⟨503.1⟩ TRIFLUOROACETIC ACID (TFA) IN PEPTIDES

INTRODUCTION

The following procedures are to be used to determine the amount of trifluoroacetic acid (TFA) in peptides. TFA/Trifluoroacetate is a common residual process impurity in the preparation of peptides or a counter ion in active pharmaceutical ingredients (API).

• **PROCEDURE**

Solution A: Add 7.0 mL of phosphoric acid and 5.0 mL of ammonium hydroxide to 900 mL of water. Mix and dilute with water to 1000 mL. [NOTE—The pH of the solution is approximately 2.5.] Pass through a filter of 0.45-µm pore size and degas. Add 20 mL of methanol, mix, and degas for an additional 2 min.

Solution B: Acetonitrile and water (50:50). Mix and degas.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
6	0	100
14	0	100
15	100	0
25	100	0

Diluent: 0.5% phosphoric acid in water (v/v)

TFA stock solution (option 1): 12 mg/mL of USP Sodium Trifluoroacetate RS in water

Calculate the concentration (C_S), in mg/mL, of TFA in the *TFA stock solution* taken:

$$C_S = 0.838 \times C$$

0.838 = molecular weight conversion factor (114.02/136.01)

C = concentration of USP Sodium Trifluoroacetate RS in the *TFA stock solution* (mg/mL)

TFA stock solution (option 2): 10 mg/mL of TFA in water prepared as follows. Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Transfer 670 µL of TFA to the flask, stopper immediately, and weigh. Dilute with water to volume.

System suitability solution: 0.025 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*

Standard solution (when TFA content is tested as a process impurity): 0.01 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*

Standard solution (when TFA content is tested as a counter ion in the API): 0.25 mg/mL of TFA in *Diluent* prepared from the *TFA stock solution*. [NOTE—The concentration can be adjusted depending on the amount of TFA expected to be present in the test material.]

Sample solution: NLT 4 mg/mL of test sample in *Diluent*. [NOTE—The sample concentration can be adjusted so that the limit (when TFA content is tested as a process impurity) or the mid-range (when TFA content is tested as a counter ion) of the amount of TFA 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. [NOTE—A guard column of 4-mm × 2-cm; 5-µm packing L1 can be used.]

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time is about 3 min for TFA.]

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of TFA in the portion of sample taken:

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

r_U = TFA peak responses from the *Sample solution*

r_S = TFA peak responses from the *Standard solution*

C_S = concentration of TFA in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Trifluoroacetate RS