Glycine

Type of Posting: Revision Bulletin, Postponement
Posting Date: 27-Jul–2018
Official Date: 01–Aug–2018
Expert Committee: Non-Botanical Dietary Supplements
Reason for Revision: Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has postponed the acceptance criteria for monochloroacetic acid and any unspecified impurity, listed in Table 2 in the test for Related Compounds in the Glycine monograph, published in the First Supplement to USP 41–NF 36.

USP has received comments regarding the implementation of the acceptance criteria for monochloroacetic acid and unspecified impurities. Additional input from stakeholders is required in order to resolve the concerns raised. Interested parties are invited to contact USP for additional information on this topic and to get involved in the dialog on the path forward for these targeted impurities in the Glycine monograph.

The Glycine Revision Bulletin supersedes the monograph becoming official in the First Supplement to USP 41–NF 36.

Should you have any questions, please contact Huy Dinh, Senior Scientific Liaison (301-816-8594 or htd@usp.org).
Glycine

C₂H₄NO₂  75.07
Glycine [56-40-6].

**DEFINITION**
Glycine contains NLT 98.5% and NMT 101.5% of glycine (C₂H₄NO₂), calculated on the dried basis.

**IDENTIFICATION**
• A. INFRARED ABSORPTION (197M)

**ASSAY**
• PROCEDURE
Sample: 150 mg of Glycine
Blank: 100 mL of glacial acetic acid

Titrmetric system
(See Titrmetry (541).)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual

Analysis: Dissolve the Sample in 100 mL of glacial acetic acid, and add 1 drop of crystal violet TS. Titrate with the Titrant to a green endpoint. Perform the Blank determination.

Calculate the percentage of glycine (C₂H₄NO₂) in the Sample taken:

\[
\text{Result} = \left( \frac{(V_s - V_b) \times N \times F}{W} \right) \times 100
\]

Where:
- \( V_s \) = Titrant volume consumed by the Sample (mL)
- \( V_b \) = Titrant volume consumed by the Blank (mL)
- \( N \) = actual normality of the Titrant (mEq/mL)
- \( F \) = equivalence factor, 75.07 mg/mEq
- \( W \) = Sample weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

**IMPURITIES**
• RESIDUE ON IGNITION (281): NMT 0.1%
• CHLORIDE AND SULFATE (221), Chloride
Standard solution: 0.10 mL of 0.020 N hydrochloric acid
Sample: 1 g of Glycine
Acceptance criteria: NMT 0.007%
• CHLORIDE AND SULFATE (221), Sulfate
Standard solution: 0.20 mL of 0.020 N sulfuric acid
Sample: 3 g of Glycine
Acceptance criteria: NMT 0.0065%

Delete the following:
•** HEAVY METALS, Method I (231): NMT 20 ppm (Official 1-Jan-2018)
• HYDROLYZABLE SUBSTANCES
Sample solution: 100 mg/mL of Glycine
Analysis: Boil 10 mL of the Sample solution for 1 min, and set aside for 2 h.
Acceptance criteria: The solution appears as clear and as mobile as 10 mL of the same solution that has not been boiled.

Change to read:

• RELATED COMPOUNDS
Solution A: Transfer 2.16 g of octanesulfonic acid sodium salt to a 1000-mL volumetric flask, add 900 mL of HPLC grade water and 2.0 mL of perchloric acid, and mix to dissolve. Adjust with 5 N sodium hydroxide solution to a pH of 2.2. Dilute with HPLC grade water to volume. Pass the solution through a membrane filter of 0.2-µm pore size.

**Solution B:** Acetonitrile
**Mobile phase:** Gradient elution. See Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
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<tr>
<td>13</td>
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<td>18</td>
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<td>10</td>
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<td>35</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Standard solution:** A mixture of 0.005 mg/mL each of USP Glycine RS, USP Diglycine RS, USP Triglycine RS, and glycine anhydride,¹ and 0.0025 mg/mL of monochloroacetic acid² in HPLC grade water.

[NOTE—Monochloroacetic acid may be omitted from the Standard solution if the article being tested does not contain this substance.]

**Sample solution:** Transfer 125 mg of Glycine into a 25-mL volumetric flask, dissolve in and dilute with HPLC grade water to volume.

**Blank:** HPLC grade water

**Chromatographic system**
(See Chromatography (621), System Suitability.)

**Mode:** LC
**Detector:** UV 200 nm
**Column:** 4.6-mm × 15-cm; 3-µm packing L1
**Column temperature:** 25°C
**Flow rate:** 1 mL/min
**Injection volume:** 20 µL

**System suitability**
**Samples:** Standard solution and Blank

[NOTE—See Table 2 for the relative retention times.]

**Suitability requirements**
**Interference peaks:** Compare the chromatogram obtained from the Standard solution with that obtained from the Blank. Any peak area from the Blank that overlaps or co-elutes with the amino acid peak from the Standard solution is NMT 2.0% of that amino acid peak area.

**Resolution:** NLT 2.0 between the diglycine and triglycine peaks, Standard solution

**Relative standard deviation:** NMT 5.0% each for the specified peaks, Standard solution

**Analysis**
**Samples:** Standard solution, Sample solution, and Blank
Separately inject the Blank, Standard solution, and Sample solution into the chromatograph. Compare the chromatogram from the Sample solution with that from the Blank. Disregard any peak observed in both the Sample solution and the Blank. Identify the amino acid impurities in the Sample solution by comparing with those specified in the Standard solution. Separately calculate the percentage of each specified impurity in the portion of Glycine taken:

\[
\text{Result} = \left( \frac{t_d - t_i}{C_d - C_i} \right) \times \left( \frac{C_i}{C_d} \right) \times 100
\]

¹ Analytical grade with purity NLT 99.0%.
² Analytical grade with purity NLT 99.0%.

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Separately calculate the percentage of iminodiacetic acid, hexamethylenetetramine, and any unspecified impurity in the portion of Glycine taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

where:
- \( r_U \) = peak response of iminodiacetic acid, hexamethylenetetramine, or any unspecified impurity from the Sample solution
- \( r_S \) = peak response of glycine from the Standard solution
- \( C_S \) = concentration of USP Glycine RS in the Standard solution (mg/mL)
- \( C_U \) = concentration of Glycine in the Sample solution (mg/mL)

Acceptance criteria: See Table 2. [Note—Disregard any impurity peak less than 0.05%.

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iminodiacetic acid</td>
<td>0.60</td>
<td>0.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>Diglycine</td>
<td>1.70</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglycine</td>
<td>1.80</td>
<td>0.1</td>
</tr>
<tr>
<td>Hexamethylenetetramine</td>
<td>2.47</td>
<td>0.1</td>
</tr>
<tr>
<td>Any unspecified impurity</td>
<td>—</td>
<td>— (Postponed on 1-Aug-2018)</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*The limit should be controlled as per International Council for Harmonisation (ICH) M7.

*b The limit should be based on the maximum daily dose (MDD) of the drug products.

### Specific Tests

- **Loss on Drying (731)**
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.2%

### Additional Requirements

- **Packaging and Storage:** Preserve in well-closed containers at room temperature. 15 (USP41)

- **USP Reference Standards (11)**
  - USP Diglycine RS 15 (USP41)
  - USP Glycine RS
  - USP Triglycine RS 15 (USP41)