

# HPLC Purity Determination for Short Peptides

Rev. 3 · Effective 2025-01 · Quality Assurance Department

## 1. Scope

This standard operating procedure describes the determination of chromatographic purity for synthetic peptides of 5–40 residues using reverse-phase high-performance liquid chromatography (RP-HPLC) with UV detection at 220 nm.

## 2. Principle

Peptides are separated on a C18 stationary phase using a binary gradient of water and acetonitrile, both modified with 0.1% trifluoroacetic acid (TFA). Purity is calculated as the integrated peak area at 220 nm relative to the total integrated absorbance, excluding the injection front and system peaks.

## 3. Instrumentation & Reagents

Item	Specification
HPLC system	Quaternary pump, autosampler, DAD detector
Column	C18, 4.6 × 250 mm, 5 µm, 100 Å
Mobile phase A	0.1% TFA in HPLC-grade water
Mobile phase B	0.1% TFA in HPLC-grade acetonitrile
Detection	UV at 220 nm (210 nm secondary)
Flow rate	1.0 mL/min
Column temperature	30 °C

## 4. Gradient Program

Time (min)	% B
0.0	5
2.0	5
22.0	65
24.0	95

26.0	95
27.0	5
32.0	5 (re-equilibration)

## 5. Sample Preparation

Reconstitute approximately 1.0 mg of lyophilized peptide in 1.0 mL of 0.1% TFA in water:acetonitrile (90:10). Vortex briefly and centrifuge at 12,000 × g for 2 minutes. Transfer 100 µL of the supernatant to an autosampler vial. Inject 20 µL.

## 6. Acceptance Criteria

Reported purity is the percentage area of the principal peak relative to the total integrated peak area between 2.0 and 26.0 minutes. Catalog material is released only when purity is  $\geq 95.0\%$  by area at 220 nm. Each lot is tested in duplicate; results must agree within  $\pm 0.5\%$ .

## 7. System Suitability

Prior to each batch run, the column is qualified using a reference standard mixture (angiotensin II, bradykinin, oxytocin). Resolution between adjacent peaks must be  $\geq 1.5$ ; tailing factor  $\leq 1.5$ ; theoretical plates  $\geq 8,000$ .

## 8. Documentation

Each chromatogram is archived in PDF format together with the integration table and is referenced in the lot's Certificate of Analysis. Raw data files are retained for a minimum of seven years.

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