charles river

Summary
Process- and product-related residual and impurity testing using sound analytical methods is important to ensure the safety of biopharmaceutical products.

BIOLOGICS TESTING SOLUTIONS

Process- and Product-Related Residuals

In the course of product manufacturing, a number of chemicals are typically added upstream as part of the bioprocess to improve on product expression and recovery. These chemicals are almost entirely removed during the downstream purification process; however, residual levels of these agents may be left within the bulk drug substance and drug product. To ensure that the level of these agents is within safe amounts, highly sensitive analytical methods need to be used for detection and quantification of such residuals.

Along with process residuals, there is also the possibility for product-related impurities to be carried through the manufacturing and purification process. These impurities, including host cell proteins, host cell DNA, N-terminal truncations and other potential modifications, may cause adverse reactions in animals and humans, and therefore, assays must be employed to ensure that any impurities present are below pre-determined acceptable levels.

While generic methods for analysis of residuals and impurities may be available, it is important that, prior to routine sample analysis, each method be evaluated for interference from buffers, excipients and protein load. Also the limit of detection (LOD) and limit of quantitation (LOQ) using the client's sample and matrices should be assessed and noted. Prior to using these assays for cGMP lot release programs, qualification and/or ICH validation of these methods is required.

Table 1: Process-Related Residuals

Residual	Method Description	LOQ (ppm)[1]	LOD (ppm)[1]
IPTG	HPLC with ELSD/CAD	6	< 1
PEI	HPLC with ELSD/Fluorescence	10/< 2	1-2/< 0.5
Kanamycin	HPLC with UV/Vis	0.03	< 0.01
Anti-foam	HPLC with ELSD/CAD	1	0.1
TFA/Acetate	HPLC with UV/Vis	1	0.1
Cyanate	HPLC with Conductivity/ECD	0.6	0.1
Imidazole	HPLC with Fluorescence	0.13	< 0.1
Triton X-100	HPLC with ELSD/CAD	5	1
Polysorbate (tween) 20/80	HPLC with ELSD/CAD	5	1
Zwittergent	HPLC with ELSD/CAD	1-5	< 1
Pluronic	HPLC with ELSD/CAD	1-5	< 1
Protein A	ELISA	0.001-0.10	< 0.001
PEG	HPLC with ELSD/CAD	1-10	< 0.5
TRIS	HPLC	1-10	< 0.5
Growth Factors (e.g., insulin, interferon)	ELISA	0.001-0.10	< 0.001

1. ppm = weight of analyte per weight of solution

Table 2: Product-Related Impurities

Impurity	Method Description	LOQ (ppm)	LOD (ppm)
Host cell proteins (HCP)	Commercial kit or customized assay	0.001-0.010	< 0.001
N-terminal truncations	N-terminal sequencing	> 10%	
Host cell DNA	PCR	Method- and product-dependent	
Oxidation/deamidation (PTMs)	Peptide mapping LC-MS or LC-MS/MS, IsoQuant HPLC		
Isoform analysis	Ion exchange HPLC, cIEF, sialic acid analysis		
Disulfide bridge scrambling (mis-folding); conformational impurities	Peptide mapping LC-MS or LC-MS/MS, Ellman's free sulfhydryl, intrinsic tryptophan fluorescence, ANS dye binding, NMR		
C-terminal integrity	Peptide mapping LC-MS or LC-MS/MS		
Aggregation	Analytical ultracentrifugation (AUC), SEC/SEC-MALLS, dynamic light scattering (DLS)		