

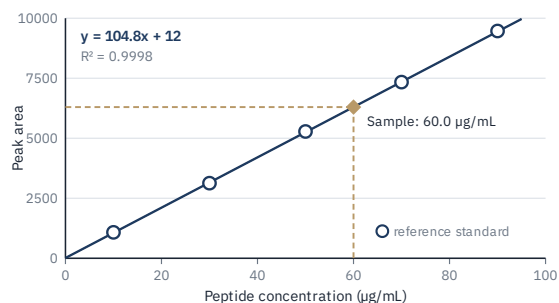
SIDEBAR

How is quantity determined?

Components are told apart by their separation in time. Figuring out *how much* of a component is present takes a **reference standard**: a precisely known amount of the peptide of interest, run through the same HPLC.

Because peak area is directly proportional to concentration, the standard's peak area lets us calculate the amount of peptide in the sample.

For high accuracy, we build a multi-point **calibration curve**. Several known concentrations of the reference standard are prepared and injected, and a straight line (linear regression) is fit through the resulting peak areas.



Five-point calibration curve. The sample's peak area is read off the line to find its concentration.

When the sample is injected, its peak area is measured and plugged into the calibration curve's equation, and the actual quantity in the sample is calculated with high accuracy.



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

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Vol 1 • 2 How Does HPLC Work?

Vol 1 • 3 Quantity vs. Net Peptide Content



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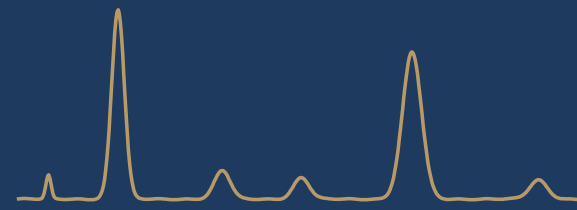
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TECHNICAL BULLETIN • VOL. 1 • NO. 2

How Does HPLC Work?

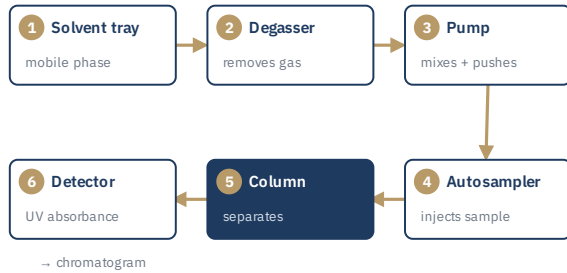
A walk through the instrument, from solvent bottle to chromatogram.



INSIDE THE INSTRUMENT

How does HPLC work?

A high performance liquid chromatograph is built from six main parts working together.



Together, these components take in a sample and output a **chromatogram**: a graph showing the sample's components (the target compound, or *analyte*, and its impurities) separated in time. In short, HPLC is a separation technique.

THE SOLVENT TRAY AND DEGASSER

The solvents that make up the **mobile phase** (e.g., water and methanol or acetonitrile) sit in individual bottles in the solvent tray atop the HPLC.

Each solvent line runs to a degasser, which removes dissolved air from the solvent before it reaches the pump, so bubbles don't form and disturb the flow or the detector signal.

THE PUMP AND AUTOSAMPLER

The solvent lines connect to a pump which, under software control, mixes the solvents in the ratio set by the test method developed for that sample. For example, the ratio might be 90% water and 10% acetonitrile, and it can stay fixed or change during the run.

The autosampler draws the sample from its vial and injects it into the flowing mobile phase, which carries it to the column.

THE COLUMN

In one sense, the column is where the "magic" happens. It's a narrow tube packed tightly with tiny silica beads. The beads carry a surface coating (C18 is common) that forms the **stationary phase**.

The mobile phase carries the sample in one end of the column. On the way through, each component (the target compound and its various impurities) has a different affinity for the stationary phase.

Think of it as different amounts of "stickiness." Each component takes a different amount of time to travel the length of the column, so they exit separated in time.

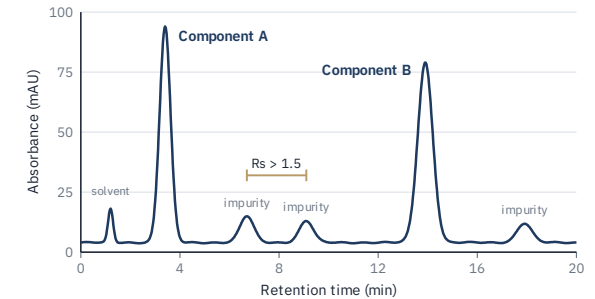
THE DETECTOR

As the mobile phase leaves the column, it passes through an ultraviolet (UV) detector. Each separated component absorbs some of the UV light as it passes through, and the detector records when it happened and how much light was absorbed.

The result is a peak. Its size reflects the amount absorbed (more compound = more absorbance), and its position is the **retention time** produced by the column's separation.

READING THE OUTPUT

An example chromatogram



Two main components plus impurity peaks, all cleanly separated.

The graph above shows two main component peaks and several smaller impurity peaks. A peptide blend, such as BPC-157 and TB-500, would look like this.

This method is well designed: the resolution (**Rs**) between neighboring peaks is above 1.5, which means baseline separation. No peaks overlap, and each one is distinct, with no visible co-elution at the column exit. (Confirming that no hidden compound sits under a peak is the job of mass spectrometry.)

How is purity determined?

The purity on your Certificate of Analysis (COA) is a relative measurement of the target peptide against its related impurities (e.g., truncated or deletion sequences). The sample is injected, and the detector measures the area under every absorbing peak: the target and any impurities.

Purity is the main peak's area divided by the total area of all the peaks. If the main peak accounts for 95% of the total peak area, purity is reported as 95%.