Millipore®
Preparation, Separation,
Filtration & Monitoring Products

White Paper

A practical approach for determining extractables in chromatography resins in the light of the upcoming USP chapter <665>

Maren Apostel, Emprove® Program Manager, Merck KGaA, Darmstadt, Germany

To improve productivity and efficiency, biopharmaceutical manufacturers have introduced innovative processes which include flexible, customizable and easy-to-install solutions such as single-use systems. In parallel with introducing new approaches to manufacturing, detailed risk assessment rules must be adhered to in order to assess all risks that might compromise patient safety. Manufacturers must then substantiate the outcomes of their assessments with reliable data provided to regulatory authorities.

Newer technologies such as single-use have led to extended risk management requirements in drug manufacturing; regulators around the world have recently supplemented or refined their respective standards, or are in the process of doing so.

The need to assure patient safety includes assessment of risks throughout the entire manufacturing process, which explicitly extends to production equipment. Traceability is therefore required for the entire supply chain of raw materials and manufacturing systems; all process steps and materials incorporated into those processes must be investigated.

In its GMP General Guidelines, the US FDA states the objective of equipment-related specifications as follows:

"Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."[1]

The US Pharmacopeia (USP) addresses the issue of production equipment and its potential influences on patient safety in a new chapter, USP <665>, titled "Plastic Components and Systems used to manufacture Pharmaceutical Drug Product and Biopharmaceutical Drug Substanceas and Products."[2], currently in draft status. USP <665> will be a new regulatory standard covering requirements for a broad range of polymeric components and systems in drug manufacturing.

When considering chromatographic steps, only columns are in scope of USP <665> draft, but as chromatography is an indispensable step in many drug production processes, it makes sense to apply the future USP <665> also to resins made up of polymeric base bead materials.

This white paper provides an overview of the upcoming USP <665> and describes our study which systematically examined chromatography resins in bulk material, assessing the risk profiles of various resins commonly used in drug manufacturing.

MERCK

Chromatography Resins in the Manufacturing Process

Separation and purification of the molecule from a complex feed stream is among the most important steps in biopharmaceutical manufacturing. Chromatography is essential for this process and to deliver the necessary purity and yield of the molecule from upstream processes. Moreover, chromatography enables robust and cost-efficient manufacturing – a vital aspect given the highly regulated environment, in which accelerated timelines and efficiency are critical.

Any component or material used at a later stage of the manufacturing process, such as chromatography resin, is considered a risk, as it could add impurities to the final drug product – and thus represent a potential risk for patient safety.

The chromatography steps used in biopharmaceutical production vary considerably, depending on the respective molecule type, chromatography technique and, most importantly, on the process stage. Most purification systems include multiple chromatography steps (see Figure 1) to achieve the level of purity needed for downstream applications. Selecting the most appropriate chromatographic methods and the sequence of the methods applied is essential to optimizing the purification process. However there are also challenges to overcome, such as finding the optimal procedure for individually packing the chromatography columns. Evaluating the risk of extractables in connection with the polymeric chromatography resins used is a further challenge that needs to be addressed.

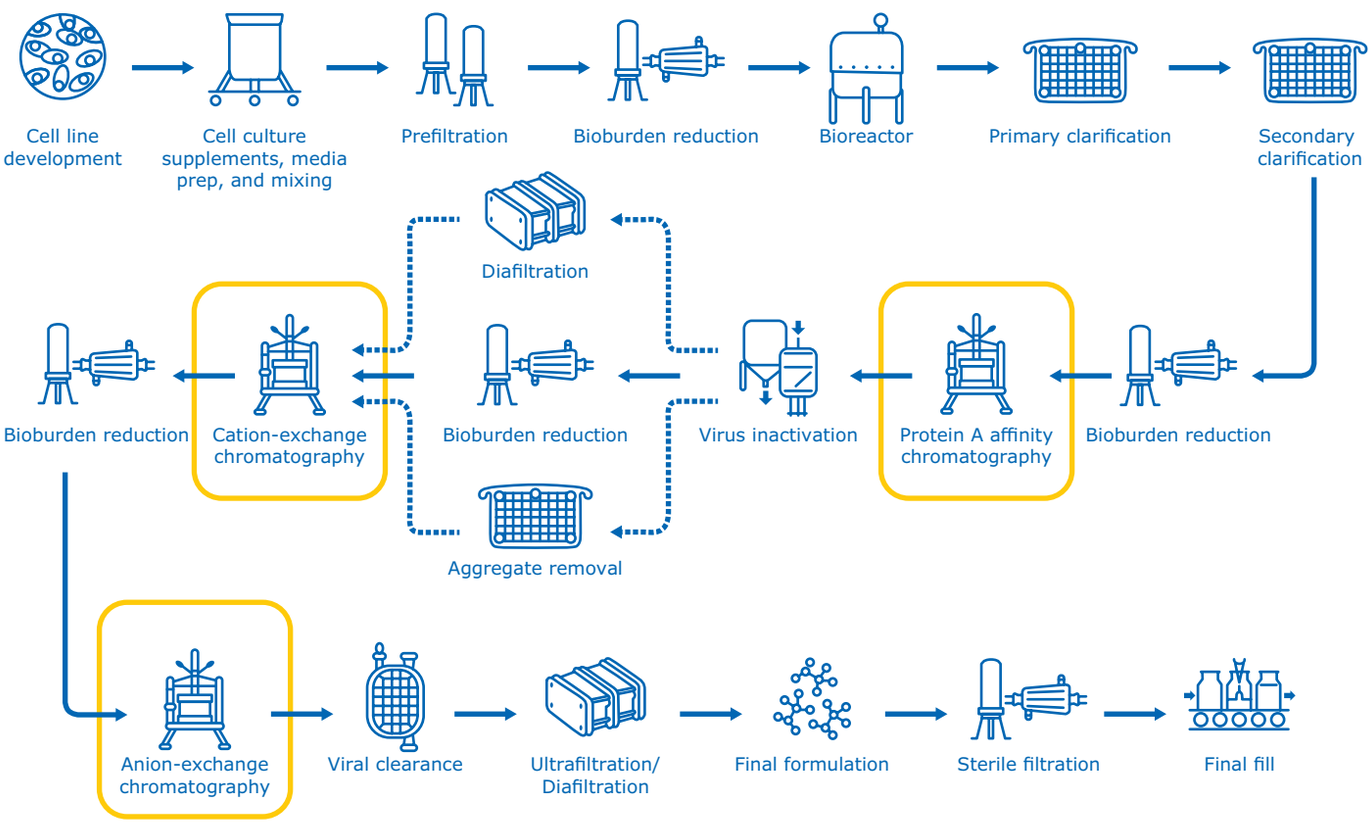

Figure 1. The manufacturing process for monoclonal antibodies (mAbs) typically includes multiple chromatography steps.

New Requirements for Plastic Materials and Components: USP <665> and USP <1665>

As shown in Figure 1, polymeric components – including chromatography resins – come into contact with the drug substance at various points in the manufacturing process. Such interactions can introduce compounds called Process Equipment Related Leachables (PERLs) which are organic or inorganic compounds that passively migrate into drug substances or drug products and that have the potential to alter their key quality attributes.

To help ensure that PERLs do not compromise patient safety, the future USP <665> defines new requirements. The chapter includes specifications on how to assess polymeric components for extractables, the chemical entities that can be released by a process system under certain conditions, such as high heat or pressure, as well as powerful acids, organic solvents and alkaline solutions. These conditions represent a worst-case scenario and do not occur in a standard manufacturing process. Leachables are typically a subset of extractables and are the chemical entities that actually migrate into a drug product under normal manufacturing conditions.

According to USP <665> draft, a manufacturing system has to be composed of materials and components that are proven safe for use with the pharmaceutical or biopharmaceutical product, including all process intermediates or process streams. The information chapter, USP <1665>[3] provides additional information for the characterization of polymeric components used in the manufacturing process. The purpose of these requirements is to differentiate between those manufacturing components or systems that should be fully evaluated for organic extractables and those that require only minimal testing. It should be noted that the specifications presented in USP <1665> offer general guidance for the testing requirements; process owners are ultimately responsible for performing and verifying their risk evaluation.

The characterization includes an initial assessment to exclude those components that do not require any testing. This step is followed by a risk assessment for the remaining components, the outcome of which will determine the level of testing required for extractables.

The Initial Assessment

As defined in USP the upcoming <665>, the initial assessment determines whether a plastic material, component or system is suitable for its intended use without further characterization. The first and second steps of the initial assessment evaluate whether there is meaningful contact between a component and a process stream, as well as the physical state of that process stream, i.e., whether it is liquid. The final step examines whether the respective component is equivalent to a comparator, that is, an equivalent system used to produce an already approved and marketed drug product. If the assessed component is isolated from the process stream, does not come into contact with a liquid, or if a comparator system can be established, then no further characterization is required (Figure 2).

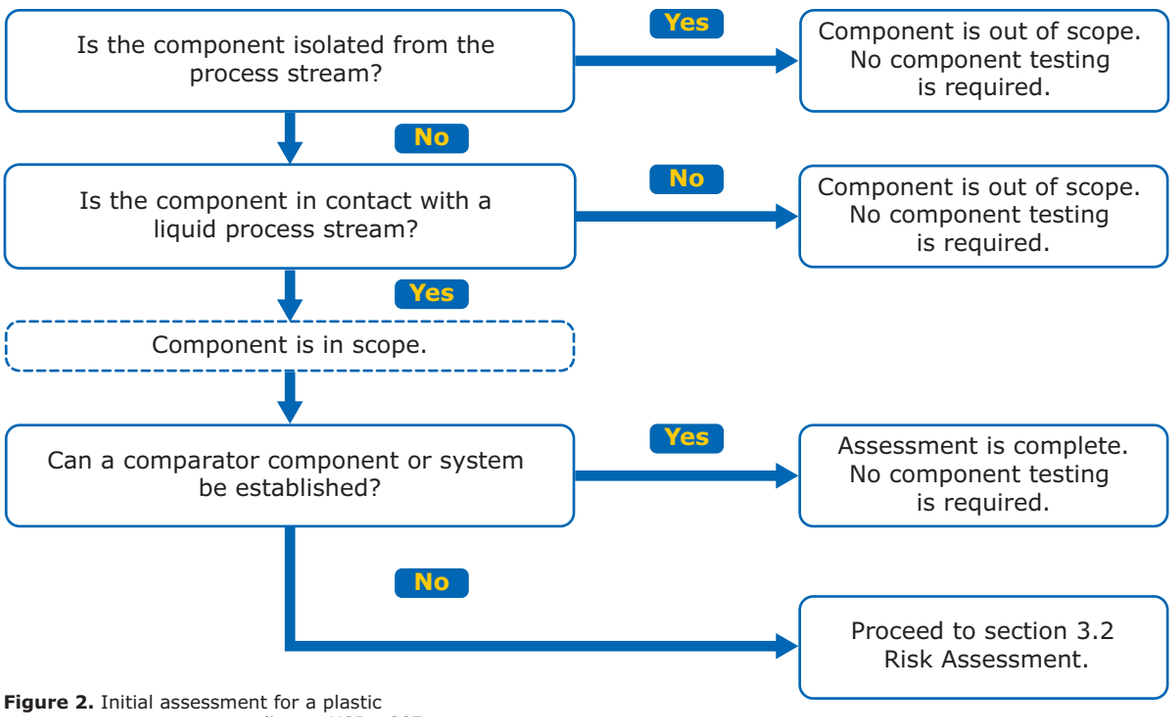

Figure 2. Initial assessment for a plastic component or system according to USP <665>.

The Risk Assessment

According to USP <1665>, the risk assessment for in-scope components or materials employs a risk evaluation matrix that assigns three risk levels and considers four aspects:

1. Duration of contact
2. Temperature of contact
3. Chemical nature of the process stream
4. Nature of the component material

The matrix considers each aspect separately, and rates it as level 1 (low risk), 2 (medium risk) or 3 (high risk). Detailed evaluation criteria are provided Tables 1–3.

Risk Level	Organic solvents (by volume)	Surfactants (by weight)	Blood/blood-derived substances (by weight)	Lipids and proteins (by weight)	pH
Aqueous Level 1	<5%	<0.1%	<1%	<1%	≥3 and ≤9
Somewhat organic Level 2	5–40%	0.1–0.5%	1–25%	1–5%	–
Highly organic Level 3	>40%	>0.5%	>25%	>5%	<3 or >9

Table 1. Risk dimensions as defined per USP <1665> for the chemical nature of the process stream.

Risk Level	Additives (by weight)	Treatment for sterilization	Processing
Inert Level 1	<0.1%	–	–
Intermediate Level 2	0.1–1%	–	chemical adhesives/ bonding of component's materials
Reactive Level 3	>1%	Irradiation/ chemical treatment	chemical adhesives/ bonding of component's materials

Table 2. Risk dimensions per USP <1665> for material of construction.

Risk Level	Temperature (°C)	Duration
Level 1	Frozen (<-10)	<24 hrs
Level 2	Refrigerated (2–8) Ambient (15–25)	1–7 days
Level 3	Elevated (>30)	>7 days

Table 3. Risk dimensions per USP <1665> for temperature and duration.

The four risk levels for the different dimensions are summarized resulting in a final risk level (low, moderate or high). By using mitigating factors, the characterization level can be adjusted by implementing additional manufacturing steps or adapting the clinical use, for example. Consequently, a high risk level can be reduced by taking into account circumstances such as the removal of PERLs via one or more manufacturing steps and modifying the clinical use of the manufactured drug product, such as the daily dose volume or the duration of clinical use. The final characterization level is then linked to the level of assessment required (Table 4).

Risk Level	Extraction Solutions for Chemical Testing	Chemical Testing of Extracts
Low	50% Ethanol (v/v)	• Non-volatile residue • UV absorbance
Moderate	50% Ethanol (v/v)	• Organic extractables profiling
High	50% Ethanol (v/v) PBS buffer pH 10 KCl solution pH 3	• Organic extractables profiling • Extractables elements (as necessary and appropriate)

Table 4. Testing requirements according to upcoming USP <665>.

Adapting the upcoming USP <665> Approach to Chromatography Resins

Components or materials used late in the process, such as for chromatographic purification, generally pose a higher risk, because at this stage, few opportunities remain for removing PERLs or other impurities. Because chromatography steps are tailored for specific molecules and processes, they can vary considerably. In addition, the exact downstream location of these steps can vary from process to process. Therefore, there is a pressing need to evaluate the risk profiles of individual chromatography resins in connection with specific process steps, and to use their extractable profiles to support a comprehensive risk assessment process.

Our team approached this task by systematically assessing our portfolio of chromatography resins. Extractables profiles were determined for bulk chromatography resins only, without the chromatographic column hardware such as housing, frits, gaskets, connectors or tubes. Therefore, slight adjustments in sample treatment and analytical procedure were required.

Another reason why the requirements had to be adapted is that chromatography resins are not used in a dry state; they are delivered in a storage solution (e.g. 20% ethanol/150 mM/L NaCl) that needs to be removed prior to use in the downstream process. In a pre-treatment step the resins have to be washed, replacing the storage solvent with water or buffer solutions, followed by a packing procedure-, as the chromatography material is used in a packed bed. Compression rates vary in a specific range. These pre-treatment steps are usually process-specific.

By applying the USP <1665> risk assessments matrix for affinity and ion exchange steps in a typical mAb process as shown in Figure 1, the chromatography steps would be considered as low to moderate risk components. Table 5 summarizes the different risk levels for each dimension and the resulting risk level. This example assessment could only give a direction for the final risk level as the specific process has to be assessed individually. Low to moderate risk level would only require extractables testing in 50% ethanol.

Product Group	Material	Duration	Tem-perature	Process Stream	Risk Level
Affinity resins	1	1	2	2–3	Low-moderate
Ion exchange resins	1	1	2	2–3	Low-moderate

Table 5. Risk evaluation examples based on rules of USP <1665>.

However, we decided to apply the full testing standards to support a worst case approach that a customer might encounter. Manufacturing processes are highly specific and chromatography steps can be – and are likely to be – located nearer to the final drug. Accordingly, data were generated for three extraction solvents – pH 3, pH 10 and 50% ethanol – as is required for a high risk level component according to USP <665> (see Table 4).

The applied pre-treatment procedure used in this study was washing with 10 bed volumes of water. Extraction buffers and solvents were used in a higher concentration to allow the dilution by the water remaining in the resin. The extraction was performed within 24 hours and at 40°C. Extracts were analysed applying the analytical methods displayed in Table 6.

Method	Extractables
HS GC/MS headspace gas chromatography-mass spectrometry	Volatile substances
GC-FID/MS gas chromatography-flame ionization detector-mass spectrometry	Semi-volatile substances
HPLC-DAD/MS high-performance liquid chromatography coupled with diode-array detection and mass spectrometry	Non-volatile substances

Table 6. Analytical methods used for extractables profiling.

In these tests, the extractable profiles of all evaluated chromatography resins resulted in only few components that could be detected, and in very small amounts. Most of the components detected could be traced back to the base matrix or to additives deriving from the packaging material. For the extractable profile (Table 7) of the Eshmuno® Q resin, for example, researchers found low amounts of acetaldehyde and 1,4 butanediol which are degradation components connected to the base matrix of the resin. Other components were found in amounts close to detection limits and included ethanol, used as shipping solution, 2-hydroxytetrahydrofuran and dichloromethane; these might derive from the base matrix under harsh conditions. Overall, the data showed that the probability of chromatography resins adding significant risk to the safety of a drug product can be considered low.

For the determination of elemental impurities, a separate worst-case approach was used, in line with ICH Q3D.

Extraction solvent (Condition procedure)	Substance	CAS	Identification level	Standard used for quantification	Method and detection mode
pH = 3	Acetaldehyde	75-07-0	confirmed	external	HS-GC-MS
pH = 3	Dichloromethane	75-09-2	confirmed	external	HS-GC-MS
pH = 10	Ethanol	64-17-5	confirmed	external	HS-GC-MS
50% aqueous ethanol (v/v)	2-Hydroxytetra-hydrofuran	5371-52-8	tentative	external	GC-FID/MS
50% aqueous ethanol (v/v)	1,4-Butanediol	110-63-4	confirmed	external	GC-FID/MS
50% aqueous ethanol (v/v)	unknown	–	unknown	external	HPLC-DAD/MS

Table 7. Extractable Profile of Chromatography Resin: Example Profile for Eshmuno® Q.

Supporting Our Customers' Risk Assessments

However, what is true for chromatography resins cannot simply be assumed to apply to the manufacturing process as a whole. After all, every customer's manufacturing process is unique and chromatography resins are just one part of it. Therefore, biopharmaceutical companies assess their entire manufacturing process. Among a multitude of other parameters, to take into account are, for example the resins in their individually packed chromatography columns, the buffers used in processing, and the resins' time of contact during the purification process.

Given this complexity, open and structured communication between drug manufacturers and chromatography resins suppliers is essential and can help ensure a successful and comprehensive risk assessment. In addition, suppliers should actively contribute to the drug manufacturer's risk assessment process by following established guidelines, fulfilling information requirements and providing documentation in a timely manner, ideally bundled in packages and transmitted electronically.

Our Emprove® Program was designed to simplify this process, help drug manufacturers overcome these challenges, and help them supply the required documentation. The Emprove® Chromatography Dossiers include:

- Material Qualification Dossier, which provide general product information.
- Quality Management Dossier, which offer supply chain information and stability data.
- Operational Excellence Dossier, which supports process optimization efforts as well as extended and safety risk assessment.

Extractable profiles of chromatography resins according to the USP <665> requirements are part of the Operational Excellence Dossier. Moreover, it includes information on elemental impurities, residual solvents, periodic product reports and analytical procedures.

This information can help pharmaceutical manufacturers efficiently implement a compliant and sustainable risk assessment process, making them optimally prepared for inspections.

Reference

1 FDA, 21 Code of Federal Regulations, Part 211, "Current Good Manufacturing Practice for Finished Pharmaceuticals".

2 USP <665> Plastic Components and Systems used to manufacture Pharmaceutical Drug Product and Biopharmaceutical Drug Substanceas and Products, September 1st, 2020.

3 USP <1665> Characterization of Plastic Components and Systems used to manufacture Pharmaceutical Drug Product and Biopharmaceutical Drug Substanceas and Products, September 1st, 2020.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

For additional information, please visit **www.MerckMillipore.com**
To place an order or receive technical assistance, please visit **www.MerckMillipore.com/contactPS**

Lit. No. MK_WP6689EN Ver 1.0
10/2020