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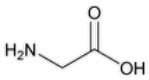
Glycine

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-glycine-20211029.



$C_2H_5NO_2$ 75.07

Glycine CAS RN®: 56-40-6.

DEFINITION

Glycine contains NLT 98.5% and NMT 101.5% of glycine ($C_2H_5NO_2$), calculated on the dried basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197M

ASSAY

• **Procedure**

Sample: 150 mg of Glycine

Blank: 100 mL of glacial acetic acid

Titrimetric system

(See *Titrimetry* ⟨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 ($C_2H_5NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

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 = equivalency 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%

• **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

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
13	90	10
18	90	10
35	100	0
45	100	0

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 acid[2] 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°

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:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glycine anhydride, monochloroacetic acid, diglycine, or triglycine from the *Sample solution*

r_S = peak response of glycine anhydride, monochloroacetic acid, diglycine, or triglycine from the *Standard solution*

C_S = concentration of glycine anhydride, monochloroacetic acid, USP Diglycine RS, or USP Triglycine RS in the *Standard solution* (mg/mL)

C_U = concentration of Glycine in the *Sample solution* (mg/mL)

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Glycine anhydride	0.25	0.1
Monochloroacetic acid	0.44	▲—▲ (RB 1-Nov-2021)
Iminodiacetic acid	0.60	0.1
Glycine	1.00	—
Diglycine	1.70	0.1
Triglycine	1.80	0.1
Hexamethylenetetramine	2.47	0.1
Any unspecified impurity	—	▲—▲ (RB 1-Nov-2021)
Total impurities	—	1.0

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.
- **USP Reference Standards** ⟨11⟩

USP Diglycine RS
USP Glycine RS
USP Triglycine RS

[1] Analytical grade with purity NLT 99.0%.

[2] Analytical grade with purity NLT 99.0%.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
GLYCINE	Fatkhulla K Tadjimukhamedov Associate Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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