used in HPLC measures the UV absorption of the elute at one or more wavelengths, or, in the case of a diode array detector, it can scan all the wavelengths simultaneously and provide a clear quantification of each separated protein. Other detection methods sometimes used with HPLC separations are evaporative light scattering and refractive index. The three most common types of HPLC are SEC, which separates based on the size or MW of the protein; ion-exchange (IEX) chromatography, which separates based on the charge of the protein; and reverse-phase (RP) chromatography, which separates based on the hydrophobicity of the protein. The RP is such a common HPLC method that when people do not specify a particular HPLC method, they are usually referring to RP-HPLC.

In RP-HPLC, separation of proteins is accomplished by differential interaction with the column matrix and the column buffer. Two buffers, called the aqueous buffer and the organic buffer (thus identifying the most important attribute of each), are used, and the separation is done with a gradient of these buffers. The most common organic buffers are based on acetonitrile, though other organic solvents, such as methanol and tetrahydrofuran, may be used. The column used for the RP-HPLC separation is most commonly a silica base, coated with hydrocarbon chains of varying sizes, such as C4, C8, and C18. The RP columns built on polymer backbones are becoming more readily available. To minimize any nonspecific interaction between the protein and the column matrix, an ion-pairing component, frequently trifluoroacetic acid, is added to both the aqueous and organic buffers. After the column is equilibrated with either the aqueous buffer or a defined mixture of the aqueous and organic buffers, the sample is loaded onto the column as an aqueous solution. Separation of the varying proteins is done by running a gradient of increasing organic buffer; proteins are resolubilized when the hydrophobic nature of the particular protein partitions into the buffer. The use of highly hydrophobic buffers for RP-HPLC usually precludes the presence of large amounts of salt, which destabilizes some proteins. In addition, proteins are denatured in RP-HPLC, and so, tertiary and quaternary structures are lost. The subunits of multisubunit proteins will usually elute separately. Multiple forms of a protein can usually be separated in RP-HPLC by their small differences of hydrophobicity and sometimes MW. The RP-HPLC is often considered to be a good method to separate related isoforms of a protein.

The IEX-HPLC separates molecules based on charge. The protein interacts with the charged moiety on the column and is then eluted with either salt or pH gradients. Elution from the column is from the weakest to the strongest bound. The protein solution is loaded onto a column that has been charged with the counterion and is then equilibrated with the starting buffer. Proteins are eluted from the column by a gradient of either salt or pH. If the column with a second buffer contains salt, this disrupts the protein interaction with the column and replaces the protein with the counterion. If the second buffer changes the pH, this alters the charge on the protein and decreases the interaction of the protein with the column. The IEX columns can be either anionic or cationic. The most common anion exchangers are quaternary ammonium, diaminoethyl, and quaternary aminoethyl, and the most common cation exchangers are sulfopropyl, methyl sulfonate, and carboxymethyl. By using buffers above or below the protein's pI, the same protein can be analyzed on both anionic and cationic columns. Because of the ionic nature of interaction between the protein and the column, the size of the protein does not affect binding. In addition, because IEX is run in an aqueous environment, the protein is not denatured and maintains