⟨503⟩ ACETIC ACID IN PEPTIDES

INTRODUCTION

This chapter provides procedures to be used to determine the amount of acetic acid in peptides. Acetic acid/acetate is a common counter ion in peptide preparations.

• **METHOD 1**

Strong sodium hydroxide solution: Dissolve 42 g of sodium hydroxide in water, and dilute with water to 100 mL.

Solution A: Add 0.7 mL of phosphoric acid to 1000 mL of water, and adjust with *Strong sodium hydroxide solution* to a pH of 3.0.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
10	50	50
20	50	50
22	95	5

Diluent: Prepare a mixture of *Solution A* and *Solution B* (95:5).

Standard solution: Dissolve an accurately weighed quantity of USP Glacial Acetic Acid RS in *Diluent* to obtain a solution having a known concentration of about 0.1 mg/mL. [NOTE—The concentration can be adjusted, depending on the amount of acetate or acetic acid expected to be present in the test material.]

Sample solution: Prepare as directed in the individual monograph. If no direction is given in the individual monograph, the sample concentration can be adjusted so that the mid-range of the amount of acetic acid stated in the specification for the test material corresponds to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Retention time of acetic acid: 3–4 min

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetic acid in the portion of test material taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glacial Acetic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

• **METHOD 2**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** See *Trifluoroacetic Acid (TFA) in Peptides* ⟨503.1⟩.

Standard solution: 0.7 mg/mL of USP Sodium Acetate Trihydrate RS in *Diluent*

Calculate the concentration of acetic acid in the *Standard solution* (C_S), in mg/mL, taken:

$$C_S = 0.441 \times C$$

0.441 = molecular weight conversion factor (60.05/136.08)
C = concentration of USP Sodium Acetate Trihydrate RS in the *Standard solution* (mg/mL)

Sample solution: About 4 mg/mL of the test sample in *Diluent*. [NOTE—The sample concentration can be adjusted so that the mid-range of the amount of acetic acid stated in the specification for the test material corresponds to that of the *Standard solution*.]

[NOTE—Alternatively, the *Standard solution* and *Sample solution* can be prepared as described in *Method 1*.]

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Analysis: Proceed as described in *Method 1*.

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Glacial Acetic Acid RS

USP Sodium Acetate Trihydrate RS