interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation-exchange chromatography (CIEC) and anion-exchange chromatography (AIEC). The ionic compound consisting of the cationic species M^+ and the anionic species B^- can be retained by the stationary phase. CIEC retains positively charged cations because the stationary phase displays a negatively charged functional group. AIEC retains anions using positively charged functional group. Note that the ion strength of either C^+ or A^- in the mobile phase can be adjusted to shift the equilibrium position and thus the retention time.

5.2.3.2 Reverse-phase chromatography (RPC) RPC, also called hydrophobic chromatography, includes any chromatographic method that uses a hydrophobic stationary phase. The hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and the hydrophilic molecules in the mobile phase will pass through the column and they are eluted first. Hydrophobic molecules can be eluted from the column by decreasing the polarity of the mobile phase using an organic (nonpolar) solvent, which reduces hydrophobic interactions. The more hydrophobic the molecule, the more strongly it will bind to the stationary phase and the higher the concentration of organic solvent that will be required to elute the molecule.

5.2.3.3 High-performance IEXC (HP-IEXC) Analytical HPLC offers a high level of resolution and precision making the technology available for identity, purity, and quantity determinations. HP-IEXC is based on highly specific analytical columns comprising monodisperse particles with a diameter of 5–10 μm separating proteins according to their electrical charge. The resolution may be comparable to that of HP-RPC, and the technology will apply to almost all types of globular proteins. The HP-IEXC is used for the detection of target protein–related compounds (e.g., des-amido forms, oxidized forms, scrambled forms, cleaved forms), which may be present in amounts from 1 ppt and upward. The resulting UV diagram provides an impurity profile within the relatively narrow window offered by the technology, but it should be kept in mind that not all impurities are detected by this or similar methods. Impurities present in 0.1% or higher should be fully characterized no later than phase 3 of the manufacture. HP-IEXC offers two separation modes: CIEC and AIEC. In CIEC, positively charged biomolecules are typically retained due to interaction with negatively charged groups (e.g., sulfonic acid) on the surface of the chromatographic resin. The buffer pH must favor a net charge of the biomolecule lower than pI in order to maintain separation. CIEC primarily retains biomolecules by the interaction with histidine, lysine, and arginine (pK_as of about 6.5, 10, and 12, respectively). In AIEC, negatively charged biomolecules are typically kept due to interaction with positively charged groups (e.g., quaternary amine) on the surface of the chromatographic resin. The buffer pH must favor a net