

Where NPC matters

Is an NPC of 75% a sign of poor quality?

No. It simply reflects the moisture and counter-ion "tax" built into the peptide manufacturing process.

— DOSING PRECISION IN IN VIVO RESEARCH

In animal studies and clinical research, doses are calculated per kilogram of body weight based on the active ingredient. Confusing gross powder weight with net peptide weight leads to systematic underdosing, which skews pharmacokinetic and pharmacodynamic data.

— QUANTITATIVE IN VITRO ASSAYS

In biochemical assays, exact molar concentration determines valid results. A researcher who assumes 100% NPC will make stock solutions weaker than planned, leading to experiments that can't be reproduced.



Independent testing for peptides & small molecules

We're a contract analytical lab in Cleveland, Ohio. Send us a sealed vial and we'll tell you what's in it, how much is there, and how clean it is.

— WHAT WE TEST

- **Identity** by LC-MS (intact mass)
- **Quantity** by HPLC-UV against a reference standard
- **Purity** by HPLC-UV (% peak area)
- Multi-component blends and GLP-class peptides
- Endotoxin testing (LAL) add-on

— COLLECT THE SERIES

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erieanalytical.com
Cleveland, Ohio

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TECHNICAL BULLETIN • VOL. 1 • NO. 3

Quantity vs. Net Peptide Content

What your COA's numbers mean, and why 10 mg of powder isn't 10 mg of peptide.



Identity, quantity and purity

A common bundle of tests requested at Erie Coast Analytical is identity, quantity and purity. What do those terms mean on your Certificate of Analysis?

— IDENTITY

Confirms that the peptide in the vial is the peptide the label claims. The test checks the peptide's molecular mass against the value expected from its amino acid sequence. It gives assurance that no manufacturing mix-up occurred: no mislabeling, no substituted compound, no vial missing its peptide entirely.

Identity is tested by liquid chromatography-mass spectrometry (LC-MS), matching the sample's measured mass-to-charge ratios (m/z) to the expected values.

— QUANTITY

Determines the amount of target peptide in the vial, in milligrams. HPLC compares the sample's UV peak area to a calibration curve built from a certified reference standard. If your vial is labeled 10 mg, quantity tells you whether it holds 10 mg or some other amount.

— PURITY

Measures the percentage of target peptide relative to the other UV-absorbing peptide material in the sample, such as truncated sequences, deletion peptides, failed synthesis byproducts or degradation products.

High purity (e.g., over 98%) means nearly all of that detectable material is your desired peptide. It also reduces the risk of unwanted biological effects, immune reactions or experimental interference from contaminants.

Purity is calculated by comparing the target compound's peak area to the total area of all peaks in the HPLC chromatogram.

IDENTITY

tells you **what** your sample is.

QUANTITY

tells you **how much** of it is there.

PURITY

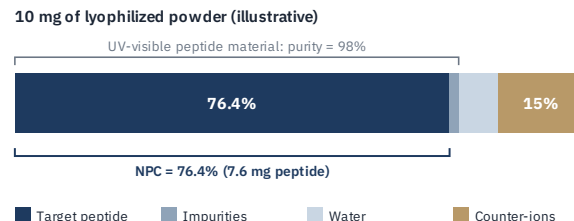
tells you **how clean** it is.

Quantity is often confused with net peptide content (NPC)

Be clear on what the quantity value (sometimes called "net content") on your COA tells you, and what it doesn't. HPLC typically uses a UV detector, which sees components by how they absorb UV light. Anything that doesn't absorb UV is effectively invisible to it.

Net peptide content is the percentage of the lyophilized powder's total weight that is actually peptide. The rest is non-peptide material the UV detector can't see: moisture, counter-ions such as trifluoroacetate (TFA) or acetate, and residual solvents.

Because the gross powder weight includes all of these, measuring NPC accurately takes specialized methods.



Two ways to measure it

The two most common ways of measuring NPC are (1) amino acid analysis and (2) the mass balance approach.

— AMINO ACID ANALYSIS (AAA)

AAA measures the absolute amount of peptide by taking the molecule apart and counting its building blocks. The sample is **hydrolyzed**: heated with acid (typically 6 N HCl at about 110 °C) to break every peptide bond.

The freed amino acids are chemically tagged to make them easier to detect, separated by HPLC, and each one is quantified against certified amino acid standards.

— MASS BALANCE APPROACH

Mass balance calculates NPC by subtracting every measured non-peptide fraction (e.g., moisture and counter-ions) from the total weight, then adjusting for purity.

- **Water:** Karl Fischer (KF) titration measures the moisture trapped in the lyophilized cake.
- **Counter-ions:** measured directly by ion chromatography, or estimated stoichiometrically.

Stoichiometric estimation calculates the theoretical counter-ion fraction from the peptide's sequence, counting its basic sites (Lys, Arg, His and the free N-terminus) and multiplying by the known salt form. NPC can also be assigned by quantitative HPLC against a reference standard of the same peptide with a certified NPC.

— PUTTING IT TOGETHER

$$\text{NPC (\%)} = \text{Purity (\%)} \times [\text{100\%} - \text{Water (\%)} - \text{Counter-ion (\%)}] \div 100$$

As the equation shows, NPC is always lower than the HPLC purity value, because it excludes moisture and counter-ion content. Purity is often above 95%, while NPC commonly lands around 70 to 85%.