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## (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](#)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
<503> ACETIC ACID IN PEPTIDES	<a href="#">Ying Han</a> Associate Science & Standards Liaison	BI012020 Biologics Monographs 1 - Peptides

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