

ACETIC ACID IN PEPTIDES

The following procedure is to be used to determine the amount of acetate or acetic acid in peptides.

Acetate is a common counterion in many peptide preparations.

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— Use methanol.

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

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

Test Solution— Prepare as directed in the individual monograph. The amount of material used can be adapted depending on the amount of acetic acid expected.

Chromatographic System— The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains not greater than 5- μ m packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0–5	95	5	isocratic
5–10	95→50	5→50	linear gradient
10–20	50	50	isocratic
20–22	50→95	50→5	linear gradient

Chromatograph the Standard Solution, and record the peak responses as directed for Procedure: the retention time of acetic acid is between 3 and 4 minutes; and the relative standard deviation for replicate injections is not more than 5%.

Procedure— Separately inject equal volumes (about 10 μ L) of the Standard Solution and the Test Solution into the chromatograph, record the chromatograms, and measure the responses for the

acetic acid peaks. Calculate the percentage of acetic acid in the portion of test material taken by the formula:

$$100(CS / M)(rU / rS)$$

in which CS is the concentration of acetic acid in the Standard Solution; M is the concentration, in mg per mL, of the Test Solution, based on the weight of test material taken and the extent of dilution; and rU and rS are the acetic acid peak responses obtained from the Test Solution and the Standard Solution, respectively.