HP-RPC, native PAGE, IEF, MS, or CE. The content accepted depends on the nature of the drug product and the dose.

5.3.4.10 Oxidized forms Oxidized forms are target protein derivatives in which one or several Met, Cys, His, Trp, and Tyr residues have been oxidized. The oxidation of cysteinyl residues results in the formation of a disulfide bond (cystinyl residue). The oxidation reaction is slow at low pH and low temperature. The formation of disulfide bonds will take place above 0 mV. Oxidized forms are removed by HP-IEC and HP-RPC. Disulfide aggregates can be removed by SEC. Oxidized forms are detected by analytical HP-IEC, HP-RPC, native PAGE, IEF, MS, or CE. The content accepted depends on the nature of the drug product and the dose.

5.3.4.11 Carbamylated forms Carbamylated forms are target protein derivatives in which one or several primary amino, sulfhydryl, carboxyl, phenolic hydroxyl, imidazole, and phosphate groups react with cyanate to form a derivative. The blocking may change the pI of the protein. Cyanate is spontaneously formed in urea solutions, which is the primary source. The formation is slow at low temperatures, but it is nevertheless strongly recommended to purify the urea solution by means of mixed ion exchangers before use. Carbamylated forms are removed by HP-IEC and HP-RPC and detected by analytical HP-IEC, HP-RPC, native PAGE, IEF, MS, or CE. The content accepted depends on the nature of the drug product and the dose.

5.3.4.12 Aggregates Aggregates are target protein derivatives in which two or more molecules is linked together either by covalent interdisulfide bonds or by hydrophobic interaction. Target protein aggregates are formed as a result of hydrophobic intermolecular reactions or because of intermolecular disulfide bond formation under oxidizing conditions. Aggregates are very often antigenic resulting in the formation of target protein antibodies. Proteins exposed to even mildly denaturing conditions may partially unfold resulting in exposure of hydrophobic residues to the aqueous solvent favoring aggregation. The aggregation process is assumed to be controlled by the initial dimerization step in a second-order reaction. Consequently, high protein concentrations will increase the aggregation rate. Intermolecular disulfide bond formation between cysteinyl residues takes place at alkaline pH under oxidizing conditions. Proteins with reactive-free thiol groups should be purified under reducing conditions (typically 1–10 mM reducing agent) in the presence of ethylenediaminetetraacetic acid. Even proteins with disulfide bonds may participate in intermolecular disulfide bond reactions due to disulfide bond shuffling at neutral and alkaline pH. The aggregation reaction based on intermolecular disulfide bond formation is prevented at pH < 6 and under reducing conditions. The hydrophobic aggregation reaction strongly depends on the hydrophobicity of the molecule. Preventive