.18)</td>
<td>≤ 0.15%</td>
<td>0.03%</td>
<td>&lt;0.03%</td>
<td>0.08%</td>
</tr>
<tr>
<td>Others (RRT 2.77)</td>
<td>≤ 0.15%</td>
<td>&lt;0.03%</td>
<td>&lt;0.03%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Sing. Unk.</td>
<td>≤ 0.15%</td>
<td>0.05%</td>
<td>0.11%</td>
<td>0.25%</td>
</tr>
<tr>
<td>Total</td>
<td>≤ 5.00%</td>
<td>1.67%</td>
<td>4.49%</td>
<td>6.32%</td>
</tr>
<tr>
<td>pH</td>
<td>report value</td>
<td>5.5</td>
<td></td>
<td>4.09</td>
</tr>
</tbody>
</table>
RT 1.22 (Solid State)

\[
\begin{align*}
\text{Tazobactam} & \quad \rightarrow \quad \text{HO}_2\text{C-CHO} + \text{HO}_2\text{C-SO}_2\text{N-N} \\
+ \text{CXA-101} & \quad \rightarrow \quad \text{Tazobactam M1}
\end{align*}
\]

Chemical Formula: C_{26}H_{32}N_{12}O_{10}S_{2}

Exact Mass: 736.18
Figure 8

NL: 1.94E5
data07#1242-1352 RT:
10.94-10.94 AV: 32 SB: 2 8.99
0.50 F: ITMS - c ESI Full ms
[100.00-1500.00]

NL: 5.71E3
data07#1242-1352 RT:
10.94-10.94 AV: 11 SB: 1
Full ms 737.28@ci@35.00
[150.00-750.00]
Figure 9

Chemical Formula: C_{26}H_{33}N_{2}O_{10}S_{2}^+  
Exact Mass: 737.19

Chemical Formula: C_{16}H_{17}N_{6}O_{7}S_{2}^-  
Exact Mass: 469.06

Chemical Formula: C_{10}H_{17}N_{6}O_{3}^+  
Exact Mass: 269.14

Chemical Formula: C_{21}H_{21}N_{10}O_{5}S_{2}^+  
Exact Mass: 607.11
CEPHALOSPORIN PHARMACEUTICAL COMPOSITIONS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 61/792,092, filed Mar. 15, 2013, and U.S. Provisional Patent Application No. 61/793,007, filed Mar. 15, 2013, both of which are incorporated by reference herein in their entireties.

TECHNICAL FIELD

This disclosure relates to antibacterial compositions.

BACKGROUND

Pharmaceutical antibiotic compositions comprising ceftolozane and tazobactam display potent antibacterial activity, including antibiotic activity against infections caused by many Gram-negative pathogens such as Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia).

Ceftolozane is a cephalosporin antibacterial agent, also referred to as CZA-101, FR264205, or by chemical names such as (6R,7R)-3-[[5-amino-4-[[2-aminoethyl]carbamoyl]amino]-1-methyl-1H-pyrazol-2-ium-2-yl)methyl]-7-{{(Z)-2-[[5-amino-1,2,4-thiadiazol-3-yl]-2-[[1-carboxy-1-methylthioxy]imino]acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, and 7β-[[Z]-2-[[5-amino-1,2,4-thiadiazol-3-yl]-2-[[1-carboxy-1-methylthioxy]imino]acetamido]-3-[[3-amino-4-[[3-2-aminoethyl]ureido]-2-methyl-1-pyrazol] methyl-3-cephem-4-carboxylate. The antibacterial activity of ceftolozane is believed to result from its interaction with penicillin binding proteins (PBPs) to inhibit the biosynthesis of the bacterial cell wall which acts to stop bacterial replication. Ceftolozane sulfate is a pharmaceutically acceptable salt of formula (I) that can be formulated for intravenous administration or infusion.

CEFTOLOZANE can be combined (e.g., mixed) with a β-lactamase inhibitor (“BLI”), such as tazobactam. Tazobactam is a BLI against Class A and some Class C β-lactamases, with well-established in vitro and in vivo efficacy in combination with active β-lactam antibiotics. Tazobactam can be combined with ceftolozane as a free acid tazobactam form of formula (II).

Antibacterial pharmaceutical compositions can include a therapeutically effective unit dose of a pharmaceutically acceptable salt of ceftolozane formulated for intravenous administration. In particular, a pharmaceutical composition for intravenous administration can include ceftolozane and tazobactam in a 2:1 weight ratio between the amount of ceftolozane active and tazobactam acid. The pharmaceutical composition can be obtained by lyophilizing a composition comprising ceftolozane sulfate and tazobactam free acid.

SUMMARY

Compositions comprising a cephalosporin compound of formula (III) are provided herein.
The compound of formula (III) can be formed by reaction of ceftolozane and formylacetic acid. The structure of formula (III) is disclosed as a ceftolozane/tazobactam by-product identified as having an HPLC relative retention time of 1.22 and the structure of formula (III) is disclosed in FIG. 5 of co-pending PCT Patent Application No. PCT/US2013/062256. The compound of formula (III) can be formed by co-lyophilization of ceftolozane sulfate and tazobactam acid in an aqueous solution (e.g., by formation of formylacetic acid from tazobactam and subsequent reaction of formylacetic acid with ceftolozane).

[0008] Compositions comprising the compound of formula (III) in combination with a therapeutically effective amount of the compound of formula (III) and the compound of formula (II) can be obtained by a process comprising the steps of: (a) forming an aqueous solution comprising ceftolozane (e.g., in a pharmaceutically acceptable salt such as formula (I)), tazobactam (e.g., in a pharmaceutically effective form such as formula (II)), and (b) lyophilizing the aqueous solution to obtain a composition comprising ceftolozane, tazobactam and a compound of formula (III), or a pharmaceutically acceptable salt thereof. In one aspect, an antibacterial pharmaceutical composition comprises ceftolozane and a compound of formula (III) obtained by a process comprising: (a) lyophilizing a solution comprising tazobactam, and an amount of ceftolozane sulfate providing a 2:1 weight ratio of ceftolozane active to tazobactam, to obtain the lyophilized composition comprising the compound of formula (III).

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIGS. 1A and 1B are chromatograms of CXA-101 ceftolozane drug substance obtained from the lyophilization process of Example 1. The chromatograms were obtained according to the analytical method described in Example 2.
[0010] FIG. 2 is a diagram of a lyophilization process for the ceftolozane obtained according to the process described in Example 1.

[0011] FIG. 3 is a table (Table 1) of peaks for the ceftolozane prepared by the lyophilization process in Example 1 obtained by HPLC according to the analytical method of Example 2.
[0012] FIG. 4A is a schematic showing a process for making the compound of formula (III) with ceftolozane and tazobactam.
[0013] FIG. 4B is a table (Table 2) showing a first composition that can be lyophilized to form a composition comprising the compound of formula (III).
[0014] FIG. 5 is a table (Table 3) showing a second composition that can be lyophilized to form a composition comprising the compound of formula (III).
[0015] FIG. 6A is a table (Table 4) showing the amount of various materials identified in lyophilized compositions comprising the compound of formula (III) (included is the material having a relative retention time of 1.22 relative to ceftolozane (RRT)) at a first temperature (25 degrees C.) and relative humidity of 60% after one (T1) and three (T2) months as measured by HPLC peak area according to the analytical method of described in Example 2.
[0016] FIG. 6B is a table (Table 5) showing the amount of various materials identified in lyophilized compositions comprising the compound of formula (III) (included is the material having a relative retention time of 1.22 relative to ceftolozane (RRT) at a second temperature (40 degrees C.) and relative humidity of 60% after one (T1) and three (T2) months as measured by HPLC peak area according to the analytical method of described in Example 2.
[0017] FIG. 7 is a schematic showing a proposed mechanism for the formation of RRT 1.22 product.
[0018] FIG. 8 are mass spectra obtained for the RRT 1.22 compound.
[0019] FIG. 9 shows chemical structures for certain peaks in the spectra in FIG. 8.

DETAILED DESCRIPTION

[0020] Compositions comprising a novel cephalosporin compound of formula (III) are provided herein.
[0021] The compound of formula (III) can be formed by reaction of cefclozane and formylacetic acid. In one embodiment, the compound of formula (III) can be formed by co-lyophilization of cefclozane sulfate and tazobactam acid in an aqueous solution (e.g., by formation of formylacetic acid from tazobactam and subsequent reaction of formylacetic acid with cefclozane).

[0022] In an embodiment, the compound of formula (III) is substantially isolated. As used herein, “substantially isolated” refers to a compound that is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched with a compound of formula (III). Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of a compound of formula (III).

[0023] Pharmaceutical compositions comprising the cephalosporin of formula (III) can be obtained by lyophilization of a solution comprising cefclozane and formylacetic acid and/or tazobactam under conditions effective to form the compound of formula (III) (e.g., Example 1 and 3). The compound of formula (III) can be isolated by HPLC (e.g., Examples 2 and 4). Specific methods of lyophilization are described in Example 1 and Remington’s Pharmaceutical Sciences, Chapter 84, page 1565, Eighteenth Edition, A. R. Gennaro, (Mack Publishing Co., Easton, Pa., 1990). Tazobactam is a β-lactamase inhibitor of the structure of formula (IV) in its free acid form.

[0024] An antibacterial pharmaceutical composition comprising a compound of formula (III) can be prepared by a process that includes the steps of (a) dissolving cefclozane and formylacetic acid or a source of formylacetic acid in an aqueous solution and (b) lyophilizing the aqueous solution to obtain the composition comprising the compound of formula (III). The source of formylacetic acid can be tazobactam (e.g., tazobactam free acid). In one example, the aqueous solution can include cefclozane and tazobactam in a fixed 2:1 ratio between the amount of cefclozane active and the amount of tazobactam active in the aqueous solution prior to lyophilization. The aqueous solution for lyophilization may comprise other additional components including stabilizers, pH adjusting additives (e.g., buffers) and the like. Non-limiting examples of these additives include sodium chloride, citric acid and L-arginine. The aqueous solution can be lyophilized to obtain a stabilized lyophilized composition comprising the compound of formula (III), cefclozane sulfate, sodium chloride, L-arginine and citric acid. The compound of formula (III) can be isolated from the lyophilized composition by HPLC (e.g., as described in Example 2).

[0025] Pharmaceutical antibiotic compositions comprising cefclozane, tazobactam and a compound of formula (III) can be obtained by methods that include the step of co-lyophilizing the cefclozane and tazobactam (e.g., in a 2:1 weight ratio between the amount of cefclozane active and the amount of tazobactam active). Other pharmaceutical antibiotic compositions can include cefclozane sulfate and the compound of formula (III). The amount of the compound of formula (III) can be increased in the composition as disclosed herein (e.g., by heating a sample produced by co-lyophilization of tazobactam and cefclozane in an aqueous solution, followed by heating the lyophilized product to increase the amount of the compound of formula (III)). The compound of formula (III) can also be isolated from compositions comprising cefclozane and tazobactam and then re-combined with cefclozane and/or tazobactam to form compositions with desired concentrations of the compound of formula (III). Alternatively, the compound of formula (III) can be formed without tazobactam by the chemical reaction of FIG. 7.

[0026] Pharmaceutical compositions can include less than 0.03%, 0.05%, 0.13%, 0.15%, 0.30%, 0.38%, 0.74% or 0.97% of the compound of formula (III), as measured by HPLC. Other pharmaceutical compositions can include a range from less than about 0.03% (e.g., see minimum detected amounts in Tables 4-5, FIGS. 6A-6B) to about 1.0% (e.g., maximum detected amount of RRT 1.22 in Tables 4-5, FIGS. 6A-6B) or more of the compound of formula (III) including compositions comprising 0.03%-0.05%, 0.05%-0.13%, 0.05%-0.15%, 0.03%-0.13% or 0.05%-0.13% of the compound of formula (III), where the percent of the compound (III) as measured by HPLC using a Develosil column ODS-UG-5; 5 micrometers; 250x4.6 mm, a mobile phase of sodium perchlorate buffer solution (pH 2.5)/CH₂CN 90:10 (v/v) at a 1.0 ml/min flow rate and oven temperature of 45°C. The pharmaceutical antibiotic compositions can include cefclozane or a pharmaceutically acceptable salt thereof and an amount of the compound of formula (III). Compositions can also be made comprising the compound of formula (III) in the absence of tazobactam and/or in the absence of cefclozane (e.g., by isolating the compound of formula (III) manufactured according to the synthesis described in FIG. 7). The pharmaceutical antibiotic compositions can be provided in a unit dosage form container (e.g., in a vial or bag). The unit dosage form can be dissolved with a pharmaceutically acceptable carrier, and then intravenously administered. A unit dosage form of a pharmaceutical composition can be formulated for parenteral administration for the treatment of complicated intra-abdominal infections or complicated urinary tract infections and can enclose a pharmaceutical composition comprising the compound of formula (III).

[0027] In one example, a pharmaceutical composition comprises cefclozane sulfate and tazobactam in a ratio of 1,000 mg cefclozane active per 500 mg of tazobactam active and up to about 1%, or more preferably up to about 0.03%-0.05%, of a compound of formula (III) as measured by HPLC using a Develosil column ODS-UG-5; 5 micrometers; 250x4.6 mm, a mobile phase of sodium perchlorate buffer solution (pH 2.5)/CH₂CN 90:10 (v/v) at a 1.0 mL/min flow rate and oven temperature of 45°C. The pharmaceutical composition obtained by a process comprising the steps of lyophilizing an aqueous solution comprising cefclozane sulfate, tazobactam (e.g., tazobactam free acid and/or tazobactam sodium), 125 mg to 500 mg of sodium chloride per 1,000 mg of cefclozane.

Unless otherwise indicated, tazobactam can be a free acid, a sodium salt, an arginine salt, or a hydrate or solvate thereof.
active, L-arginine and/or citric acid in an amount effective to adjust the pH of the first aqueous solution to 6-7 prior to lyophilization to obtain a first lyophilized ceflozalone composition.

[0028] Alternatively, the pharmaceutical composition comprising up to about 1% of a compound of formula (III) as measured by HPLC (e.g., with HPLC using a Develosil column ODS-UG-5; 5 micrometers; 250x4.6 mm, a mobile phase of sodium perchlorate buffer solution (pH 2.5)/CH$_3$CN 90:10 (v/v) at a 1.0 mL/min flow rate and oven temperature of 45°C) can be obtained by a process comprising the steps of:

(a) lyophilizing a first aqueous solution in the absence of tazobactam, the first aqueous solution comprising ceflozalone sulfate, 125 mg to 500 mg of sodium chloride per 1,000 mg of ceflozalone active, L-arginine and/or citric acid in an amount effective to adjust the pH of the first aqueous solution to 6-7 prior to lyophilization to obtain a first lyophilized ceflozalone composition; (b) lyophilizing a second solution in the absence of ceflozalone, the second solution comprising tazobactam being lyophilized to form a second lyophilized tazobactam composition; and (c) blending the first lyophilized ceflozalone composition, the second lyophilized tazobactam composition and a composition comprising the compound of formula (III). Pharmaceutical compositions comprising the compound of formula (III), ceflozalone and tazobactam can be formulated to treat infections by parenteral administration (including subcutaneous, intramuscular, and intravenous) administration. Pharmaceutical compositions may additionally comprise excipients, stabilizers, pH adjusting additives (e.g., buffers) and the like. Non-limiting examples of these additives include sodium chloride, citric acid and L-arginine. For example, the use of sodium chloride results in greater stability; L-arginine is used to adjust pH and to increase the solubility of ceflozalone; and citric acid is used to prevent discoloration of the product, due to its ability to chelate metal ions. In one particular embodiment, the pharmaceutical compositions described herein are formulated for administration by intravenous injection or infusion.

[0029] Pharmaceutical antibiotic compositions can include ceflozalone sulfate and the compound of formula (III). For example, pharmaceutical compositions comprising 0.13%, 0.15%, 0.30%, 0.38%, 0.74% or 0.97% of the compound of formula (III) are described in FIGS. 6A and 6B. The pharmaceutical antibiotic compositions can be provided in a unit dosage form (e.g., in a vial). The unit dosage form can be dissolved with a pharmaceutically acceptable carrier, and then intravenously administered.

[0030] In one aspect, provided herein is a method for the treatment of bacterial infections in a mammal, comprising administering to said mammal a therapeutically effective amount of a pharmaceutical composition prepared according to the methods described herein. A method for the treatment of bacterial infections in a mammal can comprise administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising ceflozalone sulfate and sodium chloride. Non-limiting examples of bacterial infections that can be treated by the methods of the invention include infections caused by: aerobic and facultative gram-positive microorganisms (e.g., Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Viridans group streptococci), aerobic and facultative gram-negative microorganisms (e.g., Acinetobacter baumannii, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter koseri, Moraxella catarrhalis, Morganella morganii, Neisseria gonorrhoeae, Proteus mirabilis, Proteus vulgaris, Serratia marcescens, Providencia stuartii, Providencia rettgeri, Salmonella enterica), gram-positive anaerobes (Clostridium perfringens), and gram-negative anaerobes (e.g., Bacteroides fragilis group (e.g., B. fragilis, B. ovatus, B. thetaiotaomicron, and B. vulgatus), Bacteroides distasonis, Prevotella melaninogena). In certain embodiments of the methods described herein, bacterial infection is associated with one or more of the following conditions: complicated intra-abdominal infections, complicated urinary tract infections (cUTI’s) and pneumonia (e.g., community-acquired, or nosocomial pneumonia). Community-acquired pneumonia (moderate severity only) can include infections caused by piperacillin-resistant, beta-lactamase producing strains of Haemophilus influenzae. Nosocomial pneumonia (moderate to severe) caused by piperacillin-resistant, beta-lactamase producing strains of Staphylococcus aureus and by Acinetobacter baumanii, Haemophilus influenzae, Klebsiella pneumoniae, and Pseudomonas aeruginosa.

[0031] As used herein, “treating,” “treat,” or “treatment” describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a pharmaceutical composition of the present invention to alleviate the symptoms or complications of a disease, condition or disorder, or to reduce the extent of the disease, condition or disorder. The term “treat” can also include treatment of a cell in vitro or an animal model.

[0032] By a “therapeutically effective amount” of a compound of the invention is meant a sufficient amount of the compound to treat the disorder (e.g., bacterial infection). The specific therapeutically effective amount that is required for the treatment of any particular patient or organism (e.g., a mammal) will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound or composition employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts (see, for example, Goodman and Gilman’s, “The Pharmacological Basis of Therapeutics”, Tenth Edition, A. Gilman, J. Hardman and L. Limbird, eds., McGraw-Hill Press, 155-173, 2001, which is incorporated herein by reference in its entirety). The therapeutically effective amount for a given situation can be readily determined by routine experimentation and is within the skill and judgment of the ordinary clinician.

[0033] As used herein, “1,000 mg of ceflozalone as ceflozalone active” refers to an amount of ceflozalone sulfate effective to provide 1,000 mg of ceflozalone. The amount of sodium per gram of ceflozalone activity in a pharmaceutical composition containing ceflozalone sulfate and sodium chloride can be calculated using the relevant molecular weights of ceflozalone, ceflozalone sulfate, sodium chloride and sodium.
4.6 mm, a mobile phase of sodium perchlorate buffer solution (pH 2.5)/CH₃CN 90:10 (v/v) at a 1.0 mL/min flow rate and oven temperature of 45°C.

Illustrative examples of selected embodiments of the invention

EXAMPLE 1

Manufacturing Procedure of Bulk (Tray) Lyophilized Cefiotolzone

The CXA-101 bulk lyophilization manufacturing process is presented below.

**Step 1: Washing**

- The washing solution is loaded onto a separate shell in the lyophilizer (and later discarded).
- The solution is lyophilized until dry.
- The product shelf is cooled to 20°C ±5°C.
- The lyophilized bulk drug product powder is milled.

**Step 2: Filtration**

- Filter the compounded solution with a sterile filter that consists of a 0.2 um polyvinylidene fluoride membrane filter (Durapore, Millipore) and a 0.1 um polyvinylidene fluoride membrane filter (Durapore, Millipore) to remove bacterial contamination and introduce the final filtrate into a sterile condition.
- Confirm the integrity of each filter before and after the filtration.

**Step 3: Washing**

- Wash a sufficient quantity of 28 mL vials with water for injection and sterilize the washed vials with a dry-air sterilizer. Then transfer the sterilized vials into a Grade A area located in an aseptic room. Wash a sufficient quantity of stoppers with water, for injection. Sterilize and dry the washed stoppers by steam sterilizer. Then transfer the sterilized stoppers into a Grade B area located in an aseptic room.

**Step 4: Sterilization**

- Sterilize a sufficient quantity of flip-off caps by steam sterilizer. Then transfer the sterilized flip-off caps into a Grade A or B area located in an aseptic room. Adjust the fill weight of the filtered compounded solution to 11.37 g (corresponds to 10 mL of the compounded solution), then start filling operation. Check the fill weight in sufficient frequency and confirm it is in target range (11.37 g±1%, 11.26 to 11.43 g). When deviation from the control range (11.37 g±2%, 11.14 to 11.59 g) is occurred, re-adjust the filling weight. Immediately after a vial is filled, partially stopper the vial with a sterilized stopper. Load the filled and partially stoppered vials onto the shelves of a lyophilizer aseptically.

**Step 5: Loading**

- After all filled and partially stoppered vials are loaded into a lyophilizer, start the lyophilization program shown in Fig. 2. Freeze the loaded vials at −40°C and keep until all vials are frozen. Forward the program to primary drying step (shelf temperature: −20°C, chamber pressure: 100 to 150 mTorr). Primary drying time should be determined by monitoring the product temperature. Forward the program to secondary drying step (shelf temperature: 30°C, chamber pressure: not more than 10 mTorr) after completion of the primary drying step. After all vials are dried completely, return the chamber pressure to atmospheric pressure with sterilized nitrogen. Then stopper vials completely.

**Step 6: Unloading**

- Unload the lyophilized vials from the chamber and crimp with sterilized flip-off caps.

**EXAMPLE 2**

Analytical HPLC Method

- A. Operative Conditions

- Column Develosil ODS-UG-5; 5 μm, 250x4.6 mm (Nomura Chemical, Japan)

- Mobile phase Sodium Perchlorate Buffer Solution (pH 2.5)/CH₃CN 90:10 (v/v)

- Flow rate 1.0 mL/min

- Wavelength 254 nm

- Injection volume 10 μL

- Oven Temperature 45°C

- Run Time 85 minutes

**Gradient Profile:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A %</th>
<th>B %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
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<tr>
<td>60</td>
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<td>100</td>
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<td>85</td>
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<td>100</td>
</tr>
<tr>
<td>85.1</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>110</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>

**Step 7:**

- B. Mobile phase preparation.

- Sodium Perchlorate Buffer Solution was made by dissolving 14.05 g of sodium perchlorate Monohydrate in 1000.0 mL of water followed by adjusting pH to 2.5 with diluted perchloric acid (1 in 20).

**Step 8:**

- Mobile Phase was then made by mixing Sodium Perchlorate Buffer Solution (pH 2.5) and acetonitrile in the ratio 90:10 (v/v).

**Step 9:**

- Sodium Acetate Buffer Solution pH 5.5 (Dihydrate) was made by dissolving 1.36 g of sodium acetate trihydrate in 1000.0 mL of water followed by adjusting to pH 5.5 with diluted acetic acid (1 in 10).

**Step 10:**

- C. Sample Preparation.

- Sample solution: dissolve 20.0 mg, exactly weighed, of Sample, in 20.0 mL of water (Prepare just before injection into HPLC system).

**Step 11:**

- System Suitability Solution (1%): take 1.0 mL of the Sample Solution (use first sample if more are present) and transfer into a 100.0 mL volumetric flask, dilute with water to volume and mix.

**Step 12:**

- D. HPLC Analysis Procedure

- 1. Inject Blank (water)

- 2. Inject System Suitability Solution and check for tailing factor and theoretical plate number for CXA-101 peak:

- The tailing factor must not be greater than 1.5

- Theoretical plates number must not be less than 10000

- 3. Inject Sample Solution

- 4. Inject System Suitability Solution and check for tailing factor and theoretical plate number for CXA-101 peak:

- The tailing factor must not be greater than 1.5

- Theoretical plates number must not be less than 10000

- 5. Identify the peaks of Related Substances in the Sample chromatogram based on the reference chromatogram reported in FIGS. 1A and 1B or, alternatively, on the basis of the RRT values reported in Table 1 (FIG. 3).
[0076] E. Calculations

[0077] I. Report for Each Related Substance its Amount as Expressed by Area Percent.

\[ C_i = \frac{A_i \times 100}{A_T + \sum A_i} \]

wherein:

[0078] \( C_i \) = Amount of related substance \( i \) in the Sample, area %

[0079] \( A_i \) = Peak area of related substance \( i \) in the Sample chromatogram

[0080] \( A_T \) = Area of CXA-101 peak in the Sample chromatogram

[0081] \( A_T + \sum A_i \) = Total peaks area in the Sample chromatogram

[0082] Consider as any Unspecified Impurity, each peak in the chromatogram except CXA-101, peaks from 1 to 11 and every peak present in the blank chromatogram and report the largest.

II. Report the Total Impurities Content as Expressed by the Following Formula:

\[ C_T = \frac{A_T \times 100}{A_T + \sum A_i} \]

wherein:

[0085] \( C_T \) = total impurities content in the Sample, area %

[0086] \( A_T \) = area of CXA-101 peak in the sample chromatogram

[0087] \( \sum A_i \) = total peak areas of impurities in the sample chromatogram

EXAMPLE 3

Manufacturing of Combination Product (Tazobactam and CXA-101) Containing the Compound of Formula (III) by Co-Lyophilization

[0089] Compositions comprising the compound of formula (III) were prepared by the process shown in FIG. 4A by (a) forming an aqueous solution comprising the components in Table 2 (FIG. 4B), and (b) lyophilizing the aqueous solution. Sodium content was approximately 78 mg/g of tazobactam in drug product after lyophilization. Water was removed during the lyophilization process and is controlled at no more than 4% by weight.

EXAMPLE 4

Identifying the compound of formula (III)

[0090] The Co-Lyophilized Combo Drug Product was prepared as described above in Example 3. The formulation composition of the Co-Lyophilized Combo drug product is shown in FIG. 5 (Table 3). This sample maintained at 25° C./RH=60% and 40° C./RH=75% after one month (T1) and three months (T2). Samples were analyzed using a HPLC method as described in Example 2. The data for analysis of the samples by HPLC is shown in the tables in FIG. 6A (Table 4: Stability data of Co-Lyo Combo Drug Product at 25° C.) and FIG. 6B (Table 5: Stability data Co-Lyo Combo Drug Product at 40° C.). The presence of the compound of Formula (III) was identified having a retention time of about 1.22 as measured by HPLC (see Example 2). RRT=1.22 was observed in co-lyophilized drug product. The compound of formula (III) is believed to be formed by a reaction between cefotaxime and formylacetic acid, which was a degradation product of tazobactam. The amount of the compound of formula (III) in a composition comprising cefotaxime and tazobactam can be increased over time at 25° C. and at 40° C.

[0091] The material obtained from the RRT 1.22 peak was analyzed by LC/MS, providing the spectra shown in FIG. 8. FIG. 9 is the corresponding structures for the peaks shown in FIG. 8. A test sample prepared from cefotaxime and tazobactam acid co-compounding solution containing RRT 1.22 impurity was used on the LC/MS experiment. Liquid chromatography separation was performed on a Zorbax SB CR, 3.5 μm, 3.0mm×150 mm column, using gradient elution with 20 mM ammonium formate containing 0.1% Heptfluorobutyric acid pH 3.2 as mobile phase A and 0.1% Heptfluorobutyric acid in acetonitrile as mobile phase B. The gradient starts from 3% (initial) to 15% mobile phase B in 20 minutes (with RRT 1.22 eluting at about 10.7 minutes). Mass detection was performed using electrospray ionization technique under positive mode. The column effluent was also monitored at 254 nm using a photodiode-array detector. MS/MS fragmentation was performed on m/z 737.3 positive ion using nitrogen as collision gas, with collision energy set at 35V.

What is claimed is:

1. An isolated compound of formula (III):

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.
2. A pharmaceutical composition comprising a compound of formula (III):

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of claim 2, further comprising ceftolozane sulfate.

4. The pharmaceutical composition of claim 2, further comprising tazobactam.

5. The pharmaceutical composition of claim 4, further comprising ceftolozane sulfate.

6. The pharmaceutical composition of claim 2, where the compound of formula (III) is obtained by a process comprising the steps of
   a. forming an aqueous solution comprising ceftolozane and tazobactam acid; and
   b. lyophilizing the aqueous solution to obtain a lyophilized composition comprising the compound of formula (III).

9. The pharmaceutical composition of claim 8, wherein the aqueous solution comprises ceftolozane sulfate and tazobactam acid in a 2:1 weight ratio between the amount of ceftolozane active and the amount of tazobactam acid.

10. The pharmaceutical composition of claim 9, wherein the aqueous solution comprises sodium chloride, ceftolozane sulfate, tazobactam acid and L-arginine.

11. The pharmaceutical composition of claim 9, wherein the aqueous solution has a pH of about 6.0 to 7.0.

12. The pharmaceutical composition of claim 9, wherein the pharmaceutical composition is formulated for parenteral administration.

13. The pharmaceutical composition of claim 8, wherein the compound of formula (III) is obtained by a process further comprising the step of performing high performance liquid chromatography (HPLC) on the lyophilized composition to isolate the compound of formula (III).

14. The pharmaceutical composition of claim 2, comprising about 0.13-0.97% of the compound of formula (III).

15. The pharmaceutical composition of claim 2, comprising up to about 0.15% of the compound of formula (III).

16. A pharmaceutical composition comprising a compound of formula (III),

![Chemical Structure](image)
the compound of formula (III) obtained by a process comprising the step of
a. forming an aqueous solution comprising tazobactam acid and ceftolozane sulfate in an amount providing 1,000 mg of ceftolozane active per 500 mg of tazobactam acid in the aqueous solution;
b. lyophilizing the aqueous solution of step (a) to obtain a lyophilized composition comprising a compound of formula (III); and
c. formulating the lyophilized composition as a pharmaceutical composition for parenteral delivery.

17. The pharmaceutical composition of claim 16, wherein the pH of the aqueous solution is 6.0 to 7.0.

18. The pharmaceutical composition of claim 17, wherein pharmaceutical composition is formulated for parenteral administration.

19. A pharmaceutical composition formulated for parenteral administration for the treatment of complicated intra-abdominal infections or complicated urinary tract infections, the pharmaceutical composition comprising a compound of formula (III) in a lyophilized composition obtained by lyophilizing an aqueous solution comprising tazobactam and an amount of ceftolozane sulfate containing 1,000 mg of ceftolozane active per 500 mg of tazobactam acid.

* * * * *