Safety and Innovation: Navigating the Challenges of Antibody-Drug Conjugates

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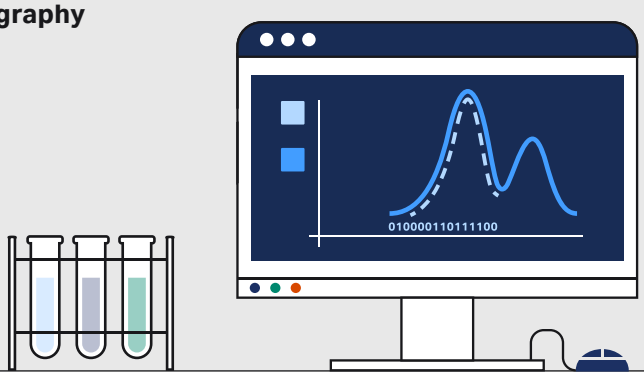

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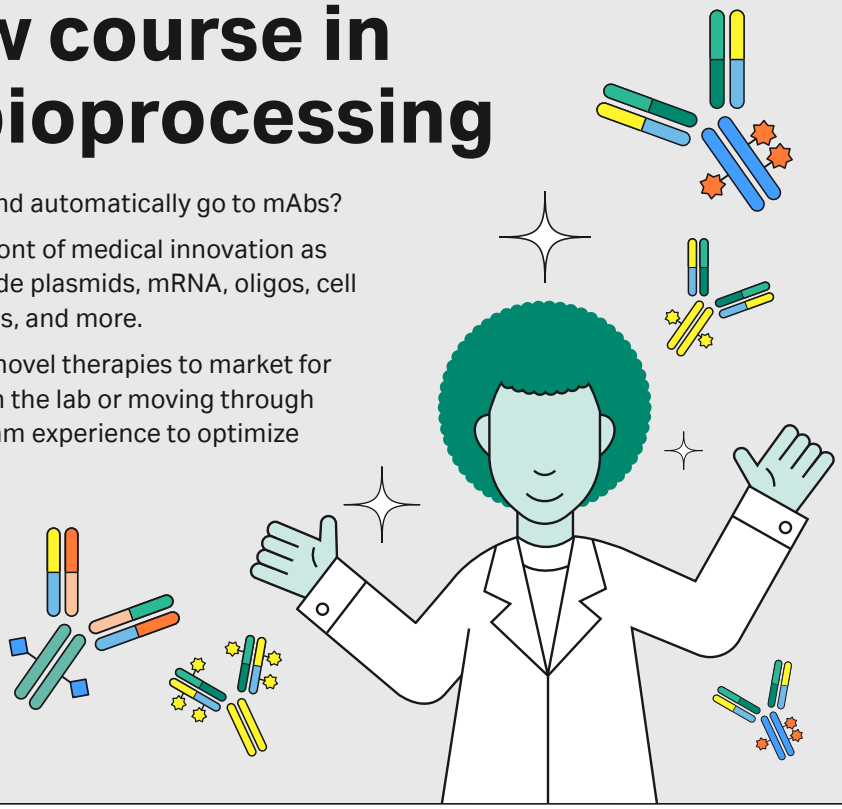

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Introduction

Antibody-drug conjugates (ADCs) are among the most advanced and innovative class of targeted cancer therapeutics, combining the high specificity of monoclonal antibodies (mAbs) with the delivery of very potent cytotoxic drugs directly to the tumor cells. At the center of the ADC is a humanized mAb engineered to selectively attach to the antigen overexpressed on the surface of target (e.g., cancer) cells. After binding to the target antigen, the ADC is internalized inside the cancer cell where it undergoes intracellular processing, ultimately releasing the cytotoxic payload. Conjugation of the antibody to the drug is usually accomplished with a cleavable linker to allow controlled release of the cytotoxic agent in the target cell. Advanced developments in the fields of antibody engineering and linker technologies offer the capability to design more stable, less cross-reactive next-generation ADCs.

However, ADCs are limited by a series of hurdles such as off-target effects, ineffective payload delivery, and treatment resistance. Current research approaches are aimed at overcoming these hurdles, especially rational design strategies that offer new avenues for achieving even greater specificity at the tumor site and increasing intracellular drug release.

ADCs are also being investigated for potential non-cancer applications including inflammatory diseases, infectious diseases, neurological disorders, autoimmune diseases, cardiovascular diseases, and rare genetic disorders.

This Expert Insights eBook begins with a report by Pradhan *et al.* [1] on how the shift from synthetic drugs to biologics, particularly ADCs, has transformed medical care with targeted delivery and reduced adverse effects. As of late 2023, there are 13 US FDA approved ADCs [2] reflecting growing interest and investment. Despite early challenges, second-generation ADCs like brentuximab vedotin and ado-trastuzumab emtansine show improved efficacy and safety over existing standard-of-care drugs in treating cancers, offering highly tumor-specific and effective therapies with reduced systemic toxicity. ADCs represent a promising strategy to address various diseases with enhanced safety and tolerability, as outlined in this comprehensive review.

Next, Ross *et al.* [3] describe how the manufacturing process of ADCs involves several critical steps, including activation of the mAb, conjugation of the mAb

with a cytotoxic agent through a linker, purification, formulation, and storage. Quality control throughout these stages is essential to maintain the safety, efficacy, and stability of the ADC. In addition, the purification and formulation stages must be designed to address the solubility and stability challenges unique to ADCs. Comprehensive characterization techniques must be employed to ensure product quality, including stability and purity, with an emphasis on monitoring critical quality attributes. The field is moving towards simplifying conjugation processes and employing next-generation chemistries to expand the therapeutic potential of ADCs, aiming for safer and more effective treatments.

Finally, Sussman *et al.* [4] discuss how the handling of ADCs raises significant safety concerns for those involved in ADC research, development, and manufacturing, necessitating rigorous hazard assessments and protective measures. Despite limited data on the safety of ADC components during handling, existing guidelines focus on evaluating hazards, controlling exposure, and ensuring worker safety through comprehensive risk assessments, containment strategies, and protective equipment. This paper details potential hazards of ADC components and the importance of controlling exposure to protect workers. It also emphasizes the need for continued vigilance and advanced containment technologies as ADCs evolve, highlighting the balance between developing ADCs' therapeutic potential and managing the risks associated with their highly potent ingredients.

Overall, this article collection offers insights not only about the design and applications of ADCs but also about the major considerations for manufacturing and handling these highly effective but also hazardous therapeutics. To explore technology options for developing your ADC process, we encourage you to visit Cytiva.

Gwen Taylor, Ph.D.,
Associate Editor

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Antibody–Drug Conjugates: Development and Applications

Adapted from Pradhan, R. *et al.* (2022)

Article Link

The focus of drug discovery has shifted towards biologics, which offer advantages such as targeted delivery, faster onset of action, and reduced adverse effects. The US FDA approved 10 biologics in 2019, including 3 antibody-drug conjugates (ADCs) for cancer treatment. As of 2024, 13 FDA-approved ADCs are commercially available. ADCs consist of a recombinant monoclonal antibody covalently bound to a drug molecule via synthetic linkers. ADCs deliver cytotoxic drugs specifically to tumor cells, minimizing systemic toxicity and adverse effects. They exploit the tumor-specificity of monoclonal antibodies, distinguishing between healthy and cancerous tissues. Despite challenges in development, second-generation ADCs like brentuximab vedotin and ado-trastuzumab emtansine show improved efficacy and safety over first-generation ADCs. This targeted approach enhances drug efficacy while reducing overall toxicity.

This article discusses ADC composition, mechanism of action, challenges, regulatory guidelines, and real-world applications, providing detailed insights into their development and potential.

Design of ADCs

ADCs consist of three components: an antibody, payload, and linker (Fig. 1), which achieve greater efficacy with reduced side effects. Administered intravenously, ADCs target specific antigens on cells. Target antigen selection is crucial in determining drug effectiveness and toxicity. Antigens should be highly expressed on target cells but minimally on healthy cells. Internalization properties aid drug efficacy, while the bystander effect enhances toxicity in nearby cells. Careful antigen selection is essential for ADC efficacy and tumor classification.

Antibody

Antibodies used in ADCs, mainly IgG, possess high specificity for target antigens on tumor cells. IgG subclasses like IgG1 and IgG4 are preferred due to their longer half-lives and immunological functions. Ideal monoclonal antibodies for ADCs should bind selectively to surface antigens, undergo receptor-mediated endocytosis, and have minimal shedding to reduce binding during circulation. They must maintain stability and pharmacokinetics after payload conjugation, with low immunogenicity and optimal binding affinity. First-generation murine antibodies faced immune reactions and penetration issues, prompting the development of second-generation humanized antibodies. However, tissue penetration limitations necessitate parenteral administration. Future research aims to improve delivery, formulations, and administration routes for optimal ADC efficacy.

Linker

Linkers are crucial for ADC efficacy and safety and must have stability in circulation with specific payload release at target sites while being nontoxic when bound to antibodies. Cleavable linkers (Fig. 2) respond to physiological stimuli like acidity, with examples like acid-labile and protease-cleavable linkers releasing drugs in lysosomes or target cells, respectively. Glutathione disulfide linkers rely on tumor cell enzymes for payload release. Non-cleavable linkers degrade in lysosomes, improving ADC stability. Various con-

jugation strategies like lysine or cysteine attachment, site-specific conjugation, and enzymatic conjugation enhance ADC specificity and homogeneity. Chemical conjugation is commonly used for its reproducibility, enabling precise drug-antibody ratios.

Payload

The payload in ADCs is crucial for their success, comprising small, potent drug molecules lacking specificity or good absorption. Ideal payloads have high solubility, low immunogenicity, longer half-lives, and allow conjugation with linkers and antibodies. They are categorized as radionuclide or highly potent drug molecule conjugates, often with anticancer mechanisms. Examples include auristatins, maytansinoids, gemtuzumab, and inotuzumab. A drug-antibody ratio (DAR) of around four ensures optimal activity, as higher DARs may decrease efficacy and cause distribution heterogeneity.

Mechanism of Action

As of 2023, approved ADCs are administered intravenously to avoid degradation in stomach acid. They bind to specific target antigens that are highly expressed in target cells (Fig. 3). After binding, they undergo receptor-mediated endocytosis. The ADC-antigen complex is internalized and undergoes recycling by FcRn receptors to prevent healthy cell death. The remaining ADCs enter the late endosome and lysosome, where cleavage releases payload, inducing cell death based on the payload type.

Pharmacokinetic Considerations for ADCs

ADCs bind to target antigens on cell surfaces and undergo receptor-mediated endocytosis, leading to lysosomal degradation and release of cytotoxic drugs, causing cell death. However, nonspecific uptake by other cells via pinocytosis can lead to unintended drug release and toxicity. The antibody component, comprising most of the ADC's molecular weight, influences its pharmacokinetics, including slow clearance, long half-life, and low volume of distribution. Though advantageous, antibodies also contribute to drawbacks like poor oral bioavailability and immunogenicity. Unconjugated and conjugated ADCs differ in drug species mixtures, while their *in vivo* behavior is determined by both antibody and cytotoxic drugs.

Figure 1

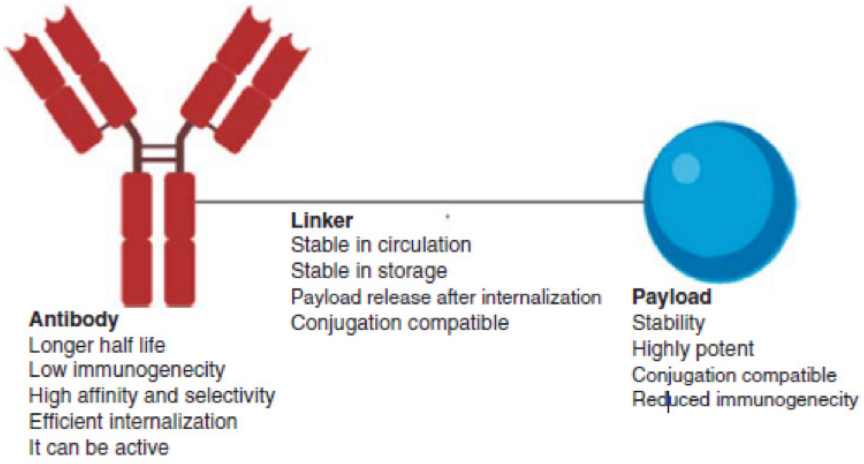

Fig. 1: *Schematic representation of an ADC, its three components, and their salient features.*

Figure 2

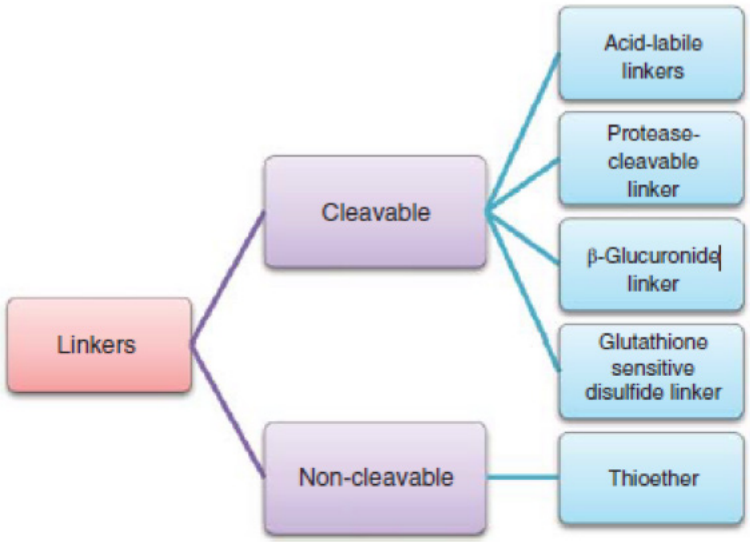

Fig. 2: *Classification of the commonly used linkers in ADCs. Modified from Tsuchikama and An (2018).*

Heterogeneity of ADCs

Heterogeneity in ADC molecules arises from manufacturing and *in vivo* processes. Manufacturing involves conjugating antibodies to drugs through chemical reactions targeting amino acid residues like lysine and cysteine, leading to varying DARs. *In vivo* processes cause deconjugation, impacting DAR and ADC efficacy. Methods to reduce heterogeneity include engineered cysteines for site specificity and DAR control. DAR is crucial for ADC design and optimization, influencing pharmacokinetics and therapeutic efficacy.

Bioanalytical Considerations for ADCs

Assays for ADCs involve measuring total antibody (Tab), conjugated drug, conjugated antibody, unconjugated antibody, and free drug. Tab concentration, crucial for PK profile and stability, is typically determined via ELISA, reflecting the antibody's behavior *in vivo*. The conjugated antibody amount, indicative of active ADC concentration, is also measured via ELISA. LC-MS/MS and ELISA are used to measure drug concentrations, while LC-MS/MS is more sensitive for unconjugated drug detection.

Figure 3

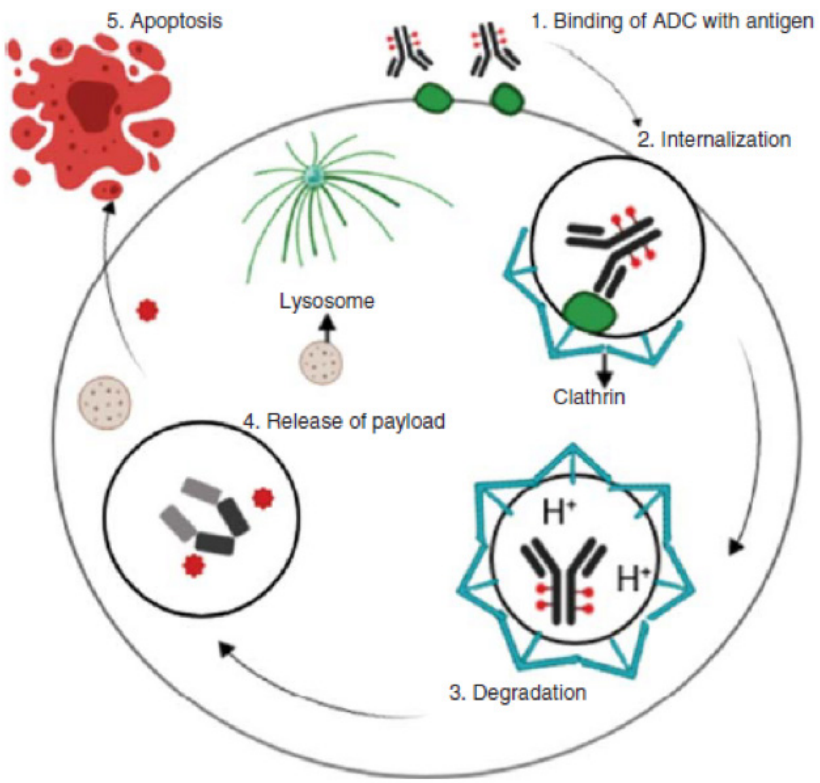

Fig. 3: *Schematic diagram of the mechanism of action of ADCs.*

Advanced technologies promise more detailed ADC characterization for improved PK understanding.

Pharmacokinetic Parameters of ADCs

The pharmacokinetic disposition of ADCs involves a rapid decrease in the concentration-time profile post-conjugation, with multi-exponential Tab profiles resembling those of antibodies. ADCs are mainly administered intravenously, and ADC absorption is the same as that of the unconjugated antibody. Distribution depends on target antigen expression and internalization into cells. Antigen shedding may lead to liver toxicity via clearance through the liver. Deconjugation of ADCs into cytotoxic drugs and antibodies occurs via enzymatic or chemical processes, influencing metabolism and elimination. Metabolites resembling small therapeutic molecules contribute to potential toxicities and drug interactions. Future research aims for targeted antibodies, stable linkers, and reduced payload-specific toxicities in ADCs.

Applications of ADCs

Currently, there are 13 approved ADCs, and more than 100 are in clinical trials. The

Table 1

Sr. No.	Generic name	Manufacturer	Brand name	Linker	Payload	Target antigen	Use
1	Gemtuzumab ozogamicin	Wyeth/Pfizer	Mylotarg	Hydrazone	*N*-acetyl gamma calicheamicin	CD33	Acute myeloid leukemia
2	Brentuximab vedotin	Seattle Genetics	Adcretris	Protease cleavable	Monomethyl auristatin E	CD30	Relapsed Hodgkin's lymphoma and relapsed anaplastic large cell lymphoma
3	Ado-trastuzumab emtansine	Roche, Genentech	Kadcyla	Non-cleavable linker	Maytansinoid DM1	HER2	Metastatic breast cancer
4	Inotuzumab ozogamicin	Wyeth/Pfizer	Besponsa	Acid labile linker	*N*-acetyl-γ calicheamicin 1,2-dimethyl hydrazine dichloride	CD22	Relapsed or refractory B cell malignancies
5	Polatuzumab vedotin	Roche, Genentech	Polivy	Protease cleavable linker	Dolastatin 10 analog monomethyl auristatin	CD79b	Relapsed or refractory (R/R) diffuse large B cell lymphoma
6	Enfortumab vedotin	Astellas Pharma/Seattle Genetics	Padoev	Protease linker	Monomethyl auristatin E	Nectin-4	Metastatic urothelial cancer
7	Trastuzumab deruxtecan	Daiichi Sankyo/ AstraZeneca	Enhertu	Cleavable linker	Deruxtecan	HER2	Metastatic HER2 positive breast cancer

Table 1: *Current marketed ADCs.*

approved ADCs are mainly used in cancer therapy, but new ADCs are being developed for the treatment of rheumatoid arthritis, bacterial infections, ocular diseases, and tuberculosis.

Use of ADCs in Rheumatoid Arthritis, Bacterial Infections, and Ophthalmology

ADCs offer a promising avenue for treating various conditions beyond cancer. In rheumatoid arthritis, tocilizumab alendronate ADCs, targeting IL-6 and inhibiting macrophages, show efficacy. For bacterial infections like *Staphylococcus aureus*, ADCs deliver antibiotics within bacteria via antigen binding, potentially overcoming antibiotic resistance. In ophthalmology, ADCs targeting unique antigens hold promise for treating choroidal neovascularization, inhibiting abnormal blood vessel growth, and posterior capsule opacification, inhibiting epithelial cell formation. While laser capsulotomy remains a primary treatment, ongoing ADC research aims to address ophthalmic diseases effectively.

Resistance of ADCs

Resistance to monoclonal-based therapeutics, due to host or tumor-related factors, poses a challenge in cancer treatment. Efflux of drugs by P-glycoprotein and cell-cycle mechanisms contributes to resistance, particularly in cells in the G0 phase. Strategies to overcome resistance include prodrug strategies, using poor P-glycoprotein substrates, or non-cleavable linkers in ADCs.

Regulatory Aspects of ADCs

The FDA evaluates ADCs and monoclonal antibodies separately, with ADCs falling under the jurisdiction of the Center for Drug Evaluation and Research (CDER) since 2003. Both undergo rigorous characterization for chemistry, manufacturing, and control (CMC) in regulatory submissions. The CDER involves the Office of Biological Products (OBP) and the Office of New Drug Quality Assessment (ONDQA) in the review process. ONDQA assesses the drug-linker, focusing on optical chirality, impurities, and potency, while OBP evaluates the monoclonal antibody, including primary and conformational structure, specificity, and post-translation modifications. Techniques such as peptide mapping and spectroscopy are utilized, ensuring batch consistency, and assessing impurities' impact on biological activity. Approval is contingent upon a joint review by OBP and ONDQA.

Conclusions and Future Directions

Research focus in medicine is shifting towards biologics, with increasing approvals of ADCs due to their effective targeting. Ongoing research aims to broaden ADC applications beyond cancer therapies.

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Antibody–Drug Conjugates: An Overview of the CMC and Characterization Process

Adapted from Ross, P.L. and Wolfe, J. (2016)

Article Link

Antibody–drug conjugates (ADCs) are a rapidly growing class of drugs that provide targeted delivery of highly potent agents and circumvent cytotoxicity problems. For currently marketed and next-generation ADCs, product development requires the integration of multiple functions spanning small molecule and protein therapeutic product development. The quality and performance of the ADC product are directly dependent on the process design and controls. Here, we will focus on processes used for the development of first-generation and next-generation ADCs. It is assumed that the design and discovery aspects of the ADC molecule and its monoclonal antibody (mAb) or small-molecule constituents have been completed and that rigorous analytical and process controls are already in place.

ADC Manufacturing Process

Starting from well-characterized mAb, linker, and cytotoxic agent, ADC development follows the steps of mAb activation, conjugation, purification, formulation, and storage for patient administration (Fig. 1). During the overall process, the components of an ADC (the antibody, toxin, and linker) all encounter hostile chemical and physical environments, which, if uncontrolled, can compromise product quality. Throughout, critical quality attributes (CQAs, Table 1) are monitored extensively to ensure product specifications, safety, and efficacy requirements are met.

Conjugation

Production of ADCs begins with conjugation, where chemical and biological starting materials are combined to yield the ADC drug substance. To ensure successful conjugation, conditions must support reactive chemistry while preventing significant degradation or cross-reactivity of reactants (Fig. 2). Ideally, the conjugation, purification, and formulation processes should not introduce new degradation or instability pathways from the mAb and cytotoxic payload intermediates. However, the ADC is intrinsically destabilized relative to its parent mAb, and measures must be taken to minimize these pathways.

Conjugation methods vary between ADC products, typically utilizing cysteine,[1] lysine,[2] tyrosine,[3] site-specific,[4] or enzyme-mediatd[5] conjugation. Competing processes during conjugation, such as succinimide or maleimide reactivity, must be managed to avoid mAb destabilization. Also, conjugation heterogeneity is influenced by global solution conditions and local pH environments within the mAb, impacting conjugation stoichiometry.

Controlled storage conditions, handling procedures, and high reagent quality are also crucial for consistent conjugation. Inadequate reductant concentration or exposure to moisture during shipping/storage can compromise conjugation efficiency, and proper mAb handling is essential to prevent excessive unconjugated species. Conjugation site and stoichiometry significantly affect ADC efficacy and safety profiles, necessitating thorough testing of these quality attributes.

Conjugation – Next-Generation Chemistry

There is a burgeoning exploration of next-generation chemistries aimed at expanding the application of ADCs as therapeutics. Some of the most active areas of development (Table 2) include the investigation of novel payloads, alternate linker technologies, and improved bioconjugation approaches that reduce heterogeneity in the ADC drug substance.

Conjugation – Novel Payloads

There are currently four cytotoxin classes of greatest relevance in next-generation ADC design.

Tubulin inhibitors, such as auristatins and maytansinoids, remain highly emphasized. However, DNA-binding and intercalation agents are also under development for ADCs. Calicheamicins are potent antitumor antibiotics that bind to the minor groove of DNA, causing double-strand breaks. Mylotarg (gemtuzumab ozogamicin) is an ADC that utilizes a calicheamicin payload which targets CD33+ acute myeloid leukemia cells. Duocarmycins are a class of cytotoxic agents that bind to the minor groove of DNA and alkylate the adenine base, leading to DNA damage and cell death. Pyrrolobenzodiazepines also target DNA by binding and cross-linking in the minor groove. Other classic cytotoxic molecules such as doxorubicin have also been examined as potential ADC payloads.

Conjugation – Linker Design

ADC linkers play a crucial role in facilitating the stable delivery of the ADC molecule and the subsequent targeted release of the cytotoxic payload. Linker design significantly influences activation and conjugation reaction conditions, as well as the handling of the ADC. They can be categorized

Figure 1

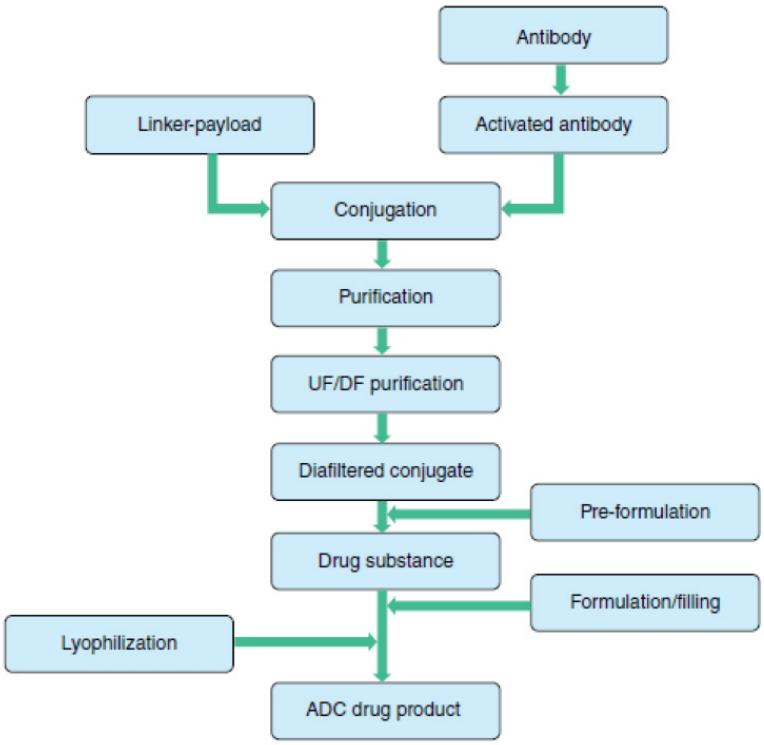

Fig. 1: *Overview of the manufacturing process for ADCs, starting from purified, well-characterized mAb, linker (activation), and cytotoxic materials. Abbreviations: DF, diafiltration; UF, ultrafiltration.*

Table1

Quality Attribute	Relevant Stage			Potential Clinical Impact			
	Conjugation	Purification	Formulation	PK	Potency	Safety	Immunogenicity
DAR (including unconjugated mAb)		X	X	X	X	X	
Free drug	X	X	X	X	X	X	
Linker stability	X	X	X	X	X	X	
mAb aggregation	X	X	X		X	X	X
Charge variants	X	X	X		X	X	X
Protein fragments							
Conjugation impurities (solvents, by-products)		X	X		X	X	
Protein concentration	X	X	X		X		
mAb posttranslational modifications	X		X		X	X	X
Particulate number	X	X	X			X	X
Residual solvents			X			X	
pH and appearance		X	X			X	
Antigen binding		X	X		X		X
Target potency	X			X	X		
Sterility/endotoxin	X		X			X	X
Host cell DNA content	X					X	X
Host cell protein content	X					X	X

Table 1: *ADC critical quality attributes, the relevant stages at which these attributes may be assessed, and the potential clinical impact associated with insufficient control of these attributes. Abbreviations: DAR, drug-to-antibody ratio; DNA, deoxyribonucleic acid; mAb, monoclonal antibody..*

Figure 2

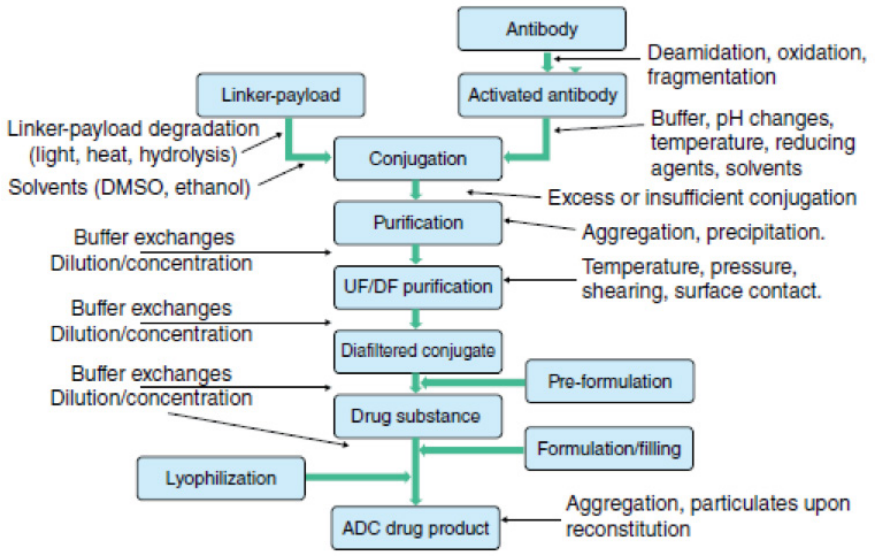

Fig. 2: *Physical and chemical forces that act upon the ADC molecule throughout the manufacturing process.*

Table 2

Method	Reactive species
Engineered cysteine	Reaction of electrophiles at thiols engineered at optimized locations
Non-natural amino acid	Engineered CHO expression system to incorporate *p*-acetylphenylalanine, which reacts with alkoxyamine linker-drug via oxime ligation
Aldehyde tagging with formylglycine	React formylglycine with hydrazide linker-drug
Transglutaminase	Conjugation of linker-drug with free amine to engineered amino acid tag (LLQG), both recognized as transglutaminase substrates
Selenocysteine incorporation	Engineered seleno-cys (Sec) insertion, reaction with maleimide or haloacetamide on linker-drug
Glycoengineering	Periodate oxidation of engineered sialic acids to aldehydes, reaction of alkoxyamine linker-drug via oxime ligation
Sortase tagging	Transpeptidation using bacterial sortase with "click" functionalized linker-drug
Thio bridge/next-gen maleimides	Bifunctional Cys-Cys bridging reagent carrying linker-payload
SPDB	Optimized bifunctional Lys linker leading to more stable hindered disulfide linkage (*N*-succinimidyl-4-(2-pyridyldithio)butyrate, SPDB linker)
Hydrophilic linkers	Charged sulfonate or noncharged PEG backbone in bifunctional linker - higher DAR for hydrophobic payloads

Table 2: *Examples of current linker-payload and conjugation strategies.*

as non-cleavable or cleavable, each with its own set of challenges and implications for process development and characterization. Cleavable linkers utilize various mechanisms such as acid-labile hydrazones, reducible disulfides, or protease-susceptible peptide motifs for controlled payload release post-internalization.

Monitoring the purity and exposure of linkers to destabilizing forces throughout manufacturing is crucial. While linker cleavage is ideally intracellular, external factors such as pH, temperature, light, and air during downstream processes can lead to low-level release of free drug, posing toxicity risks. Comprehensive characterization at all manufacturing steps is essential to detect free drug and related cleavage forms, as well as any losses of linker payload from the ADC.

mAb Engineering

The primary challenge of linker chemistries for ADCs is conjugation heterogeneity, which affects payload stoichiometry and location. Proper reaction conditions and high-quality starting materials are crucial for maintaining the drug-to-antibody ratio (DAR) and avoiding mAb destabilization. Simplification of the conjugation process is being explored (Table 2) to reduce ADC heterogeneity.

Engineered cysteine residues and non-natural amino acids are mature site-specific conjugation approaches, although they pose risks of protein structural changes. Reactive chemistry during conjugation exposes antibodies to harsh conditions, creating risks for degradation. Next-generation conjugation techniques aim to modify mAb structure or expression systems but may lack established stability compared to marketed antibody drugs.

Purification

After conjugating the antibody to achieve the desired composition, purification processes remove low-molecular-weight components. Diafiltration, typically used for purification, employs a semipermeable membrane to retain higher molecular weight ADCs while allowing smaller molecules to pass through. This process also facilitates buffer exchange and concentration adjustment. Diafiltration methods vary by scale, with small-scale operations using centrifugation or vacuum-based ultrafiltration and large-scale operations employing continuous flow systems like tangential flow filtration (TFF). TFF can create stability risks due to exposure of sensitive biomolecules to air, surface contact, and temperature changes, but many of these risks can be significantly mitigated using modern automated equipment and process strategies.

ADC purification requires specialized handling due to its hydrophobic groups that reduce solubility and stability compared to unconjugated IgG molecules.

Formulation

Formulation stabilizes the ADC after purification and supports its administration via ly-

Table 3

Process monitored	Techniques	Phase of product development
Property: chemical stability		
Drug loading	HIC, LC-MS, RP-HPLC	All
Free drug loss	RP-HPLC-UV, LC-MS	Purification, formulation
Charge state distribution, deamidation	iCIEF, IEX chromatography	Conjugation, purification, extended characterization
Drug distribution	RP-HPLC, peptide map by LC-UV or LC-MS	Conjugation, extended characterization
mAb fragmentation	Reducing and nonreducing SDS-PAGE	All
mAb oxidation, deglycosylation	LC-MS/MS	Conjugation, purification, extended characterization
Property: conformational stability		
Aggregation	SEC, SEC-MALS	Formulation
Particulate formation, denaturation	DLS, UV-vis absorbance, microflow imaging	
Thermodynamic changes	DSC, spectroscopic techniques including UV-vis, fluorescence, or CD spectroscopy	Formulation development and extended characterization
Property: solution stability		
Protein concentration	UV-vis absorbance, amino acid analysis	Purification, formulation
Particulate formation	DLS, MALS	Purification, formulation

Table 3: *Stability properties of ADCs and characterization techniques involved.*

ophilization and IV injection. Formulation is the link between the purified ADC molecule and the ADC product and can vary from preclinical to later clinical development according to ADC development specifications.

ADC formulation is pivotal in manufacturing, influencing stability and reconstitution. Buffering agents, sugars, and surfactants are key components, differing from mAb formulations in concentration range and linker-payload stability consideration. Formulation development requires vigilance against aggregation and instability, necessitating diverse analytical techniques (Table 3). Stability assessment methods must be appropriate for each stage and remain consistent and robust for long-term comparative testing across manufacturing locations.

Characterization

The following overview describes the analytical and biophysical techniques used to characterize ADC drug substances, emphasizing the overall ADC development process and where techniques are most usefully applied.

Quality and Stability Testing

Characterization of ADCs involves examining the heterogeneity of the active molecule starting from the conjugation phase. Key challenges include chemical stability, conformational stability, colloidal stability, and general solution stability, as well as characterizing and controlling linker–payload stoichiometry and site occupancy (Fig. 3)

Solution-based chromatographic and electrophoretic methods and mass spectrometry play a central role in quantitatively measuring ADC chemical purity, stability, and conformational stability (Fig. 4). Dynamic light scattering (DLS) and size exclusion chromatography (SEC) coupled with multi-angle light scattering are both used to monitor aggregation. Hydrophobic interaction chromatography (HIC) is increasingly used to provide qualitative and quantitative DAR distribution information, particularly for cysteine-based ADCs. Differential scanning calorimetry (DSC) is used to assess the thermal stability of ADCs by measuring the heat change associated with protein denaturation of the antibody component. Finally, circular dichroism (CD) is a spectroscopic technique that can provide information about protein conformational changes and stability.

UV-vis spectroscopy and LC-MS are used for quantitation, with the latter being especially important for lysine conjugates due to their greater heterogeneity.

Imaging capillary isoelectric focusing and ion exchange chromatography (IEX) provide information on charge distribution and heterogeneity. The same electrophoresis platforms also support reducing and nonreducing SDS-PAGE sizing analysis for the detection of protein fragmentation. HIC, SEC, and IEX can also be performed under non-denaturing conditions, allowing the isolation of specific components for further analysis or scale-up.

Reverse-phase HPLC with MS detection is essential for quantifying small-molecule species and identifying linker-toxin degradants. Quantification of residual solvents like DMSO or DMF is also crucial due to their toxicity concerns.

Biochemical and Microbiological Testing

ELISA is used to assess the antigen-binding potency of the ADC, ensuring no affinity loss post-conjugation. Residual impurities from expression systems (e.g., host-cell DNA and protein) are quantified using PCR and ELISA, essential for quality control pre-conjugation. Next-gen ADCs based on engineered components may require assay re-optimization as their plasma stability and pharmacokinetic properties may deem them new molecular entities.

Extended Characterization

The characterization of the ADC drug product is crucial to mitigate development risks and ensure market approval. A comprehensive approach involves confirming identity, purity, and stability throughout the product's lifecycle. Techniques such as peptide mapping via reverse-phase HPLC and MS provide detailed structural analyses, including posttranslational modifications. Specialized MS tools are essential for comprehensive structural analysis, while glycan analysis ensures integrity post-processing. Spectroscopic methods like circular dichroism and fluorescence spectroscopy assess conformational and colloidal stability, vital for understanding mAb behavior under different conditions. Differential scanning calorimetry

Figure 3

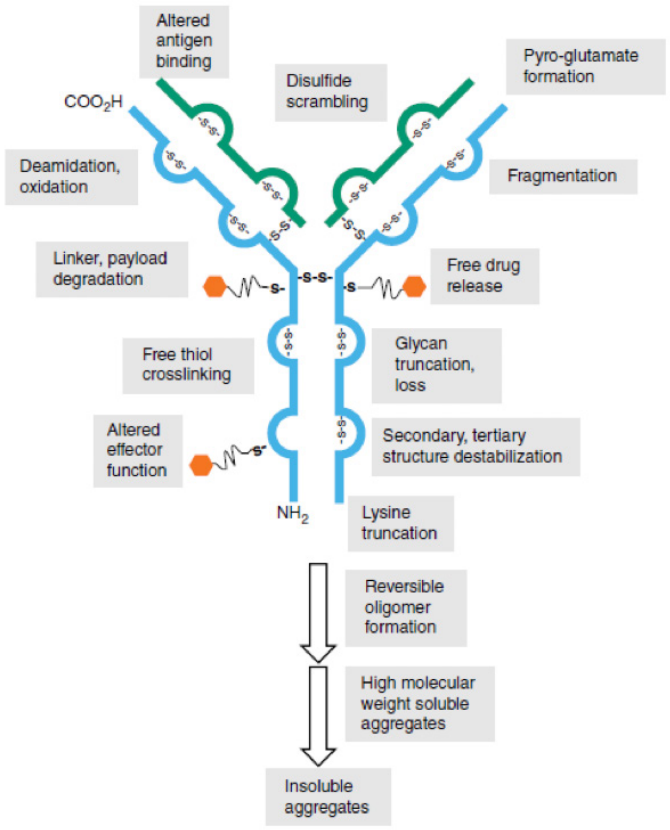

Fig. 3: *Common chemical and physicochemical degradation pathways that are encountered by ADCs throughout the manufacturing process.*

Figure 4

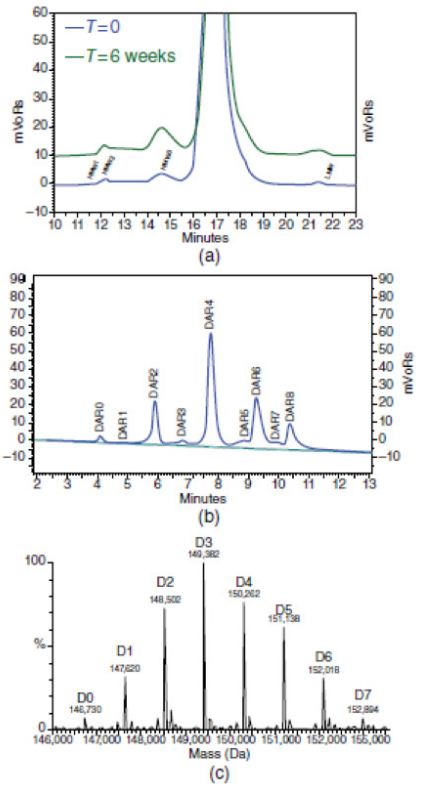

Fig. 4: *Example data from commonly used ADC characterization techniques; (a) size-exclusion chromatogram before and after stability testing, (b) hydrophobic interaction chromatogram of a cysteine-conjugated ADC showing 0-8 linker drugs, (c) size exclusion-ESI-MS of an ADC.*

and dynamic light scattering are emerging as tools for thermodynamic assessment. These methods are crucial post-purification to monitor linker-payload effects on mAb conformation and during formulation optimization to identify stabilizing excipients.

Comparability

Comparability analysis evaluates the impacts of manufacturing changes on ADC safety and efficacy. Maintaining physicochemical and functional consistency across suppliers, sites, and equipment is crucial. The process starts with establishing controls and acceptance criteria for mAb and drug/linker materials pre-conjugation. Full structural, purity, stability, and biological assessments are conducted for mAb intermediates, while linker/drug molecules undergo structural, elemental characterization, impurity profiling, and stability assessments through conjugation, purification, and formulation to ensure comparability between manufacturing lots.

Concluding Remarks

Production of ADCs combines many of the complexities of cytotoxic small molecule and mAb drug development, with the potential benefit being improved patient outcomes. It is anticipated that conjugation process simplifications and improved ADC process understanding, along with stage-appropriate characterization tools, will lead to safer, more efficacious, and more cost-effective therapeutic solutions.

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Occupational Health and Safety Aspects of ADCs and Their Toxic Payloads

Adapted from Sussman, R. and Farris, J. (2016)

Article Link

Antibody-drug conjugates (ADCs) enable targeted delivery of potent materials to diseased tissues, with minimal off-target toxicity. However, handling these potent drug substances poses significant hazards. This article outlines ADC's background, identifies associated hazards, discusses hazard evaluation methods, and proposes measures for controlling and verifying safety protocols when handling these compounds.

Background

ADCs offer a novel approach to treating cancer by delivering potent drugs to specific target cells using antibodies as carriers. Typically comprising a humanized antibody, active drug (payload), and linker (Fig. 1), ADCs selectively bind to disease targets like cancer cells, releasing the drug upon internalization. This targeted delivery may improve efficacy and reduce systemic toxicity compared to the unconjugated drug, especially in aggressive or chemotherapy-resistant cancers. Ideally, ADCs target proteins or carbohydrates overexpressed on cancer cell surfaces, triggering internalization and payload release. Examples include CD19, CD22, CD33, HER2, and PSMA, found in various cancers like lymphoma, leukemia, and prostate and breast cancers.

Payloads

ADC technology has revived interest in potent drugs previously deemed too toxic for clinical use. Maytansinoids, derived from an East African shrub, were among the first ADC payloads developed for cancer therapy due to their potent antimitotic activity. Maytansine, the prototype, showed promising anticancer activity in preclinical studies but caused dose-limiting toxicities in clinical trials. Conjugating maytansinoids with antibodies decreased systemic toxicity significantly.

New potent payloads including immune-modulating agents, proteolysis-targeting chimeric molecules (PROTACs), and dual payload constructs are being explored for ADCs. While mostly investigated for oncological purposes, ADCs with potent antioxidant properties are being explored for neurological indications like Alzheimer's disease.

Linker Technologies

ADC linkers must balance stability during circulation in the blood with rapid payload release inside tumor cells. Current linkers are primarily cleavable (e.g., disulfide) or non-cleavable (e.g., thioether) (Fig. 2). Cleavable linkers are designed to be sensitive to conditions such as low pH or high concentrations of certain enzymes like proteases or glutathione, ensuring that the drug is released only when the ADC reaches its target. Non-cleavable linkers generally rely on the complete degradation of the antibody component of the ADC to release the payload.

Linkers have also been developed that can incorporate increasingly potent cytotoxic payloads. This involves the use of hydrophilic linker chemistries that mitigate the inherent hydrophobicity of many potent cytotoxic drugs, which can lead to aggregation and increased immunogenicity. Such advancements in linker technologies not only expand the range of potential payloads but also improve the therapeutic index of ADCs.

Other technologies include engineered antibodies for covalent conjugation, flexible polymer linkers for greater drug loading, and endogenous amino acids.

However, toxicity assessment is crucial. ADCs with reducible disulfide bonds may exert effects on antigen-negative cancer cells via a "bystander effect." Non-cleavable linkers offer less off-target toxicity and generate fewer toxic metabolites compared to cleavable linkers.

Antibodies

Antibodies in ADCs are generally of low toxicity and have limited bioavailability by routes other

Figure 1

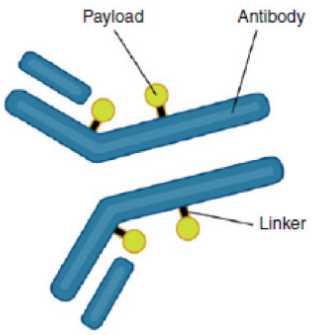

Fig. 1: *Components of a typical ADC molecule.*

skin absorption is low, with large antibodies having <5% bioavailability through inhalation. The antibody's role in ADCs is to specifically deliver the payload to the target. The payload is then released, leading to cell death. ADC catabolism occurs via proteolysis in lysosomes, with minimal involvement of cytochrome P450 enzymes, demonstrating specificity and limited cytotoxicity to antigen-negative cells. Catabolites are excreted in bile with minimal elimination in urine.

Occupational Hazard Assessment of ADCs and Their Components

Hazard is inherent to materials regardless of quantity or form. Occupational exposure limits (OELs) are set to protect workers from hazardous substances, including ADCs, based on concentrations unlikely to cause harm. Toxicologists establish OELs using a traditional approach involving a point of departure from animal or human studies and adjustment factors for data robustness. Limited data for ADCs may lead to conservative OELs. Once antibodies are conjugated and purified, toxicity risks are limited, but understanding the release and activity of payloads is crucial for safety assessments. A conservative approach is recommended until payload activity is fully understood.

Occupational Implications and Uncertainties

The risk of injury from exposure to chemicals during manufacture and handling depends on the occupational toxicity of the material and the exposure potential. Routes of occupational exposure include inhalation, subcutaneous transfer, skin absorption, mucous membrane contact, and ingestion. Despite the lower toxicity of complete ADCs to patients compared to the payload, the potency of the linked payload remains hazardous. Unconjugated components in the final formulation also pose safety concerns.

The binding efficiency of the payload to the antibody, influenced by linker technology and antibody specificity, affects the specificity of ADCs to target cells. Finally, local effects in the lung, such as off-target or cleaved linkers in pulmonary fluids, must also be considered, warranting a conservative approach to risk assessment.

General Guidance for Material Handling

The process steps in ADC activities vary, requiring specific risk assessments for each unit operation to account for extreme toxicity and low airborne concentrations. Procedures for handling payloads and ADCs should be established with precautions and controls for laboratory, pilot plant, and clinical manufacturing. A comprehensive safety program should include employee selection, training, personal protective equipment (PPE), work practices, spill response, and medical surveillance. Facility designs should ensure effective containment, ventilation, and PPE usage to minimize exposure risks. Good work practices are crucial, with attention to minimizing chemical handling, maximizing containment, and utilizing ventilation effectively. Powder handling should occur in isolators or ventilated containment systems, while solutions must be handled inside containment systems or with local exhaust ventilation to prevent aerosolization. Prompt cleanup of residues is essential to reduce subsequent exposure risks.

Facility Features and Engineering Controls

Recommendations for facility features in ADC handling vary based on the stage of production and type of operation. For powder-handling areas, a separate HVAC system with negative air pressure and airlocks for personnel flow are recommended. Air changes should meet occupancy standards, and airflow should minimize turbulence. Room exhaust air recirculation is discouraged to prevent particle reintroduction. HEPA filtration is mandated for manufacturing areas, and safe change exhaust filtration systems are required to prevent exposure during maintenance. Changing facilities with PPE storage are preferred, while clinical and commercial operations necessitate adjoining gowning and degowning areas. Designated areas for

Figure 2

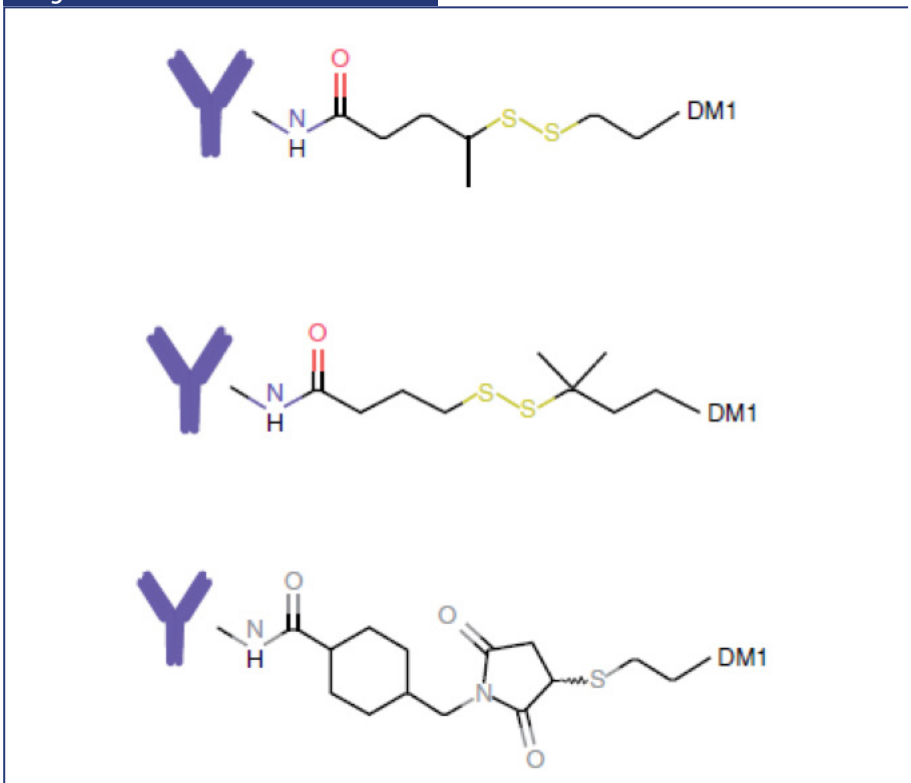

Fig. 2: *ADC linkers. Cleavable disulfide linkers (top and middle) and non-cleavable thioether linker (bottom).*

pharmaceutical compounds must have easily cleanable surfaces and restricted access. Double-door pass-through chambers are suggested for sample transfer and waste removal to limit personnel traffic.

Specific Operational Guidance

In payload synthesis, safety measures include conducting procedures within isolators or well-ventilated enclosures based on aerosolization risk. Chemical synthesis should adhere to good laboratory practices within isolators or ventilated containment systems verified by industrial hygiene monitoring. Organic solvent processes require chemical hoods or effective ventilation. Milling and size reduction for powders are discouraged, but if needed, enclosed methods must be used. Conjugation involves isolator use for powder handling and chemical hoods or biosafety cabinets for liquids. Filling procedures vary by scale, with manual filling in biosafety cabinets for laboratories and isolators for clinical and commercial operations. Lyophilization control should match the ADC's physical form, potentially integrating isolators for light powders. Cleaning activities must minimize toxin exposure through careful equipment assessment and cleaning technique development.

Personal Protective Equipment

Chemical protective clothing requirements depend on the specific task and must be determined after a task-specific risk assessment. Gloves should consist of two layers of latex, nitrile, or neoprene, with selection based on reagent chemicals and operations performed. Gloves should be changed upon suspicion of puncture, abrasion, or contamination, and procedures should be rigorously followed to minimize contamination risks. Eye protection is mandatory, with safety glasses or chemical goggles required based on the nature of the task. Respiratory protection, determined by trained professionals, requires a written program including equipment selection, fit testing, and maintenance. Until confirmed otherwise, employees handling payloads and ADCs should wear NIOSH-rated powered air-purifying respirators or supplied-air respirators, with confirmation through air sampling of unit operations.

Training

Employees must receive training on the toxicity and signs of overexposure to handled drugs, adhering to OSHA (or international) standards. Training must address health effects specific to the ADC and its payload, including bone marrow suppression, gastrointestinal issues, and reproductive toxicity. Signs of overexposure must be reported to supervisors and health personnel. Pregnant employees should be aware of developmental toxicity risks. A focused training program on potent compound safety is essential, covering detection challenges and safe handling practices. Specialized training in ADC synthesis and handling is also necessary.

Industrial Hygiene Monitoring

Baseline industrial hygiene air monitoring is crucial for activities involving payload and ADC handling, using validated methods developed by experienced pharmaceutical analysis laboratories. Sampling strategies should include breathing zone and area air sampling to verify the effectiveness of engineering controls and work practices. Methods typically involve portable air sampling pumps pulling air through Teflon filters, with careful handling to prevent contamination. Breathing zone monitoring assesses worker exposure, while area monitoring evaluates airborne levels at specific locations. Surface monitoring, using wipe sampling, is valuable for assessing contamination on surfaces. Detailed sampling plans are necessary, including sample type, location, and dimensions. After sampling, filters are sent to labs for analysis. For surface sampling, wearing two pairs of gloves is essential, and a sampling plan should specify sample type, location, and area dimensions.

Medical Surveillance Program

Health surveillance for employees in pharmaceutical laboratories and pilot plants, particularly those working with ADCs, is crucial. Initial surveillance should focus on hematologic, gastrointestinal, hepatic, neurological, and reproductive systems. Baseline evaluations, including complete blood count (CBC) and liver enzyme tests, are recommended. Pregnancy status should be determined, aligning with organizational policies. Depending on the findings, periodic surveillance or referrals may be necessary. Trend analysis helps monitor overall employee health over time, adjusting protective measures as needed.

Summary and Future Directions

ADCs offer a promising approach to treating various tumors with ingredients previously deemed too toxic. However, synthesizing these drugs involves handling potent genotoxic moieties. While ADCs are less toxic to patients, their off-target effects in healthy individuals are unclear. Understanding occupational exposure risks is crucial, especially as payloads become more potent, requiring advanced containment technology.

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Extractables in single-use systems used in ADC manufacturing

We performed extractables studies on ReadyToProcess™ columns, ÄKTA readyflux™ flow kits, and ÄKTA ready™ flow kits using common solvents in ADC manufacturing, DMA and DMSO.

Single-use (SU) systems have great potential for use in antibody-drug conjugate (ADC) manufacturing. The use of organic solvents in the ADC process might, however, raise questions about potential leachables from the plastic and elastomeric materials of single-use components. To address those concerns, we performed extractables studies on a disposable chromatography column housing, and two different disposable flow paths. The extractables studies were performed with two solvents commonly used in the ADC cytotoxin conjugation step: DMA and DMSO.

The studies were designed to ensure that conditions were exaggerated compared with existing ADC manufacturing processes. Extractable organic compounds and trace elements from the single-use components were identified and semi-quantitated with a complementary set of analytical techniques. The low levels of extractables found in this study support the use of ReadyToProcess™ columns, ÄKTA readyflux™ flow kits, and ÄKTA ready™ flow kits in ADC processes.

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Introduction

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ADCs are biotherapeutic molecules consisting of a cytotoxin coupled to a monoclonal antibody (mAb) by a linker. The target specificity of the mAb enables delivery of the toxic drug to cancer cells, while minimizing collateral damage to healthy cells. mAbs used in ADC production are typically manufactured according to traditional processes, including purification via protein A-platform processes (1, 2). Before coupling the linker, the mAb needs to be transferred to a suitable solution. This solvent exchange is normally performed by an ultrafiltration/diafiltration (UF/DF) operation. After the linker coupling reaction, the next step is the conjugation reaction, which couples the cytotoxic drug. Figure 1 provides an example workflow for manufacturing an ADC from a bulk mAb product. Conjugation reactions are typically performed in a solvent containing either N,N-dimethylacetamide (DMA) or dimethyl sulfoxide (DMSO) (3) at concentrations from 10% to 15%.

SU systems are well suited to ADC manufacturing for several reasons. Importantly, SU systems minimize operator exposure to toxins, while also protecting the product from the operator and environment. The high potency of ADCs means that relatively small amounts of products need to be produced per batch. The small batch sizes are well suited for incorporation of SU technology, which provides a cost-efficient solution for multi-product manufacturing. In addition to the lower upfront capital cost compared with reusable systems, SU technologies are quicker to start using because they are supplied ready to use. Cleaning and cleaning validation between manufacturing campaigns is unnecessary, and the risk of carryover of cytotoxin from one batch to another is minimized. Because cleaning is not performed, SU technologies minimize the volume of contaminated waste that must be handled and disposed of.

To support the adoption of SU technologies in ADC production, relevant extractables information is needed. Therefore, extractables studies were performed on three SU products: the ReadyToProcess™ 1 L column housing, disposable ÄKTA ready™ low flow kit for chromatographic systems, and ÄKTA readyflux™ flow kit for tangential flow filtration (Fig 2 and 3).

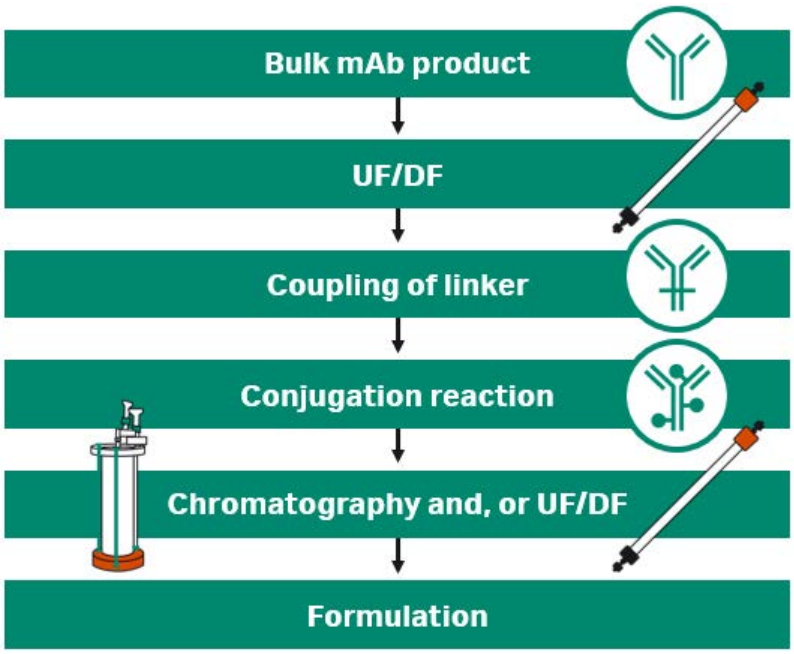

Fig 1. Simple workflow for preparing an ADC from bulk mAb.

Fig 2. ÄKTA ready™ system with a 20 L ReadyToProcess™ column and ÄKTA ready™ flow kit. In this study, 1 L column housings were used.

Fig 3. ÄKTA readyflux™ tangential flow filtration system with flow kit.

Materials and methods

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The goal of the extraction studies was to characterize extractables profiles with equipment and conditions relevant to current ADC manufacturing processes. The studies were designed and performed with advice from customers who use disposables in their ADC processes.

Extractables study design

The extractables studies were designed with consideration for test conditions representing a worst-case scenario and for appropriate analytical techniques (3). Solvent concentrations used in typical cytotoxin conjugation reactions were exaggerated, as were surface area-to-volume ratios, temperatures, and contact times (Table 1). The experiments were set up to ensure contact with all wetted parts. Control samples of DMSO and DMA solution that had been stored at the same conditions but not in contact with the test article were included as blank references.

Table 1. Study design parameters compared with standard conditions

	Standard process chromatography	**ReadyToProcess™ column ÄKTA ready™ flow kit**	**ÄKTA readyflux™ flow kit**
Solvent concentration	10% to 15%	15%	30%
Temperature	20°C to 25°C	30°C	30°C
Contact time	6 to 8 h	24 h	24 h
Surface area to volume ratio	Flow velocity at running conditions will yield a large volume	Highest possible area to volume ratio. Recirculation or dynamic extraction on orbital shaker.	Highest possible area to volume ratio. Dynamic extraction on orbital shaker.

Extraction solutions

A 30% or 15% (v/v) solution of N, N-dimethylacetamide (DMA) was prepared in ultrapure water at neutral pH. A 30% or 15% dimethyl sulfoxide (DMSO) solution was prepared the same way.

ÄKTA readyflux™ flow kit

Complete flow kits, manufactured with the standard method and gamma irradiated, were used. See Table 2 for a list of the materials in the wetted parts. Most wetted surface of the ÄKTA readyflux™ flow kit are made of TPE, silicone, and PP. Additionally, other materials are present in small components or in subassemblies. During extraction, additional silicone gaskets of the type recommended for this product were used to connect all open ends.

Table 2. Materials in wetted parts of ÄKTA readyflux™ flow kit

Materials	**Sources**
Silicone	Tubings, gaskets, connectors
Thermoplastic elastomer (TPE)	Tubing, Pump head, UV-sensor
Ethylenepropylenediene monomer (EPDM)	Gaskets, Pump head, pH-sensor
Polypropylene (PP)	Connections, Pump head, sensors
High-density polyethylene (HDPE)	pH-sensor
Polysulfone (PSU)	Sensors
Polycarbonate (PC)	Sensors, connectors
Gold	Conductivity sensors
Ceramic	pH-sensor

ReadyToProcess™ 1 L column

Column housings, assembled at the manufacturing site, were used. See Table 3 for a list of the materials in the wetted parts. Additional tubing needed for the experimental setup with the column was polytetrafluoroethylene (PTFE).

Table 3. Materials in wetted parts of ReadyToProcess™ columns

Materials	Sources
Polypropylene (PP)	Tube, lids, TC outlet, TC and hose connections, nets, net rings, support nets, hose
Polytheretherketone (PEEK)	Plug, net holder, nozzle tube
Polyolefin (POE)	Hose
Ethylenepropylenediene monomer (EPDM)	TC gasket
Fluorocarbon rubber (FPM/PKM)	O-rings

ÄKTA ready™ low flow kit

Complete flow kits, manufactured with the standard method, were used. This method includes gamma irradiation of the parts except for the pump tubing, which is autoclaved. See Table 4 for a list of the materials in the wetted parts. Additional EPDM gaskets and TC clamps were used for the experimental setup with flow kits to connect the column tubing and the six inlets with the outlets.

Table 5. Materials in wetted parts of ÄKTA ready™ flow kit

Materials	Sources
Polypropylene (PP)	Connections, housings, and other parts
Polyetheretherketone (PEEK)	Plug, T- and Y-connections
Ethylenepropylenediene monomer (EPDM)	TC gasket, pressure membranes, O-rings
Polyamide (PA)	Airtrap housing
Thermoplastic elastomer (TPE)	UV cell, double mold
Polymethylpentene (PMP)	Flowmeter parts
Silicone (Si)	Hose
Titanium (Ti)	Conductivity cell
Polytetrafluoroethylene/silicone (PTFE/Si)	Pump hose

Process setup for ÄKTA readyflux™ flow kit

Two ÄKTA readyflux™ flow kits were tested, using the smallest size of the flow kit (1/4 in. flow kit TC) to ensure the highest possible surface area-to-volume ratio, representing a worst case. The wetted materials of construction are listed in Table 2. The ÄKTA readyflux™ flow kits were filled with extraction solvent (30% DMSO and 30% DMA in water) placed on a shaker (50 rpm) and then incubated at 30°C for 24 h. The conditions chosen targeted the highest levels of solvent that might be use during ADC manufacturing. All open ends were closed by connecting to each other using TC clamps and silicone gaskets. The vent filters were excluded from the extraction study by placing a metal clamp on the tubing prior to the filters (Fig 4). Control samples were made and incubated simultaneously using similar preparation devices, but without contact with the flow kit.

Fig 4. ÄKTA readyflux™ flow kit with connections to obtain a closed loop. The red circles mark the clamps used to exclude the vent filters.

Process setup for column housings

The test articles were two 1 L ReadyToProcess™ column housings (Fig 5). Because of the wide variety of resins that could be used, this study was limited to the column hardware. The smallest size ReadyToProcess™ column was selected to ensure the highest possible surface area-to-volume ratio, representing a worst case.

The wetted materials of construction are listed in Table 3. Tubing was connected to the inlet of each column. The other end of each piece of tubing was placed into a volumetric cylinder containing 1 L of either 15% DMA or 15% DMSO. The extraction solutions were pumped into the columns using a peristaltic pump. After filling, the tubing was removed, and the inlet and outlet tubes of the columns were clamped.

The filled columns were placed upright on an orbital shaker (19 mm orbit diameter) and incubated at 100 revolutions per minute (rpm) for 24 h at 30°C. After incubation, the clamps from the inlet and outlet tubes were removed. The extracts were transferred to separate bottles by applying nitrogen pressure.

The control samples were prepared by pumping 1 L of each solution through two pieces of tubing from a volumetric cylinder into a glass bottle, using a peristaltic pump. The bottles were placed on an orbital shaker alongside the filled columns and agitated at 100 rpm for 24 h at 30°C.

After incubation, extraction solutions from the test articles and control samples were collected for analysis, divided into separate containers for the different analytical techniques, and stored at 5°C.

Fig 5. ReadyToProcess™ 1 L column. Column housings without chromatography resin were used for this study.

Process setup for ÄKTA ready™ low flow kit

The test articles were two ÄKTA ready™ low flow kits. The smallest size flow kit was selected to ensure the highest possible surface area-to-volume ratio, representing a worst case. The wetted materials of construction are listed in Table 4. The open ends of the tubing of a low flow kit were connected to each other using EPDM gaskets and TC 25 clamps. The pump tubing of each flow kit was connected to a peristaltic pump, and the pump was used to fill the flow kit with 700 mL of either 15% DMA or 15% DMSO. During filling, the air trap of the flow kit was mounted at the highest position to let the air escape from the flow kit and to make sure that all surfaces were wetted with extraction solvent. Subsequently, the solution was circulated through the flow kit for 24 h at 30°C in an incubator (Fig 6).

Fig 6. Filling and circulation procedure of ÄKTA ready™ low flow kit in an incubator. The peristaltic pump is placed behind and connected to the pump tubing. The bottle with the control sample is seen to the right. Control samples were prepared by filling a glass bottle with 500 mL of either extraction solution and placed in the incubator at 30°C for 24 h. After incubation, extraction solutions from the test articles and control samples were collected for analysis, divided, and stored in the same manner as the solutions from the column housing and ÄKTA readyflux™ flow kit studies.

Analytical methods

Two classes of extractable compounds were analyzed: organic compounds and a spectrum of elements. Organic compounds were identified, and semi-quantitative results obtained with liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) methods. Test methods for volatile (VOC), semi-volatile (SVOC), nonvolatile compounds (NVOC), and elements are listed in Table 5. Analyses of organic compounds were performed by Nelson Labs Europe in Leuven, Belgium. Elemental analysis was performed by ALS Scandinavia AB in Luleå, Sweden by inductively coupled plasma/sector field mass spectrometry (ICP-SFMS).

Table 5. Overview of chemical analyses performed

Analysis	Target compounds	Typical compounds that could be detected if present
HS-GC-MS	Volatile organic compounds (VOC)	Residual monomers and solvents, small polymer degradation products
GC-MS	Semi-volatile organic compounds (SVOC)	Process lubricants, plasticizers, antioxidants, polymer degradation products, high boiling solvents
LC-MS positive and negative mode	Nonvolatile organic compounds (NVOC)	Antioxidants, fillers, plasticizers, polymerization or hydrogenation catalysts, polymer additives, and nonvolatile degradation products of those compounds
ICP-SFMS	Elements	Metals in fillers, pigments, catalyst residues

* MS = mass spectrometry; GC = gas chromatography; HS = headspace; APCI = atmospheric pressure chemical ionization; ICP-SFMS = inductively coupled plasma/sector field mass spectrometry; UPLC = ultra performance liquid chromatography

Sample preparation

Prior to GC-MS and LC-MS, liquid/liquid extraction was performed on samples of the test and control solutions to transfer organic compounds to a low boiling point organic solvent. Dichloromethane (DCM) was used as extraction solvent for the DMSO samples, while hexane was used as extraction solvent for the DMA samples because of the solubility of DMA in DCM.

Liquid/liquid extractions were performed at three different pHs. The combined extracts of different pH were concentrated under nitrogen flow with a concentration factor of 10.

Differential peaks were determined and identified for VOC analysis. The concentration of detected SVOC was estimated for VOC, except that a different internal standard was used for calculations. The reporting limit was set at 50 µg/L.

NVOC analysis using LC-MS APCI, positive and negative modes

The liquid/liquid extraction sample prep described for GC-MS was used also for LC-MS. An internal standard (Tinuvin 327) was added to a sample of each test or control solution.

Separation was performed on a 3 × 100 mm 1.7 µm C18 column with a water:methanol gradient from 20% to 100% methanol. High resolution mass spectrometry (HRMS) detection was performed in alternating full scan polarity-switching mode (positive and negative APCI, 100 to 1500 amu).

Differential analysis was performed with a software to find differences between the extract and control sample. For each differential peak, retention time, accurate mass of the molecular ion, and mass spectrum were matched against a database to allow identification. Identification level was assigned as: identified compound, most probable compound, tentatively identified compound, or unidentified.

The quantitation of a detected NVOC assigned as identified compound was performed with the compound-specific relative response factor (RRF) available for identified compounds. For other compounds, the response was compared with the response of the internal standard. The reporting limit was set at 50 µg/L.

Elemental analysis

The analysis with ICP-SFMS targeted 25 elements (aluminum, arsenic, barium, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, palladium, potassium, silicon, silver, strontium, sulfur, titanium, vanadium, zinc, and zirconium). Detection limit was in the range of 0.1 to 10 µg/L for all elements except for calcium (100 µg/L), iron (30 µg/L), magnesium (30 µg/L), potassium (100 µg/L), silicon (500 µg/L), sulfur (10 mg/L), and zinc (30 µg/L).

Results and discussion

ÄKTA readyflux™ flow kit

In the extract from the ÄKTA readyflux™ flow kit incubation, five organic compounds were found above reporting limit for each solvent (30% DMSO and 30% DMA). All compounds identities were assigned confirmed and the majority of them were related to antioxidants and silicone material. Some differences between the solvents could be seen, but the total amount of extractables found did not differ significantly. The compound with the highest abundance was a carboxylic acid, semi-quantified to 430 µg per ÄKTA readyflux™ flow kit.

For ICP-SFMS, the results were aligned between the solvents: four elements were above the reporting limit, the same elements in both solvents, with potassium having the highest abundance (3.8 mg/flow kit in 30% DMA).

ReadyToProcess™ column housings

No organic extractable compounds were found above the reporting limit with HS-GC-MS or GC-MS in the extraction samples with 15% DMA or 15% DMSO. The results with LC-MS showed one organic compound that was present in both 15% DMA and 15% DMSO. The extractable compound was assigned a confirmed identity level as an identified compound related to a curing agent used with elastomeric material. The concentration was estimated below 200 µg/L (ppb) in both extraction solvents (that is, less than 200 µg/ReadyToProcess™ column).

Analysis with ICP-SFMS showed low levels of a few extractable elements. The most abundant were calcium (< 100 µg/L) and magnesium (< 25 µg/L), followed by zinc (< 10 µg/L) and barium (< 2 µg/L). The extractable elements were found at a similar level in both 15% DMA and 15% DMSO.

ÄKTA ready™ low flow kit

The results showed five organic extractable compounds above the reporting limit with HS-GC-MS, GC-MS, and LC-MS. Two of the compounds were found in 15% DMA, and three compounds were found in 15% DMSO.

All five extractable compounds were assigned a confirmed identity level as identified compounds related to polyamide and silicone materials and one solvent.

The highest abundant extractable compound was present at a concentration below 600 µg/L (that is, 410 µg/ ÄKTA ready™ low flow kit, because the extraction volume was 700 mL).

Analysis with ICP-SFMS showed that the most abundant extractable element was silicon, which was present below 9 mg/L (ppm). Additionally, calcium, barium, zinc, and copper were found at lower levels. The extractable elements were found at a similar level in both 15% DMA and 15% DMSO.

Assessment of results

A general toxicity and safety evaluation of extractable compounds as a worst case was conducted. The evaluation can only be general, because the specific details regarding the route of administration, dosage level, or toxicity of the proposed drug compounds will differ between different ADCs.

Toxicological information and a derived risk index (RI) for eight out of the ten identified extractable compounds were listed in a reference containing compiled safety impact information (4). In that reference, risk indices were obtained by subjecting toxicological safety data such as no observed effect levels (NOELs), no observed adverse effect levels (NOAELs), lowest published toxic dose (TDLOs), and others to a systematic evaluation process using appropriate uncertainty factors. An RI value represents a daily intake value for life-long intravenous administration. Two additional RI values were derived for the final identified extractable compounds from a reported NOAEL value and appropriate uncertainty factors.

Assumptions for the assessment were:

- Single-use equipment included in this assessment comprise the column housing, the chromatography flow kit, and the tangential flow filtration flow kit. All extractables from these disposables end up as impurity in the ADC product.
- Batch size is 5 g, considered a small batch, representing a worst case.
- Dosage is 3.6 mg/kg given every three weeks (21 d cycle), that is, 3.6 mg/kg × 70 kg bodyweight (bw)/21 d = 12 mg/person/d.

Potential exposure to extractables was calculated from the highest result on extractables divided with the batch size of ADC multiplied with the daily dosage of ADC (Table 6).

Table 6. Calculations for the safety assessment of the eight extractable compounds (highest concentration chosen if reported more than once)

Extractable compound	Highest results on extractables (µg/system)	Batch size ADC (g)	(mg/d)	extractables (µg/d)	(µg/d) (4)
Related to antioxidant[1]	68	5	12	0.16	210
Related to antioxidant[1]	65	5	12	0.16	14 000
Related to antioxidant[1]	180	5	12	0.43	19 100
Related to antioxidant[1]	74	5	12	0.18	14 000
Related to polyamide[3]	410	5	12	0.98	21 000
Related to curing agent[2]	190	5	12	0.46	560
Related to silicone[1,3]	430	5	12	1.03	1750
Solvent[1,3]	25	5	12	0.06	50 000
Related to silicone[1,3]	8	5	12	0.02	700
Related to silicone[1,3]	7	5	12	0.02	11 200

1ÄKTA readyflux™ flow kit

2ReadyToProcess™ column

3ÄKTA ready™ flow kit

Assessment of the results according to the listed assumptions shows that the potential exposure to extractables is well below the RI for each extractable compound. Therefore, extractables from the ÄKTA readyflux™ flow kit, ReadyToProcess™ column housing, and ÄKTA ready™ flow kit should pose no safety concern for use in ADC manufacturing within the conditions of this study.

Conclusions

he low levels of extractables found in this study demonstrate the chemical compatibility of ÄKTA readyflux™ flow kits, ReadyToProcess™ columns, and ÄKTA ready™ flow kits with two organic solvents typically employed in ADC manufacturing processes: DMSO and DMA. Detailed results of these studies are available in the validation guides for these SU components to supplement existing data generated with aqueous solvents and ethanol. Along with other SU components and systems, the ÄKTA readyflux™ flow kits, ReadyToProcess™ columns, and ÄKTA ready™ flow kits offer a solution to some of the main challenges in ADC manufacturing.

Resources

Access validation guides via Cytiva Regulatory Support (registration required)

ÄKTA ready™ flow kits

ÄKTA readyflux™ flow kits

Learn more about ReadyToProcess™ columns

cytiva.com

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Further Reading and Resources

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- **Welcoming the "Era of ADCs"**
- **Getting CMC Right for Emerging Technologies**
- **Following the Science to Develop Best-in-Class Cancer Drugs**
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