AAPS PharmSciTech (2026) 27:224
https://doi.org/10.1208/s12249-026-03457-9

MINI-REVIEW-THEME

Integrated Strategies in CMC Development of Antibody Drug Conjugates

Antibody–Drug Conjugates Drug Product Formulation and Process Development, Scalability and Stability Considerations

Léa Sorret[1] · Mamiko Ninomiya[2] · Nils Hillebrandt[3] · David Schmitt[1] · Arundhati Chattopadhyay[3] · Orla McGarvey[3]

Received: 6 February 2026 / Accepted: 8 May 2026

Abstract

Antibody–drug conjugates (ADCs) combine the target specificity of monoclonal antibodies with the potency of cytotoxic small molecules, offering enhanced therapeutic potential. However, their inherent structural complexity, arising from the interplay of antibody, linker, and payload, introduces unique challenges for drug product (DP) development, stability, and scalability requiring tailored approaches to maintain the quality target product profile throughout the product lifecycle. This review summarizes key formulation and process development considerations for ADCs, with an emphasis on aspects that are particularly distinctive for ADC drug products. Critical degradation risks, such as photo- and heat-induced degradation, are discussed in the context of formulation screening, formulation excipients and primary packaging selection, and DP process design. Basic considerations on analytical tools used to identify and monitor critical quality attributes are provided. Lyophilization strategies, essential for the stability of commercialized ADCs, are recommended using Design of Experiments and modeling approaches. Furthermore, robust DP process control strategies during scale-up are reviewed with a focus on lyophilization, the application of Quality by Design (QbD) principles, and risk-based methodologies to address ADC-specific challenges. Finally, the review explores the transition from early development to late-phase clinical and commercial stages, underscoring the need for evolving control strategies. Overall, it provides an overview of current practices and challenges in ADC formulation and DP process development, offering strategies to navigate these complexities based on literature and insights from approved ADCs.

Keywords antibody–drug conjugates · drug product · formulation development · lyophilization · process development

Introduction

Antibody Drug Conjugates (ADCs) represent a rapidly evolving class of biotherapeutics that combine the specificity of monoclonal antibodies (mAbs) with the potency of cytotoxic small molecules. ADCs are an evolving class of therapeutic agents in oncology with multiple U.S. Food and Drug Administration (FDA)-approved products across various types of malignancies, and several more in late-stage clinical development [1]. ADCs achieve selective tumor cell necrosis while minimizing collateral damage to healthy tissues [2]. This unique mode of action is achieved through a complex interplay of three critical components: (1) the mAb specific for a tumor antigen, (2) the cytotoxic payload, and (3) the linker that links the mAb to the payload. While the payload aims to kill the targeted cancer cells, the linker ensures stability in systemic circulation while enabling the payload's controlled release within the target cell. The interplay among these elements, along with the conjugation chemistry, site of attachment, and drug-to-antibody ratio (DAR) introduces unique physicochemical and biological challenges that distinguish ADCs from their mAb parent molecule and its individual components [3, 4].

Communicated by Purbasa Patnaik and Mysore Ramprasad.

✉ Orla McGarvey
orla.mcgarvey@lonza.com

[1] Drug Product Formulation Development, Lonza AG, Hochbergerstrasse 60G, 4057 Basel, Switzerland

[2] Preclinical Development Bioconjugates, Lonza AG, Rottenstrasse 6, 3930 Visp, Switzerland

[3] Drug Product Process Development, Lonza AG, Hochbergerstrasse 60G, 4057 Basel, Switzerland

Published online: 04 June 2026

Since the approval of the first ADC, Mylotarg® in 2000, the field has witnessed significant progress, with 16 ADCs approved globally and over 300 in clinical development as of 2025 [5]. Early generation ADCs were limited by the use of labile linkers, such as acid-cleavable hydrazone, and stochastic lysine or cysteine conjugation, often resulting in suboptimal stability and safety profiles [6]. Subsequent generations have incorporated more robust linkers and site-specific conjugation strategies, improving the therapeutic index and reducing off-target toxicity [7]. Despite these advancements, ADCs remain inherently less stable than their parent mAb, necessitating tailored solutions during formulation and DP process development and scale-up, while considering an increased safety level [8].

Formulation development of ADCs should address instability typically introduced by conjugating the mAb to the linker and payload, such as linker cleavage or increased aggregation [9–11]. In addition, instability caused by either physical and chemical stresses associated with external factors such as storage temperature, mechanical stress (i.e., shaking), light exposure, and oxidative stress can further compromise the ADC's stability and should be addressed during formulation and process development [10, 12–14]. Chemical liabilities from linker instability can result in premature, non-specific release of the free drug, causing systemic side effects due to exposure of the potent cytotoxic payload to healthy tissues. Consequently, to mitigate some of these stability risks, most marketed ADCs are supplied as lyophilized powders to enhance drug product's (DP) shelf-life and minimize degradation [15]. However, lyophilization introduces its own complexity, requiring careful optimization of excipients, cycle parameters, and reconstitution stability [16, 17]. Moreover, ADC stability can be further impacted during scale-up and fill-and-finish operations, making it essential to implement tailored process development steps and well defined control strategies to ensure product integrity and consistency [8, 18].

This article presents a comprehensive review of ADC stability considerations, formulation development and DP process development considerations including control strategy. The latter are discussed with a particular emphasis on lyophilization, while other unit operations are addressed only with regard to ADC-specific risks. Drawing data from approved ADC products and the available literature, this review aims to inform on best practices for developing robust, clinically effective, and commercially viable ADC DPs. Although the landscape of biologic conjugates is rapidly expanding, encompassing diverse combinations of biologic-linkers-payloads architectures, such as radio-conjugates and oligonucleotide conjugates, this review focuses on molecules containing solely a mAb, linker, and cytotoxic payload construct and other molecule types are considered out of scope as their construction could alter their stability and influence the overall final DP formulation and process approach.

ADC Stability

Contributing Factors to ADC Stability

ADC stability is inherently more complex than mAb stability due to the incorporated linker-payload moiety which introduces additional chemical liabilities and perturbs the mAb's physicochemical properties [19, 20]. The conjugation of a typically hydrophobic payload can alter surface charge, hydrophobicity, and colloidal and conformational properties, leading to reduced solubility, increased aggregation, and impact on pharmacokinetics [3]. Moreover, the linker-payload itself is prone to chemical degradation under stress conditions. Consequently, ADC stability should be considered as an integrated property of the entire conjugate rather than as the sum of the individual components – the mAb, linker, and payload. Stability risks generally fall into two broad categories: (i) physical stability, including aggregation, conformational perturbation, and (ii) chemical stability, encompassing chemical modifications to the mAb, linker or payload.

Figure 1 lists the cause of ADC instability and consequences to the molecule undergone during development, shelf-life stability, and in-use stability. The causes of instabilities arise from both intrinsic, conjugation-induced perturbations and extrinsic stress factors encountered throughout the ADC lifecycle as shown in Fig. 1. Beyond careful selection of linker and payload, tailored formulation design is critical to mitigate physicochemical instability across the product's shelf-life.

Intrinsic, Conjugation-induced Instability of ADCs

ADCs require payloads with high potency because the mAb-based drug delivery system limits the absolute amount of cytotoxic agent reaching the target cell necessitating that the drug be effective at concentrations several orders of magnitude lower than those used in traditional chemotherapy with small molecules [21–23]. The small-molecule payloads used in most ADCs, such as Auristatins (MMAE), Maytansinoids (DM1), and Camptothecin derivatives (Exatecan and Deruxtecan), are highly potent, non-polar and inherently hydrophobic [24, 25]. The attachment of those hydrophobic molecules changes the surface charge, increasing its overall hydrophobicity and thereby reducing overall colloidal and conformational stability [9, 10, 26]. Differential scanning fluorimetry and dynamic light scattering experiments have shown the correlation between the destabilization of the native mAb structure and the increasing hydrophobicity of

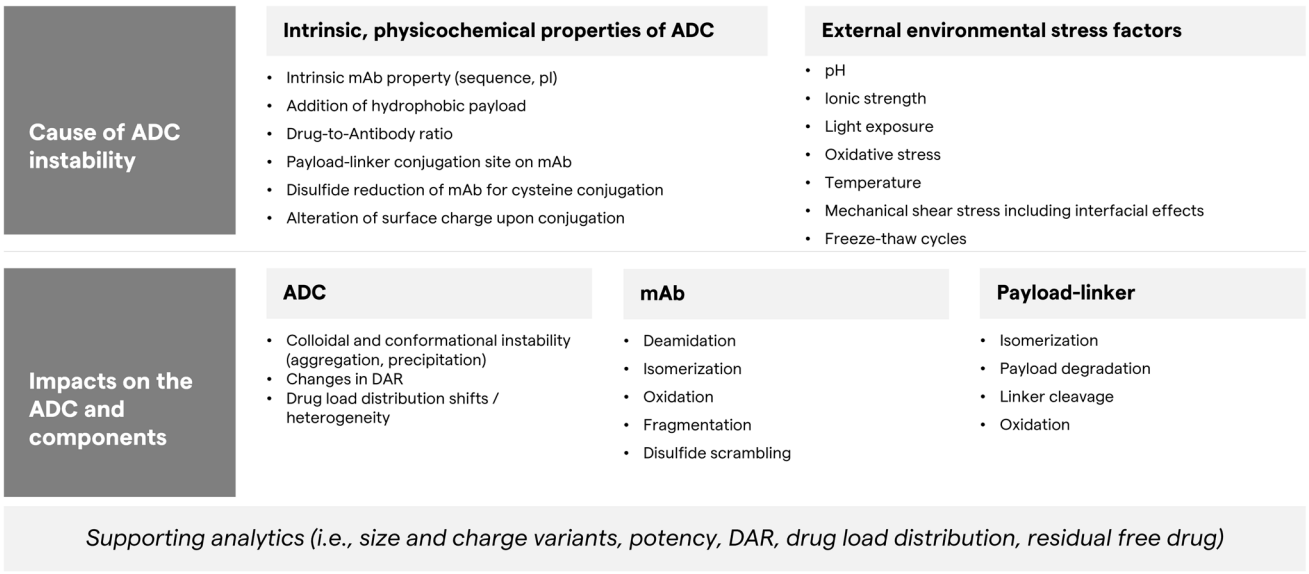

Fig. 1 Key stability considerations and stress factors in ADC formulations

the payload [27]. Although higher drug loading can theoretically enhance the drug's efficacy, excessive hydrophobicity associated with high DARs has been linked to increased aggregation [9, 10, 25, 28]. Accordingly, the DAR of most commercially approved ADCs is typically within a range of 2 to 4, with few exceptions as shown in Table I. However, the success of Enhertu® with a DAR of 8 has driven a trend toward the development of ADCs with higher DAR, most often using topoisomerase I inhibitor payloads (SN-38, Dxd). This shift toward higher DARs may introduce additional stability challenges, requiring careful selection of the linker and formulation [29].

The conjugation chemistry and the attachment site significantly influence the physical stability of ADCs, affecting aggregation as well as thermal stability [30–32]. For example, the conventional lysine conjugation method can reduce surface charge through the removal of the positive lysine charge, increasing aggregation risk [33, 34]. Similarly, cysteine-maleimide conjugation, which involves reducing interchain disulfides, introduces hydrophobic linker and payloads. Guo et al. reported that such modification decreased the parent mAb stability by decreasing the overall structure compactness and increasing hydrophobicity, despite retaining the secondary or tertiary structures [26]. Site-specific conjugation strategies, such as engineered cysteines or enzymatic methods, enable rational site selection to optimize stability which is critical as the location of the conjugation site can modulate hydrophobicity, aggregation propensity, and conformational stability. For instance, conjugation in buried regions can shield hydrophobic payloads and reduce aggregation, whereas conjugation near solvent-exposed areas often increases hydrophobicity and aggregation [31, 35–37].

Linker design in ADCs has evolved not only to control payload release but also to modulate the conjugate's hydrophobicity and stability. The different linkers commonly used have been reviewed previously [38, 39] and are shown in Table I for each commercialized ADC. Early linker designs were selected primarily based on their cleavage mechanism and systemic stability. The linker types can be broadly divided into four categories: acid-labile, redox-labile, enzyme-labile, and non-cleavable [38]. In addition to those cleavage mechanisms, more recently, linker architectures have been refined to incorporate hydrophilic or charged motifs, such as poly(ethylene glycol) or amino acid residues, to enhance solubility and minimize ADC aggregation associated with the conjugation of hydrophobic payloads [38, 40].

Instability of ADCs Induced by External Stresses

Extrinsic stress factors introduced during the development and product lifecycle, such as thermal, pH, freeze–thaw, or mechanical stress and light exposure can lead to the degradation of ADCs [11]. The impact of those external stresses on the parent mAb molecule can lead to physicochemical changes such as protein aggregation, fragmentation, and denaturation and these degradation pathways have been extensively reviewed [41–45]. In contrast, the systematic investigation of these environmental stress factors on ADCs have been far less reported in literature. One study by Wakankar et al. demonstrated that elevated temperature can impact the physicochemical stability of the ADC in comparison to the parent mAb [46]. ADCs generally exhibit low thermal stress tolerance, which can destabilize and expose hydrophobic regions, increasing aggregation propensity.

Table I Summary of approved ADCs on the market as of October 2025, information gathered using either product's SmPC or Package Insert when available

Name[a]	Approval Year[b]	Antibody	Conjugation	Linker (Cleavable or Non-cleavable)	Payload	DAR	Dosage Form	DP conc.[c] (mg/mL)	Buffer	pH	Excipient 1	Excipient 2	Excipient 3	DP primary container
Gemtuzumab ozogamicin (Mylotarg®)	2000	IgG4	Lysine	Hydrazone (acid-labile)	Calicheamicin	2–3	Lyophilized	1	Sodium phosphate	7.5	Sucrose	NaCl	Dextran 40	Amber glass vial
Brentuximab vedotin (Adcetris®)	2011	IgG1	Cysteine	MC-VC-PABC (enzyme-labile	Auristatin (MMAE)	4	Lyophilized	5	Sodium citrate	6.6	Trehalose	-	0.02% PS80	Clear glass vial
Tratuzumab emtansine (Kadcyla®)	2013	IgG1	Lysine	MCC (non-cleavable)	Maytansine (DM1)	3.5	Lyophilized	20	Sodium succinate	5	Sucrose	-	0.02% PS20	Clear glass vial
Inotuzumab ozogamicin (Besponsa®)	2017	IgG4	Lysine	Hydrazone (acid-labile)	Calicheamicin	6	Lyophilized	0.25	Tris	8	Sucrose	NaCl	0.01% PS80	Amber glass vial
Polatuzumab vedotin (Polivy®)	2019	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	Auristatin (MMAE)	3.5	Lyophilized	20	Sodium succinate	5.3	Sucrose	-	0.12% PS20	Clear glass vial
Enfortumab vedotin (Padcev®)	2019	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	Auristatin (MMAE)	4	Lyophilized	10	Histidine	6	Trehalose	-	0.02% PS20	Clear glass vial
Trastuzumab deruxtecan (Enhertu®)	2019	IgG1	Cysteine	MC-GGFG (enzyme-labile)	Camptothecin (DXd)	8	Lyophilized	20	Histidine	5.5	Sucrose	-	0.03% PS80	Amber glass vial
Sacituzumab govitecan (Trodelvy®)	2020	IgG1	Cysteine	CL2A (MCC-PEG-carbonate) (acid-labile)	Camptothecin (SN-38)	7–8	Lyophilized	10	MES	6.5	Trehalose	-	0.01% PS80	Clear glass vial
Belantamab mafodotin (Blenrep®)	2020	IgG1	Cysteine	MC (non-cleavable)	Auristatin (MMAF)	4	Lyophilized	50	Sodium citrate	6.2	Trehalose	EDTA	0.02% PS80	Clear glass vial
Loncastuximab tesirine (Zynlonta®)	2021	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	PBD (SG3199)	2.3	Lyophilized	5	Histidine	6	Sucrose	-	0.02% PS20	Clear glass vial
Tisotumab vedotin (Tivdak®)	2021	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	Auristatin (MMAE)	4	Lyophilized	10	Histidine	6	Sucrose	Mannitol	-	Clear glass vial
Disitamab vedotin (Aidixi™)	2021 (China)	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	Auristatin (MMAE)	4	Lyophilized	10	Histidine	N/A[d]	Sucrose	Mannitol	PS80	N/A[d]
Mirvetuximab soravtansine (Elahere®)	2022	IgG1	Lysine	sulfo-SPDB (redox-labile)	Maytansine (DM4)	3.4	Liquid	5	Sodium acetate	5	Sucrose	-	0.01% PS20	Clear glass vial

Table I (continued)

Name[a]	Approval Year[b]	Antibody	Conjugation	Linker (Cleavable or Non-cleavable)	Payload	DAR	Dosage Form	DP conc.[c] (mg/mL)	Buffer	pH	Excipient 1	Excipient 2	Excipient 3	DP primary container
Sacituzumab tirumotecan (Jiataile™)	2024 (China)	IgG1	Cysteine	sulfonyl-pyrimidine CL2A-carbonate linker (acid-labile)	Camptothecin (Belotecan-derivative)	7.4	Lyophilized	20	Histidine	N/A[d]	Sucrose	-	PS20	Glass vial
Datopotamab deruxtecan-dlnk (Datroway®)	2025	IgG1	Cysteine	MC-GGFG (enzyme-labile)	Camptothecin (DXd)	4	Lyophilized	20	Histidine	6	Sucrose	-	0.03% PS80	Amber glass vial
Telisotuzumab vedotin (Emrelis®)	2025	IgG1	Cysteine	MC-VC-PABC (enzyme-labile)	Auristatin (MMAE)	3	Lyophilized	20	Histidine	6	Sucrose	-	0.01% PS80	N/A

[a]Only molecules with defined structures consisting of mAb + linker + cytotoxic payload are included, as such Lumoxiti® is not shown, Akalux® and Zevalin® are also excluded because their payloads are not cytotoxic: Akalux® is an imaging agent, and Zevalin® carries a radioligand. Trastuzumab rezetecan (approved in May 2025 in China) is not shown due to insufficient official data in English as of October 2025.

[b]FDA or EMA approval year unless specified otherwise.

[c]Concentrations after reconstitution for lyophilized products.

[d]Not available in Chinese package insert or other documentation.

Abbreviations: *ADC* Antibody–drug conjugate; *conc* concentration, *DAR* drug-to-antibody ratio, *DM1* mertansine, *DM4* soravtansine, *DP* Drug product, *DXd* deruxtecan, *EDTA* Ethylenediaminetetraacetic acid; *GGFG* Gly-Gly-Phe-Gly, *IgG* Immunoglobulin G, *N/A* Not available, *MC* Maleimidocaproyl, *MCC* Maleimidomethyl cyclohexane-1-carboxylate, *MES* 2-(N-morpholino) ethanesulfonic acid, *MMAE* Monomethyl auristatin E, *MMAF* Monomethyl auristatin F, *PABC* Para-aminobenzyloxycarbonyl, *PBD* Pyrrolobenzodiazepine, *PEG* Polyethylene glycol, *PS20* Polysorbate 20, *PS80* Polysorbate 80, *SmPC* Summary of product characteristics, *SPDB* N-succinimidyl 4-(2-pyridyldithio)butanoate, *Tris* Tris(hydroxymethyl)aminomethane, *VA* valine-alanine, *VC* Valine-citrulline.

The extent of thermal sensitivity varies with linker type and payload hydrophobicity [9]. Mohamed et al. assessed the stability of lysine-linked ADC Trastuzumab emtansine in comparison to its parent Trastuzumab as a function of agitation, freeze–thaw, and pH stress, in addition to thermal stress [11]. In general, the aggregation of the ADC, measured by size-exclusion chromatography (SEC), dynamic light scattering, and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, was increased relative to its parent mAb as a function of the applied stresses, even at temperatures as low as 25°C, whereas no significant changes were observed as a result of freeze–thaw stress. This degradation was attributed to the presence of the drug-linker, which resulted in the alteration of the reversed-phase liquid chromatography profile as well as the decrease in conformational stability measured by differential scanning calorimetry (DSC), impacting the molecule's overall stability during applied external stresses [11, 46].

The presence of the linker and payload can introduce additional pH-dependent chemical instability in ADCs, beyond the well-characterized pH effects on the parent mAb, such as isomerization and deamidation [45, 47]. Such sensitivity can lead to premature cleavage of the cytotoxic payload [48] (Fig. 1). Specifically, the hydrazone moiety containing acid-cleavable linker has a short half-life at pH 5.0 [49], leading to the premature release of payload when the pH of the DP formulation buffer is not suitable. Currently often used enzymatically cleavable dipeptide-based linkers (e.g., valine-citrulline or valine-alanine) are designed to remain largely stable under formulation and storage conditions and undergo efficient cleavage only after internalization and lysosomal protease processing.

Some of the small molecule payloads used in ADCs are light-sensitive due to their chemical structure and reactive functional groups required for their cytotoxic activity [14, 50]. A study by Thiess et al. demonstrated that an ADC exhibited photo-degradation, including oxidation, under relatively mild exposures (36 Wh/m^2 UV and 56 kLux•h vis), not observed in the mAbs-only counterpart [51]. This photosensitivity was strongly dependent on payload type, with the greatest impact on ADCs conjugated to Exatecan, a Camptothecin derivative, for which a strong presence of reactive oxygen species (ROS) was measured. Based on other studies for other Camptothecin derivatives, the photodegradation involves lactone ring opening, suggesting that the pH might be one of the important factors for the light-sensitivity of ADCs with Camptothecin derivatives [14, 50, 52–55].

Furthermore, oxidative stress can also be induced by the presence of ROS generated through metal oxidants, such as Cu(II) and Fe(II/III) and/or introduced during the manufacturing process, for example via fermentation media, buffers/excipients including surfactants, isolator sterilization, container closure systems [13, 56–59]. Glover et al. demonstrated that these metal-catalyzed reactions not only lead to oxidation but also to changes in aggregation and fragmentation, for both cysteine- and lysine-conjugated ADCs [13]. The results demonstrate that the conjugation did not make the ADC more susceptible to chemical modifications relative to the parent mAb however increased aggregation was observed upon exposure to additional oxygen relative to the parent mAb. This review focuses on the stability related to the DP development up to in-use stability. Therefore, any liabilities after administration such as premature release of free-payload linker from thiol-maleimide conjugation by retro-Michael reactions are out of scope of this review.

The degradation pathways aforementioned can negatively impact critical quality attributes (CQAs) related to immunogenicity, pharmacokinetics, and potency, and must be minimized through rational ADC design and an optimized formulation. The liabilities outlined underscore the need for controlled formulation and DP manufacturing process environments, coupled by robust analytical strategies tailored to monitor ADC-specific quality attributes. Comprehensive analytical tools are thus essential for monitoring ADC stability throughout development and long-term stability to achieve the targeted shelf-life [60, 61]. Furthermore, analytical comparability studies are required for the implementation of manufacturing and formulation changes throughout the product lifecycle[8, 62]. Beyond the standard analytical methods employed to assess the stability and purity of mAbs such as size variants analysis by SEC and capillary electrophoresis SDS, charged variant analysis by imaged capillary isoelectric focusing or ion-exchange chromatography, and potency analysis, ADCs require additional analytics to evaluate ADC specific quality attributes. These include methods to determine the average DAR, drug load distribution, residual drug by for example chromatographic methods [63]. Analytical strategies around ADCs is outside the scope of this review and will not be further discussed here, it has been well documented in the literature [8, 60, 61, 63–65].

Formulation development of ADCs

Formulation Review of Marketed ADCs

The landscape of the 16 marketed ADCs offers valuable insight into formulation strategies of ADCs as detailed compositional data of most clinical stage ADCs remains largely publicly undisclosed. Table I summarizes the formulation profiles of marketed ADC DP as of October 2025, based on information from the Package Leaflet (FDA), the Summary of Product Characteristics (SmPCs) (European Medicines Agency, EMA) or equivalent regulatory document provided by other national authorities (i.e., Aidixi™ and Jiataile™ approved only in China by the National Medical

Products Administration (NMPA)). Given the scope of this review, ADCs that have been withdrawn or granted only provisional approval were also included. In contrast, molecules which lacked the typical linker-cytotoxic payload architecture of ADCs, such as Lumoxiti® sometimes referenced in other published work were excluded [66]. As shown in Table I, the majority of approved ADC formulations contain a buffering and a stabilizing agent, and a non-ionic surfactant, typically polysorbate, and are formulated at concentrations below 20 mg/mL, except for Blenrep®, reported at 50 mg/mL. The relatively low DP concentrations reflect the low dosage required for these potent drugs (often much below 10 mg/kg) and their intravenous administration route, which avoids the volume limitations associated with subcutaneous delivery, increasing in popularity for many mAb therapeutics [67]. Finally, only one of the marketed ADC drugs listed in Table I is presented in liquid form, Elahere®, an anti-Folate Receptor-α mAb (IgG1) conjugated via a cleavable linker to cytotoxic payload DM4. Although unusual to have an approved liquid ADC DP, this may be indicating a trend in recent years towards liquid formulation ADC products [51]. Interestingly, Elahere® has a long shelf-life stability of 60 months at 2–8°C [68], likely enhanced by the chemically robust amide linkages, despite the heterogeneity of the lysine conjugation [69]. Despite the advantages of liquid dosage forms (avoiding the need for reconstitution, resulting in faster administration in the clinic), lyophilized DP formats predominate presumably to enhance shelf-life and stability.

As shown in Table I, all excipients are common excipients found in mAb-based DP formulations and fall within a typical range for intravenous parenteral biological products [70, 71]. Although histidine buffer remains the predominant choice (found in 50% of disclosed ADC formulations), the overall distribution of buffer type and pH range is more heterogeneous compared to conventional mAb formulations [71]. While mAbs are generally formulated at pH 5–7 (average around 5.8, with no significant trend between low and high concentration products [71]), ADC formulations span a broader pH range from pH 5.0 to 8 (Fig. 2). This broader distribution likely reflects the additional stability constraints imposed by the conjugated linker-payload in addition to the mAb itself. Among two major conjugation methods used in the commercial ADCs (Table I), cysteine-maleimide conjugated ADCs, are typically formulated under slightly acidic conditions, between 5.3 and 6.6, presumably to prevent the hydrolysis of thio-succinimide ring and maintaining ADC's physical stability for long-term storage. In contrast, lysine-conjugated ADCs formulation buffer pH range both acidic (pH 5) and basic (pH > 7.5) pH, reflecting the reduced products susceptibility to hydrolysis. Specifically the slight basic formulation from lysine conjugated products corresponds to acid-labile hydrazone linker containing ADCs (Mylotarg® and Besponsa®) to avoid the release of payload in the formulation buffer.

Most approved ADCs contain one or more excipients commonly found in parenteral biologics. Notably, all contain a disaccharide, such as sucrose or trehalose, with 12 of 16 containing sucrose, while the remaining contain trehalose (Table I). Mannitol, a saccharide-derivative, is also present in Aidixi™ and Tivdak®. These sugars have been reported to contribute to stability during storage and reconstitution and are especially important for cake quality and product stability in lyophilized presentations [17]. Disaccharides enhance the conformational stability of mAbs through preferential exclusion by favoring the folded state of the

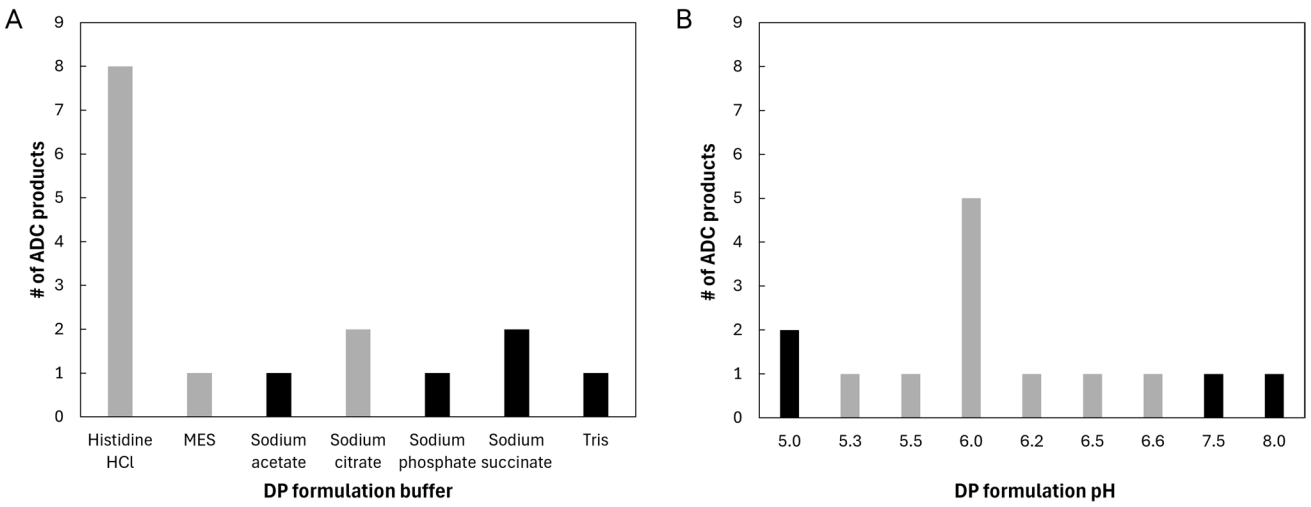

Fig. 2 Number of commercialized ADC product from Table I by (**A**) DP formulation buffer and (**B**) DP formulation pH. Black bars represent lysine conjugation and gray bars cysteine conjugation technology. pH values for Aidixi™ and Jiataile™ not available in Chinese package insert or other official documentation, therefore excluded from graph

protein [72] and act as cryoprotectants during freeze–thaw and lyophilization. While these mechanisms have been well described for mAbs, their role on ADCs stability remains limited. Additional stabilizers appear in 2 of the 16 marketed ADCs, Dextran 40 (Mylotarg®), and NaCl (100 mM NaCl in Mylotarg®, and 10 mM NaCl in Besponsa®). Dextran 40, similarly to the sucrose already present in Mylotarg®, can help stabilize and provide cryo-protection. Interestingly, Adem et al. reported that formulation with high ionic strength (> 50 mM NaCl) generally contributed to increased aggregation and fragmentation of an ADC compared to its parent mAb, attributing this to hydrophobic interactions induced by the conjugation, in particular at increased DAR [24]. Furthermore, the excipient NaCl is generally avoided in lyophilized formulations as it tends to crystallize during freezing and drying which can compromise cake integrity, making it unlikely in general to be included as an excipient in ADC products [17].

Although ADCs containing Camptothecin payloads are susceptible to photodegradation, none of the currently marketed ADC products include antioxidants commonly used in photo-sensitive mAb formulations, such as methionine [73]. Recent studies by Luo et al. demonstrated that optimizing buffer and excipient selection can mitigate light-induced ADC degradation compared to the parent mAb [14]. For example, histidine buffer can act as a scavenger, although pH shifts under light stress were observed. Additional protection was achieved by incorporating excipients such as methionine, ascorbic acid, or sucrose. Instead, mitigation strategies at the DP level, shown in Table I, rely primarily on protective packaging, such as amber vials. Additional protective measures may be implemented during development and manufacturing, however, such details are not publicly disclosed in product labeling. In regards to metal-catalyzed oxidative degradation, only one product, Blenrep®, contains a chelators (ethylenediaminetetraacetic acid, EDTA), to protect its auristatin payload. EDTA can play a protective antioxidant role by chelating metal ions that catalyze the formation of ROS [71].

Non-ionic surfactants, in particular polysorbate 20 (PS20) and polysorbate 80 (PS80) are ubiquitous in ADC formulations, serving to prevent aggregation, including agitation-induced aggregation [74] and unwanted surface adsorption which can be more pronounced due to ADC's increased hydrophobicity relative to unconjugated parent mAb. The distribution of surfactant type mirrors recent trends observed in mAb formulations: while earlier mAb products before 2015 predominantly used PS80 (~ 60%), smaller proportions used PS20 (~ 20%) or no surfactant (~ 20%), newer trends indicate a shift toward PS20, now present in 34% of mAb formulations while only 2% lack surfactants [75]. ADCs DP exhibit a similar pattern, with 50% containing PS80, while 37.5% contain PS20, and 12.5% with no surfactant consistent with evolving formulation preferences. Surfactant concentrations in ADCs (typically 0.02–0.1% w/v) are comparable to those used in mAbs.

Although the limited number of commercially approved ADCs restricts robust statistical or trending analysis, the available, albeit fragmentary, information suggests that ADC formulation strategies both overlap with and diverge in certain respects from classical mAb formulation norms. Core excipients such as disaccharides and non-ionic surfactants remain common to both modalities; however, ADCs introduce additional considerations driven by linker-payload moiety. These constraints manifest into broader pH ranges, narrower buffer and excipient choices. Recognizing these trends early is critical for designing robust and adapted ADC formulations that balance mAb stability principles and liabilities from the ADC linker-payload conjugation.

ADC Formulation Development Strategy

Figure 3 depicts a suggested DP pre-clinical formulation and process development strategy for ADCs. While many principles align with mAbs development, such as physicochemical stability screens within a Quality by Design (QbD) framework supported by relevant analytical and statistical tools [61, 76–78], ADC development introduces additional complexity due to the linker-payload conjugation [79]. These unique attributes demand tailored approaches, without compromising development timelines, to ensure a stable DP with the desired shelf-life [20, 61]. Furthermore, the emergence of next-generation ADC technologies aimed at improving the therapeutic window and reducing toxicity adds further challenges, including diverse conjugation chemistries and more varied mAb scaffolds [2].

A risk-based approach is essential to prioritize CQAs and identify formulation variables most likely to impact ADC stability and safety. Early screening, or 'developability' assessments as described in Jarasch et al., are critical to both mAb and ADC candidates to inform on lead selection [80]. These screens, leveraging *in-silico* and *in-vitro* tools, can identify stability liabilities and inform formulation design, although *in-silico* computational approaches are less mature for novel constructs such as ADCs than for mAbs [80–83]. The formulation development of the mAb unconjugated intermediate should consider the intended storage conditions (i.e., ≤ −65°C) which contrasts with the final ADC DP or typical mAb-only DP formulations, typically stored at 2–8°C [15]. Furthermore, excipient compatibility with the conjugation steps should be considered to minimize buffer exchanges and avoid components that interfere with coupling reactions, for example, primary amine excipients (e.g., histidine) in lysine-based conjugation [15, 61]. Surfactants should also be excluded, as they are difficult to remove and may mask reactive sites, thereby disrupting conjugation chemistry [84].

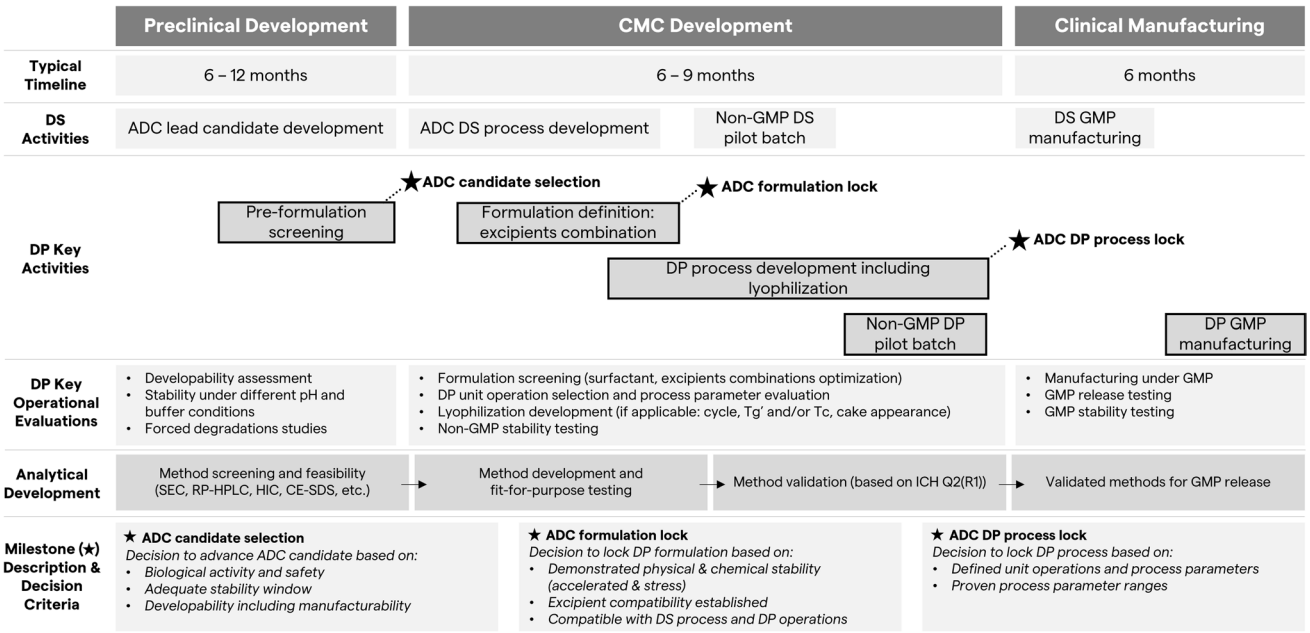

Fig. 3 Example of an ADC DP preclinical formulation and process development workflow from ADC lead candidate development to clinical manufacturing. Stages involving DP activities are highlighted with a black outline. Stars indicate major development milestones for DP. Abbreviations: ADC, antibody–drug conjugate; CE-SDS, capillary electrophoresis-sodium dodecyl sulfate; CMC, chemistry, manufacturing, and controls; DP, drug product; DS, drug substance; GMP, good manufacturing practice; HIC, hydrophobic interaction chromatography; ICH, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; RP-HPLC, reversed-phase high-performance liquid chromatography; SEC, size-exclusion chromatography; Tc, collapse temperature; Tg′, glass transition temperature of the maximally freeze-concentrated solution

Optimizing the parent mAb formulation early can accelerate timelines and mitigate supply constraints, as mAb material is often available ahead of conjugation and process development. High-throughput, plate-based screening methods should be employed when feasible, to conserve material and accelerate development [85].

Conjugation frequently compromises molecular stability, therefore acceptable mAb stability does not ensure ADC stability [20, 84]. To mitigate ADC-specific liabilities, a tailored formulation development should incorporate excipients such as sugars, surfactants, as discussed previously [79, 84]. A systematic strategy should include screening of pH and buffer systems as well as excipient evaluation under accelerated and stressed conditions [86, 87]. Given the hydrophobic nature of ADCs and the typical lower DP concentration, thereby increasing the risk of surface adsorption, surfactant selection should be carefully evaluated. Here, polysorbate degradation is a known challenge in liquid dosage forms, as even trace levels of lipases from host cells can drive degradation. A careful monitoring using orthogonal analytical methods alongside identification and lipolytic activity assays can be performed [88, 89]. Higher polysorbate concentrations can be used, whilst balancing the impact of peroxide and fatty acid generation from polysorbate degradation [58]. With the strong preference for lyophilized presentations for early-stage formulations and in the current commercialized ADC products, lyophilization prevents time-dependent polysorbate degradation and particle formation. On the other hand, formulation parameters should be optimized for successful lyophilization, particularly in anticipation of the commercial presentation. This includes for example avoiding high salt concentrations and phosphate buffers, which can lead to risks of crystallization and poor cake structure, or volatile buffer species such as acetic acid [16] (see Lyophilization strategies for further details). However, frozen liquid DP presentations as applied for other immunoconjugates [15], may represent a viable alternative, particularly for early clinical trials. Finally, primary packaging considerations, typically glass vials for ADCs, should be integrated early in development (see Process Scalability and Control Strategy for further considerations).

Lyophilization Strategies

Despite recent advances in liquid dosage forms (e.g. Elahere®), linker-related degradation pathways mentioned in early sections and shown in Fig. 1, including hydrolysis and premature payload release, remain major stability liabilities [90]. For ADCs with linkers that are particularly sensitive

to degradation in aqueous environments, lyophilization can provide substantial stability benefits by minimizing hydrolysis and other stress factors encountered in liquid formulations (Fig. 1) and can be included in the DP development workflow (Fig. 3).

Lyophilization Cycle Development

When developing a lyophilization cycle, conducting a comprehensive analysis of the thermal properties of the final formulated ADC is crucial to ensure correct parameters for the lyophilization process are selected. The glass transition temperature of the maximally freeze-concentrated solution (T_g') and the collapse temperature (T_c), are critical product properties [16]. T_g' is determined using appropriate thermal analytical techniques such as modulated temperature DSC [91, 92]. A conservative safety margin should be applied below the measured T_c by light transmission freeze-drying microscopy to avoid a microcollapse of the lyophilized cake [93]. Therefore, the target product temperature (T_p) during primary drying is typically set at approximately 2–5°C below T_c (or below T_g' for amorphous systems), also to account for process variability and edge vial effects [94, 95]. To the best of our knowledge, no trends in the thermal behavior (T_g' or T_c) of ADCs compared to antibodies are reported in literature. Moreover, the thermal behavior of a formulation is predominantly influenced by its excipients and their concentrations as well as the concentration of the protein [96, 97], implying that common lyophilization strategies for proteins are also applicable to ADCs.

However, ADCs present additional challenges due to the conjugation of the payload. Conjugation, especially via certain chemistries such as thiol-maleimide, can reduce the structural stability of the antibody scaffold when subjected to thermal stress [26, 98]. Buecheler et al. describe how increasing payload hydrophobicity can also contribute to reduced ADC conformational thermal stability as well as an enhanced propensity to self-associate [90]. This additional complexity of the ADC molecule, as also mentioned in the previous section on ***ADC stability***, highlights the need to experimentally determine the stability of the molecule from the start of lyophilization until the completion of reconstitution.

Safety Considerations

The high potency of ADC payloads and the potential for airborne particle generation during lyophilization necessitate stringent safety measures to protect laboratory personnel and ensure safe handling. However, isolators and containment strategies applied at manufacturing scale may not always be available in development laboratories [99, 100]. Alternatively, protective bags can be used to mitigate this risk. However, protective bags were shown to hinder vapor flow leading to extended drying times which should be considered during scale-up [101, 102], e.g. through modeling as discussed below. Furthermore, to reduce the risk of handling highly potent active material during small-scale laboratory studies and to conserve active material, surrogate formulations can be employed. Such surrogate formulations should have similar physical attributes to the DP, including total solids content, viscosity, surface tension, density, and product temperature profile during processing [103, 104]. Such surrogates can consist of a high molecular weight substance replacing the active, e.g. Dextrans, and at least a bulking agent[104]. Nevertheless, it is important to include representative active vials in strategic locations across the lyophilizer shelf to generate product quality readouts. Surrogate vials then serve the purpose of simulating a full lyophilizer load which is an important factor during cycle development and optimization [94]. In addition, as highlighted earlier, the majority of ADCs are formulated at low active concentration, further justifying the use of surrogate as a suitable readout of key attributes such as cake appearance and T_p during lyophilization cycle development. As disclosed by McGarvey et al. [105], comparable cake appearance, moisture content and reconstitution time was demonstrated between a formulated mAb-MMAE conjugate active at 10 mg/mL and its corresponding placebo after lyophilization in the same cycle. Thermocouples were placed in placebo vials to support tracking the endpoint of primary drying which was shown to correlate well with other in-process monitoring tools (i.e., thermal conductivity, pressure and relative humidity).

Beyond these practical approaches, strategies such as design of experiments and modeling can support lyophilization process development [106–109]. Although not necessarily specific to conjugated mAbs, these same approaches benefit more complex ADC lyophilization development, ensuring minimal quantities of highly potent ADC active material need to be handled for any given study, while still generating a robust dataset suitable for cycle optimization and subsequent scale-up to manufacturing.

Process Scalability and Control Strategy

Although ADCs require tailored analytical strategies for each component as highlighted earlier [4, 110], the overall DP manufacturing process is largely similar to that of unconjugated mAbs, which is well described in recent literature [77].

As outlined in the previous section on lyophilization strategies, the use of surrogate during lyophilization process development and characterization is advantageous, particularly during scale-up, when lyophilizer load is key to verify

cycle transferability, and where it is important to study the impact of a fully loaded lyophilizer at scale. 'Seeding' the surrogate loaded lyophilizer shelves with active vials is an accepted approach [111, 112]. Moreover, it is highly desirable to minimize the number of runs needed for a successful cycle scale-up through the use of modeling tools and by preferentially performing small scale runs [94]. As highlighted by Ramprasad, a cycle optimization, i.e. the primary drying shelf temperature, can be significantly accelerated through dynamic approaches, e.g. in certain pilot scale freeze-dryers using SMART™ technology [78], or via mechanistic models [113]. Mechanistic models also provide benefit in scaling up predictions of small-scale trial runs and statistical tools can further fill knowledge gaps, as reported in a conference poster by Koci et al. [114]. Furthermore, design space approaches are particularly valuable in identifying optimal processing conditions and a safe operating window to avoid undesirable outcomes such as product collapse or an impact on critical quality attributes [107, 113, 115]. Scalability considerations for all other unit operations during DP manufacturing are well described by Das et al., including compounding, homogenization, filtration, filling and the associated CQAs which may be impacted by the applied stresses [116].

As previously described in the stability section of this review, ADCs can be photo-sensitive. To address this sensitivity, mitigation measures during DP large scale manufacturing should be implemented, as described in the recent literature [14]. Some of the measures include installation of safer light sources, such as yellow, amber or red light as well as LED lighting. Additionally, for the final DP packaging, reduced UV transmittance, multi-layer cyclic olefin polymer (COP) vials and amber glass vials have been proven to offer protection from photodegradation, with COP providing a moderate protection compared to amber vials, which demonstrated a substantial mitigation of photodegradation, preventing changes in both high-molecular weight species and pH. Mylotarg®, Besponsa®, Enhertu® and Datroway® are examples of marketed ADC products which are packaged in amber vials as shown in Table I.

Vial fogging, a largely unpredictable phenomenon, has been observed more frequently in lyophilized ADC vials [117–120], likely driven by the combination of the reduced surface tension from surfactants and the hydrophobic payloads, with conjugation increasing molecular hydrophobicity, exposing hydrophobic domains that preferentially migrate to air–liquid and container-liquid interfaces [121]. Approaches reported to reduce and control vial fogging include the use of hydrophobic-surface vials, specific processing conditions (e.g., adjustment of the depyrogenation parameters, incorporating a prolonged pre-freezing hold or an annealing step), and formulation adjustments (e.g., increasing viscosity or increasing surface tension by omitting a surfactant) [119, 121]. A minor degree of fogging is generally regarded as a cosmetic defect while fogging in the neck region is considered critical due to a potential impact on container closure integrity [122], which was, however, recently shown to be limited [123].

During the transition from early clinical phases to late and commercial phases, process knowledge gained from early development will influence the process design phase and Stage 1 process validation, which should account for the constraints of commercial-scale manufacturing limitations (Fig. 4). Stage 2 focuses on the qualification of facilities, equipment, and utilities as well as process performance qualification (PPQ), ensuring that the design established in Stage 1 will be reproducible in commercial-scale manufacture [124]. For ADCs specifically, it is an expectation to apply QbD principles, risk-based containment, and integrated control strategies for both biologic and small

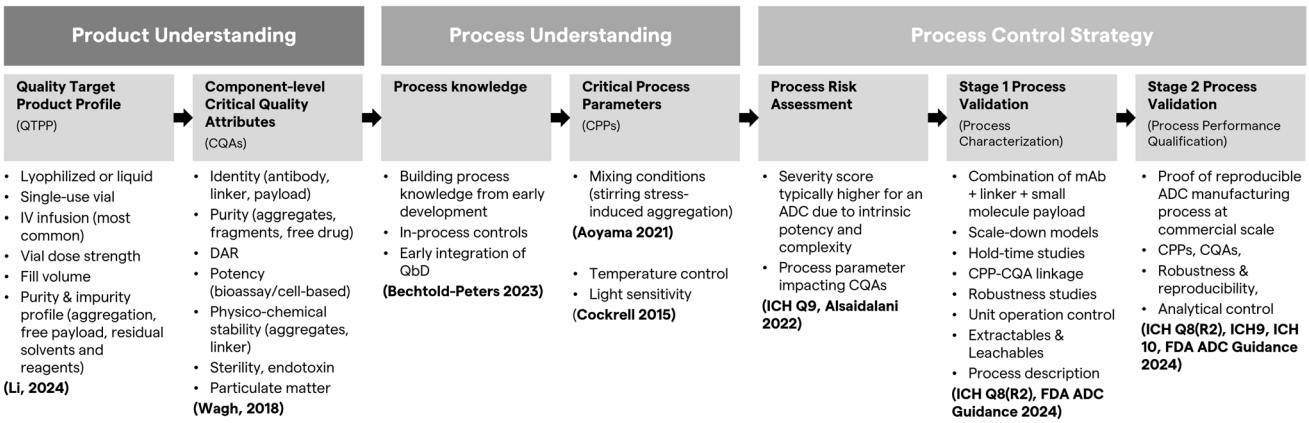

Fig. 4 A science and risk-based DP process control strategy with considerations from recent ADC literature. Abbreviations: IV, intravenous; DAR, drug-to-antibody ratio; QbD, Quality by Design; ADC, antibody–drug conjugate; CQA, critical quality attribute; CPP, critical process parameter; mAb, monoclonal antibody

molecule components based on ICH guidelines [125–127]. It is important to highlight that there is not yet a harmonized approach to CMC strategies for ADCs [8], and local authority expectations differ across regions, with for example FDA following a cross-center approach in a single BLA submission, with the Center for Biologics Evaluation and Research (CBER) leading the review for mAb, conjugation, DP quality and sterility and Center for Drug Evaluation and Research (CDER) experts involved for small-molecule payload and linker chemistry aspects. A consistent and controllable process requires upfront assessment of risk and process characterization studies to establish criticality of process parameters and proven acceptable ranges. An overview of considerations when defining the control strategy for an ADC, with references to recent relevant ADC reviews and articles [8, 12, 63, 64, 125–130] is presented in Fig. 4.

Bechtold-Peters et al. provide an in-depth analysis of the complexities with ADCs, highlighting control strategies, critical quality attributes that are specific to ADC products, including free drug impurities, unconjugated mAb, DAR, conjugate variants, potency and charged species [8]. The paper compares traditional linear validation strategies versus more modern process validation approaches. The former poses timeline challenges specifically for ADCs, where the DP PPQs use validated drug substance (in turn prepared from a validated mAb intermediate and a separately validated drug-linker intermediate) knowledge. More forward-looking considerations, using QbD principles, involving risk-based and data-driven approaches, are increasingly accepted for ADCs by the regulatory agencies and support accelerating clinical-to-commercial transitions without compromising quality [131].

Conclusion

Conjugation-induced instability and increased hydrophobicity from cytotoxic payloads makes ADCs more vulnerable to environmental stresses, leading to increased risks such as aggregation, linker hydrolysis, and premature payload release impacting both drug efficacy and safety. This review highlights how to address these liabilities through tailored formulation and DP process development relying on published works and on the data available from marketed ADCs. The latter demonstrates the reliance mostly on more traditional excipients (sugar, buffer, surfactant) with formulation pH values tailored to linker and payload chemistry and the predominance of lyophilized formats to tackle instability issues. Although ADCs introduce analytical and manufacturing complexities, the overall DP process remains largely analogous to that of the parent mAb. Risk-based strategies, including QbD principles, are essential to define critical process parameters and mitigate ADC-enhanced risks, such as photodegradation and vial fogging during lyophilization. Robust scale-up and commercialization workflows depend on well-integrated control strategies, predictive modeling, and design space approaches to ensure an efficient development of reliable and robust processes. While regulatory expectations for ADCs remain non-standardized, science-driven and flexible validation strategies are recommended. Looking forward, it is expected that the molecular complexity of the ADC constructs will increase, driven by the growing diversity of each component and the expansion of therapeutic targets. Furthermore, alternative routes of administration (e.g. subcutaneous), higher doses, or a shift towards liquid or frozen dosage forms will be explored for specific therapies. During this evolution, pharmaceutical development will benefit from the presented strategies and may require even more innovative formulation and process solutions to ensure stability, manufacturability, and safety. Addressing these complexities would enable the development of next-generation ADCs that are clinically effective, commercially viable, and safe throughout their lifecycle.

Acknowledgements The authors sincerely thank Virginie Le Brun and Nina Hentzen for their thorough and constructive review of this manuscript and Abbas Razvi for the invaluable support in facilitating the cross-departmental collaboration required for the successful completion of this paper.

Author Contributions *Léa Sorret*. Paper leadership and substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. *Mamiko Ninomiya*. Substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. *Nils Hillebrandt*. Substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. *David Schmitt*. Substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. *Arundhati Chattopadhyay*. Substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and

resolved. *Orla McGarvey*. Paper leadership and substantial contributions to the conception and design of the work; acquisition, analysis, or interpretation of data for the work; and drafting the work and revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

Funding All authors are paid employees of Lonza AG.

Declarations

Conflict of Interest All authors are employees of Lonza AG.

References

1. Walsh G, Walsh E. Biopharmaceutical benchmarks 2022. Nat Biotechnol. 2022;40:1722–60. https://doi.org/10.1038/s41587-022-01582-x.
2. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody–drug conjugates. Nat Rev Drug Discov. 2017;16:315–37. https://doi.org/10.1038/nrd.2016.268.
3. Ross PL, Wolfe JL. Physical and chemical stability of antibody drug conjugates: current status. J Pharm Sci. 2016;105:391–7. https://doi.org/10.1016/j.xphs.2015.11.037.
4. Sasso JM, Tenchov R, Bird R, Iyer KA, Ralhan K, Rodriguez Y, et al. The evolving landscape of antibody–drug conjugates: in depth analysis of recent research progress. Bioconjugate Chem. 2023;34:1951–2000. https://doi.org/10.1021/acs.bioconjchem.3c00374.
5. Maecker H, Jonnalagadda V, Bhakta S, Jammalamadaka V, Junutula JR. Exploration of the antibody–drug conjugate clinical landscape. MAbs. 2023;15:2229101. https://doi.org/10.1080/19420862.2023.2229101.
6. Chalouni C, Doll S. Fate of antibody-drug conjugates in cancer cells. J Exp Clin Cancer Res. 2018;37:20. https://doi.org/10.1186/s13046-017-0667-1.
7. Jackson DY. Processes for constructing homogeneous antibody drug conjugates. Org Process Res Dev. 2016;20:852–66. https://doi.org/10.1021/acs.oprd.6b00067.
8. Bechtold-Peters K, Ruggiero A, Vriezen N, Ihle N, Klein A, Morgan C, et al. CMC regulatory considerations for antibody-drug conjugates. J Pharm Sci. 2023;112:2965–80. https://doi.org/10.1016/j.xphs.2023.09.007.
9. Mills BJ, Kruger T, Bruncko M, Zhang X, Jameel F. Effect of linker-drug properties and conjugation site on the physical stability of ADCs. J Pharm Sci. 2020;109:1662–72. https://doi.org/10.1016/j.xphs.2020.01.029.
10. Beckley NS, Lazzareschi KP, Chih H-W, Sharma VK, Flores HL. Investigation into temperature-induced aggregation of an antibody drug conjugate. Bioconjug Chem. 2013;24:1674–83. https://doi.org/10.1021/bc400182x.
11. Mohamed HE, Mohamed AA, Al-Ghobashy MA, Fathalla FA, Abbas SS. Stability assessment of antibody-drug conjugate Trastuzumab emtansine in comparison to parent monoclonal antibody using orthogonal testing protocol. J Pharm Biomed Anal. 2018;150:268–77. https://doi.org/10.1016/j.jpba.2017.12.022.
12. Cockrell GM, Wolfe MS, Wolfe JL, Schöneich C. Photoinduced aggregation of a model antibody–drug conjugate. Mol Pharm. 2015;12:1784–97. https://doi.org/10.1021/mp5006799.
13. Glover ZK, Wecksler A, Aryal B, Mehta S, Pegues M, Chan W, et al. Physicochemical and biological impact of metal-catalyzed oxidation of IgG1 monoclonal antibodies and antibody-drug conjugates via reactive oxygen species. mAbs. 2022;14:2122957. https://doi.org/10.1080/19420862.2022.2122957.
14. Luo S, Bulos J, Uroza R, Zhao Y, Pan X, Su Y, et al. Insights into photo degradation and stabilization strategies of antibody–drug conjugates with camptothecin payloads. Pharmaceutics. 2025;17:1397. https://doi.org/10.3390/pharmaceutics17111397.
15. Duerr C, Friess W. Antibody-drug conjugates- stability and formulation. Eur J Pharm Biopharm. 2019;139:168–76. https://doi.org/10.1016/j.ejpb.2019.03.021.
16. Cheng Y, Duong HTT, Hu Q, Shameem M, Tang X. Practical advice in the development of a lyophilized protein drug product. Antibody Ther. 2024;8:13–25. https://doi.org/10.1093/abt/tbae030.
17. Kasper JC, Winter G, Friess W. Recent advances and further challenges in lyophilization. Eur J Pharm Biopharm. 2013;85:162–9. https://doi.org/10.1016/j.ejpb.2013.05.019.
18. Patnaik P. ADC manufacturing's biggest CMC Challenges and ways to approach them. Bioprocess Online. 2025; https://www.bioprocessonline.com/doc/adc-manufacturing-s-biggest-cmc-challenges-and-ways-to-approach-them-0001. Accessed 5 Jan 2026.
19. Adem YT. Antibody-drug conjugates, methods and protocols. Methods Mol Biol. 2019;2078:301–11. https://doi.org/10.1007/978-1-4939-9929-3_21.
20. Razinkov VI, Treuheit MJ, Becker GW. Accelerated formulation development of monoclonal antibodies (mAbs) and mAb-based modalities: review of methods and tools. SLAS Discov. 2015;20:468–83. https://doi.org/10.1177/1087057114565593.
21. Chari RV, Miller ML, Widdison WC. Antibody–drug conjugates: an emerging concept in cancer therapy. Angew Chem Int Ed Engl. 2014;53:3796–827. https://doi.org/10.1002/anie.201307628.
22. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Signal Transduct Target Ther. 2022;7:93. https://doi.org/10.1038/s41392-022-00947-7.
23. Conilh L, Sadilkova L, Viricel W, Dumontet C. Payload diversification: a key step in the development of antibody–drug conjugates. J Hematol Oncol. 2023;16:3. https://doi.org/10.1186/s13045-022-01397-y.
24. Adem YT, Schwarz KA, Duenas E, Patapoff TW, Galush WJ, Esue O. Auristatin antibody drug conjugate physical instability and the role of drug payload. Bioconjug Chem. 2014;25:656–64. https://doi.org/10.1021/bc400439x.
25. Nakada T, Sugihara K, Jikoh T, Abe Y, Agatsuma T. The latest research and development into the antibody–drug conjugate, [fam-] Trastuzumab Deruxtecan (DS-8201a), for HER2 cancer therapy. Chem Pharm Bull. 2019;67:173–85. https://doi.org/10.1248/cpb.c18-00744.
26. Guo J, Kumar S, Prashad A, Starkey J, Singh SK. Assessment of physical stability of an antibody drug conjugate by higher

order structure analysis: impact of thiol-maleimide chemistry. Pharm Res. 2014;31:1710–23. https://doi.org/10.1007/s11095-013-1274-2.
27. Buecheler JW, Winzer M, Tonillo J, Weber C, Gieseler H. Impact of payload hydrophobicity on the stability of antibody–drug conjugates. Mol Pharm. 2018;15:2656–64. https://doi.org/10.1021/acs.molpharmaceut.8b00177.
28. Hamblett KJ, Senter PD, Chace DF, Sun MMC, Lenox J, Cerveny CG, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. Clin Cancer Res. 2004;10:7063–70. https://doi.org/10.1158/1078-0432.ccr-04-0789.
29. Nakada T, Masuda T, Naito H, Yoshida M, Ashida S, Morita K, et al. Novel antibody drug conjugates containing exatecan derivative-based cytotoxic payloads. Bioorg Med Chem Lett. 2016;26:1542–5. https://doi.org/10.1016/j.bmcl.2016.02.020.
30. Kaempffe A, Dickgiesser S, Rasche N, Paoletti A, Bertotti E, Salve ID, et al. Effect of conjugation site and technique on the stability and pharmacokinetics of antibody-drug conjugates. J Pharm Sci. 2021;110:3776–85. https://doi.org/10.1016/j.xphs.2021.08.002.
31. Strop P, Liu S-H, Dorywalska M, Delaria K, Dushin RG, Tran T-T, et al. Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates. Chem Biol. 2013;20:161–7. https://doi.org/10.1016/j.chembiol.2013.01.010.
32. Ohri R, Bhakta S, Fourie-O'Donohue A, dela Cruz-Chuh J, Tsai SP, Cook R, et al. High-throughput cysteine scanning to identify stable antibody conjugation sites for maleimide- and disulfide-based linkers. Bioconjug Chem. 2018;29:473–85. https://doi.org/10.1021/acs.bioconjchem.7b00791.
33. Johann F, Wöll S, Gieseler H. "Negative" impact: the role of payload charge in the physicochemical stability of auristatin antibody–drug conjugates. J Pharm Sci. 2024;113:2433–42. https://doi.org/10.1016/j.xphs.2024.04.023.
34. Gandhi AV, Randolph TW, Carpenter JF. Conjugation of emtansine onto trastuzumab promotes aggregation of the antibody–drug conjugate by reducing repulsive electrostatic interactions and increasing hydrophobic interactions. J Pharm Sci. 2019;108:1973–83. https://doi.org/10.1016/j.xphs.2019.01.029.
35. Coumans RGE, Ariaans GJA, Spijker HJ, Verkerk PR, Beusker PH, Kokke BPA, et al. A platform for the generation of site-specific antibody–drug conjugates that allows for selective reduction of engineered cysteines. Bioconjug Chem. 2020;31:2136–46. https://doi.org/10.1021/acs.bioconjchem.0c00337.
36. Sarvepalli S, Pasika SR, Verma V, Thumma A, Bolla S, Nukala PK, et al. A review on the stability challenges of advanced biologic therapeutics. Pharmaceutics. 2025;17:550. https://doi.org/10.3390/pharmaceutics17050550.
37. Yang Q, Liu Y. Technical, preclinical, and clinical developments of Fc-glycan-specific antibody–drug conjugates. RSC Med Chem. 2024;16:50–62. https://doi.org/10.1039/d4md00637b.
38. Lei Y, Zheng M, Chen P, Ng CS, Loh TP, Liu H. Linker design for antibody drug conjugates: a comprehensive review. ChemMedChem. 2025;e202500262. https://doi.org/10.1002/cmdc.202500262
39. Matsuda Y, Chang JR, Mendelsohn BA. Advanced antibody–drug conjugates design: innovation in linker chemistry and site-specific conjugation technologies. ChemBioChem. 2025. https://doi.org/10.1002/cbic.202500305.
40. Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, et al. Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. Nat Biotechnol. 2015;33:733–5. https://doi.org/10.1038/nbt.3212.
41. Chi EY, Krishnan S, Kendrick BS, Chang BS, Carpenter JF, Randolph TW. Roles of conformational stability and colloidal stability in the aggregation of recombinant human granulocyte colony-stimulating factor. Protein Sci. 2003;12:903–13. https://doi.org/10.1110/ps.0235703.
42. Chi EY, Krishnan S, Randolph TW, Carpenter JF. Physical stability of proteins in aqueous solution: mechanism and driving forces in nonnative protein aggregation. Pharm Res. 2003;20:1325–36. https://doi.org/10.1023/a:1025771421906.
43. He F, Woods CE, Becker GW, Narhi LO, Razinkov VI. High-throughput assessment of thermal and colloidal stability parameters for monoclonal antibody formulations. J Pharm Sci. 2011;100:5126–41. https://doi.org/10.1002/jps.22712.
44. Roberts CJ. Therapeutic protein aggregation: mechanisms, design, and control. Trends Biotechnol. 2014;32:372–80. https://doi.org/10.1016/j.tibtech.2014.05.005.
45. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. Pharm Res. 2010;27:544–75. https://doi.org/10.1007/s11095-009-0045-6.
46. Wakankar AA, Feeney MB, Rivera J, Chen Y, Kim M, Sharma VK, et al. Physicochemical stability of the antibody–drug conjugate trastuzumab-DM1: changes due to modification and conjugation processes. Bioconjug Chem. 2010;21:1588–95. https://doi.org/10.1021/bc900434c.
47. Peters B, Trout BL. Asparagine deamidation: pH-dependent mechanism from density functional theory. Biochemistry. 2006;45:5384–92. https://doi.org/10.1021/bi052438n.
48. Bargh JD, Isidro-Llobet A, Parker JS, Spring DR. Cleavable linkers in antibody–drug conjugates. Chem Soc Rev. 2019;48:4361–74. https://doi.org/10.1039/c8cs00676h.
49. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2003;21:778–84. https://doi.org/10.1038/nbt832.
50. Zhou T, Dong X, Yu J, Song C, Liu Q, He L, et al. Photostability of topoisomerase I inhibitor–conjugated IgG1 antibody–drug conjugates: characterization study and degradation mechanism analysis. Bioconjug Chem. 2025;36:2645–54. https://doi.org/10.1021/acs.bioconjchem.5c00485.
51. Thiess CO, Mäder K, Mahler H-C, Wöll S. Payload-dependent photostability of antibody-drug conjugates. Eur J Pharm Biopharm. 2025;216:114867. https://doi.org/10.1016/j.ejpb.2025.114867.
52. Dodds HM, Craik DJ, Rivory LP. Photodegradation of irinotecan (CPT-11) in aqueous solutions: identification of fluorescent products and influence of solution composition. J Pharm Sci. 1997;86:1410–6. https://doi.org/10.1021/js970110c.
53. Fassberg J, Stella VJ. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. J Pharm Sci. 1992;81:676–84. https://doi.org/10.1002/jps.2600810718.
54. Lown JW, Chen H-H. Studies on the effects of the antitumor agent camptothecin and derivatives on deoxyribonucleic acid mechanism of the scission of deoxyribonucleic acid by photoactivated camptothecin. Biochem Pharmacol. 1980;29:905–15. https://doi.org/10.1016/0006-2952(80)90221-x.
55. Lau UY, Benoit LT, Stevens NS, Emmerton KK, Zaval M, Cochran JH, et al. Lactone stabilization is not a necessary feature for antibody conjugates of camptothecins. Mol Pharm. 2018;15:4063–72. https://doi.org/10.1021/acs.molpharmaceut.8b00477.
56. Nassar MN, Chandrasekharan S, Sadineni V. Implications of trace levels of redox-active metals in drug-product formulation. BioPharm International. 2014. https://www.biopharminternational.com/view/implications-trace-levels-redox-active-metals-drug-product-formulation. Accessed 5 Jan 2026
57. Klair N, Kim MT, Lee A, Xiao NJ, Patel AR. Stress temperature studies in small-scale Hastelloy® drug substance containers lead

to increased extent of and increased variability in antibody–drug conjugate and monoclonal antibody aggregation: evidence for novel oxidation-induced crosslinking in monoclonal antibodies. J Pharm Sci. 2021;110:1615–24. https://doi.org/10.1016/j.xphs.2020.09.052.
58. Weber J, Buske J, Mäder K, Garidel P, Diederichs T. Oxidation of polysorbates – an underestimated degradation pathway? Int J Pharm X. 2023;6:100202. https://doi.org/10.1016/j.ijpx.2023.100202.
59. Meleties M, Cooper BM, Marcano-James D, Bhalla AS, Shameem M. Vaporized hydrogen peroxide sterilization in the production of protein therapeutics: uptake and effects on product quality. J Pharm Sci. 2023;112:2991–3004. https://doi.org/10.1016/j.xphs.2023.09.012.
60. Wakankar A, Chen Y, Gokarn Y, Jacobson FS. Analytical methods for physicochemical characterization of antibody drug conjugates. MAbs. 2011;3:161–72. https://doi.org/10.4161/mabs.3.2.14960.
61. Galush WJ, Wakankar AA. Antibody-drug conjugates. Methods Mol Biol. 2013;1045:217–33. https://doi.org/10.1007/978-1-62703-541-5_13.
62. Yu W, Ramprasad MP, Pal M, Chen C, Paruchuri S, Skidmore L, et al. Analytical comparability to evaluate impact of manufacturing changes of ARX788, an anti-HER2 ADC in late-stage clinical development. PLoS One. 2023;18:e0284198. https://doi.org/10.1371/journal.pone.0284198.
63. Wagh A, Song H, Zeng M, Tao L, Das TK. Challenges and new frontiers in analytical characterization of antibody-drug conjugates. MAbs. 2018;10:222–43. https://doi.org/10.1080/19420862.2017.1412025.
64. Li M, Zhao X, Yu C, Wang L. Antibody–drug conjugate overview: a state-of-the-art manufacturing process and control strategy. Pharm Res. 2024;41:419–40. https://doi.org/10.1007/s11095-023-03649-z.
65. Li W. Analytical development and testing strategy of antibody–drug conjugates. AAPS PharmSciTech. 2026;27:150. https://doi.org/10.1208/s12249-026-03402-w.
66. Jameel F, Mills BJ, Moussa EM, Sisodiya V, Cano T, Haight AR. Development of biopharmaceutical drug-device products. AAPS Adv Pharm Sci Ser. 2020;27–48. https://doi.org/10.1007/978-3-030-31415-6_2
67. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. J Pharm Sci. 2004;93:1390–402. https://doi.org/10.1002/jps.20079.
68. European Medicines Agency, Elahere: EPAR – Product Information. https://www.ema.europa.eu/en/documents/product-information/elahere-epar-product-information_en.pdf. Accessed 4 May 2026
69. Haque M, Forte N, Baker JR. Site-selective lysine conjugation methods and applications towards antibody–drug conjugates. Chem Commun. 2021;57:10689–702. https://doi.org/10.1039/d1cc03976h.
70. Rao VA, Kim JJ, Patel DS, Rains K, Estoll CR. A comprehensive scientific survey of excipients used in currently marketed therapeutic biological drug products. Pharm Res. 2020;37:200. https://doi.org/10.1007/s11095-020-02919-4.
71. Mieczkowski CA. The evolution of commercial antibody formulations. J Pharm Sci. 2023;112:1801–10. https://doi.org/10.1016/j.xphs.2023.03.026.
72. Sudrik CM, Cloutier T, Mody N, Sathish HA, Trout BL. Understanding the role of preferential exclusion of sugars and polyols from native state IgG1 monoclonal antibodies and its effect on aggregation and reversible self-association. Pharm Res. 2019;36:109. https://doi.org/10.1007/s11095-019-2642-3.
73. Wang X, Zhang X, Xu J, Xu S, Huang K, Ni Q, et al. High-concentration L-methionine as a potent antioxidant for oxidation resistance and stability enhancement in high-concentration antibody therapeutics. Int J Pharm X. 2025;10:100385. https://doi.org/10.1016/j.ijpx.2025.100385.
74. Khan TA, Mahler H-C, Kishore RSK. Key interactions of surfactants in therapeutic protein formulations: a review. Eur J Pharm Biopharm. 2015;97:60–7. https://doi.org/10.1016/j.ejpb.2015.09.016.
75. Martin KP, Grimaldi C, Grempler R, Hansel S, Kumar S. Trends in industrialization of biotherapeutics: a survey of product characteristics of 89 antibody-based biotherapeutics. MAbs. 2023;15:2191301. https://doi.org/10.1080/19420862.2023.2191301.
76. Basle YL, Chennell P, Tokhadze N, Astier A, Sautou V. Physicochemical stability of monoclonal antibodies: a review. J Pharm Sci. 2020;109:169–90. https://doi.org/10.1016/j.xphs.2019.08.009.
77. Sampathkumar K, Kerwin BA. Roadmap for drug product development and manufacturing of biologics. J Pharm Sci. 2024;113:314–31. https://doi.org/10.1016/j.xphs.2023.11.004.
78. Ramprasad MP. Analytical and formulation development road map for protein therapeutics. In: Comprehensive Medicinal Chemistry III. Elsevier; 2017. p. 198–215. https://doi.org/10.1016/b978-0-12-409547-2.12412-6.
79. Wen L, Zhang Y, Sun C, Wang SS, Gong Y, Jia C, et al. Fundamental properties and principal areas of focus in antibody–drug conjugates formulation development. Antibody Ther. 2025;8:99–110. https://doi.org/10.1093/abt/tbaf005.
80. Jarasch A, Koll H, Regula JT, Bader M, Papadimitriou A, Kettenberger H. Developability assessment during the selection of novel therapeutic antibodies. J Pharm Sci. 2015;104:1885–98. https://doi.org/10.1002/jps.24430.
81. Obrezanova O, Arnell A, de la Cuesta RG, Berthelot ME, Gallagher TR, Zurdo J, et al. Aggregation risk prediction for antibodies and its application to biotherapeutic development. MAbs. 2015;7:352–63. https://doi.org/10.1080/19420862.2015.1007828.
82. Kim J, McFee M, Fang Q, Abdin O, Kim PM. Computational and artificial intelligence-based methods for antibody development. Trends Pharmacol Sci. 2023;44:175–89. https://doi.org/10.1016/j.tips.2022.12.005.
83. Almeida V, Soares MBP, Santos-Filho OA. Exploring experimental and in silico approaches for antibody–drug conjugates in oncology therapies. Pharmaceuticals. 2025;18:1198. https://doi.org/10.3390/ph18081198.
84. Ji JA, Liu J, Wang YJ. Antibody–drug conjugates, the 21st century magic bullets for cancer. AAPS Adv Pharm Sci Ser. 2015;79–95. https://doi.org/10.1007/978-3-319-13081-1_5
85. Capelle MAH, Gurny R, Arvinte T. High throughput screening of protein formulation stability: practical considerations. Eur J Pharm Biopharm. 2007;65:131–48. https://doi.org/10.1016/j.ejpb.2006.09.009.
86. Cleland JL, Powell MF, Shire SJ. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation. Crit Rev Ther Drug Carr Syst. 1993;10:307–77.
87. Shire SJ. Formulation and manufacturability of biologics. Curr Opin Biotechnol. 2009;20:708–14. https://doi.org/10.1016/j.copbio.2009.10.006.
88. Jahn M, Zerr A, Fedorowicz FM, Brigger F, Koulov A, Mahler H-C. Measuring lipolytic activity to support process improvements to manage lipase-mediated polysorbate degradation. Pharm Res. 2020;37:118. https://doi.org/10.1007/s11095-020-02812-0.
89. Zarei M, Jonveaux J, Razvi A, Jahn M. Integrated strategy for deep profiling of host cell proteins in downstream processing of therapeutic monoclonal antibodies: novel approach to

isolate and digest host cell proteins. Eur J Pharm Biopharm. 2024;201:114369. https://doi.org/10.1016/j.ejpb.2024.114369.
90. Buecheler JW, Winzer M, Weber C, Gieseler H. Alteration of physicochemical properties for antibody–drug conjugates and their impact on stability. J Pharm Sci. 2020;109:161–8. https://doi.org/10.1016/j.xphs.2019.08.006.
91. Chang L, Milton N, Rigsbee D, Mishra DS, Tang X, Thomas LC, et al. Using modulated DSC to investigate the origin of multiple thermal transitions in frozen 10% sucrose solutions. Thermochim Acta. 2006;444:141–7. https://doi.org/10.1016/j.tca.2006.03.006.
92. Pansare SK, Patel SM. Practical considerations for determination of glass transition temperature of a maximally freeze concentrated solution. AAPS PharmSciTech. 2016;17:805–19. https://doi.org/10.1208/s12249-016-0551-x.
93. Greco K, Mujat M, Galbally-Kinney KL, Hammer DX, Ferguson RD, Iftimia N, et al. Accurate prediction of collapse temperature using optical coherence tomography-based freeze-drying microscopy. J Pharm Sci. 2013;102:1773–85. https://doi.org/10.1002/jps.23516.
94. Tchessalov S, Shalaev E, Bhatnagar B, Nail S, Alexeenko A, Jameel F, et al. Best practices and guidelines (2022) for scale-up and technology transfer in freeze drying based on case studies. Part 2: past practices, current best practices, and recommendations. AAPS PharmSciTech. 2023;24:96. https://doi.org/10.1208/s12249-023-02553-4.
95. Tchessalov S, Maglio V, Kazarin P, Alexeenko A, Bhatnagar B, Sahni E, et al. Practical advice on scientific design of freeze-drying process: 2023 update. Pharm Res. 2023;40:2433–55. https://doi.org/10.1007/s11095-023-03607-9.
96. Depaz RA, Pansare S, Patel SM. Freeze-drying above the glass transition temperature in amorphous protein formulations while maintaining product quality and improving process efficiency. J Pharm Sci. 2016;105:40–9. https://doi.org/10.1002/jps.24705.
97. Haeuser C, Goldbach P, Huwyler J, Friess W, Allmendinger A. Excipients for room temperature stable freeze-dried monoclonal antibody formulations. J Pharm Sci. 2020;109:807–17. https://doi.org/10.1016/j.xphs.2019.10.016.
98. Duerr C. Studies on the physicochemical stability of antibody conjugates [PhD thesis]. Ludwig-Maximilians-Universität München. https://edoc.ub.uni-muenchen.de/22387/7/Duerr_Corinna.pdf. (2018). Accessed 5 Jan 2026.
99. Denk R, Flückiger A, Kisaka H, Krause S, Maeck R, Restetzki L, et al. Preventing cross-contamination during lyophilization: GMP and occupational cleaning requirements for nonproduct and indirect product-contact parts. PDA J Pharm Sci Technol. 2019;73:487–95. https://doi.org/10.5731/pdajpst.2018.009530.
100. Procopiou G, Mason-Home J. Occupational Health and Safety Considerations for the Handling and Manufacture of Antibody–Drug Conjugate (ADC) Payloads. In: Cytotoxic payloads for antibody–drug conjugates. 2019. p. 439–60. https://doi.org/10.1039/9781788012898-00439.
101. Cherry CLA, Millward H, Cooper R, Landon J. A novel approach to sterile pharmaceutical freeze-drying. Pharm Dev Technol. 2014;19:73–81. https://doi.org/10.3109/10837450.2012.752388.
102. Chamberlain R, Schlauersbach J, Erber M. Freeze-drying in protective bags: characterization of heat and mass transfer. Eur J Pharm Biopharm. 2020;154:309–16. https://doi.org/10.1016/j.ejpb.2020.07.013.
103. Cullen S, Walsh E, Gervasi V, Khamar D, McCoy TR. Technical transfer and commercialisation of lyophilised biopharmaceuticals — application of lyophiliser characterisation and comparability. AAPS Open. 2022;8:14. https://doi.org/10.1186/s41120-022-00059-0.
104. Boksa K, Walsh P, Shah A. Case study in the design of a surrogate solution for use in biopharmaceutical drug product process development. AAPS PharmSciTech. 2021;22:32. https://doi.org/10.1208/s12249-020-01881-z.
105. McGarvey O, Ratnaswamy G, Sun Y, Van Schravendijk MR. Pharmaceutical compositions comprising anti-191P4D12 antibody drug conjugates and methods of use thereof. US Patent 12,257,340 B2. 2025.
106. Pardeshi SR, Deshmukh NS, Telange DR, Nangare SN, Sonar YY, Lakade SH, et al. Process development and quality attributes for the freeze-drying process in pharmaceuticals, biopharmaceuticals and nanomedicine delivery: a state-of-the-art review. Future J Pharm Sci. 2023;9:99. https://doi.org/10.1186/s43094-023-00551-8.
107. Patel SM, Pikal MJ. Lyophilization process design space. J Pharm Sci. 2013;102:3883–7. https://doi.org/10.1002/jps.23703.
108. Radhakrishnan V, Davis P, Hiebert D. Challenges in protein product development. AAPS Adv Pharm Sci Ser. 2018;441–71. https://doi.org/10.1007/978-3-319-90603-4_20
109. Patel SM, Jameel F, Sane SU, Kamat M. Quality by design for biopharmaceutical drug product development. AAPS Adv Pharm Sci Ser. 2015;303–29. https://doi.org/10.1007/978-1-4939-2316-8_14
110. Kishore KH. The ABC of ADCs (antibody–drug conjugates): a comprehensive review of technical, regulatory, and clinical challenges. Adv Chem Eng Sci. 2023;13:363–81. https://doi.org/10.4236/aces.2023.134025.
111. Tchessalov S, Warne N. Lyophilization: cycle robustness and process tolerances, transfer and scale up. 2008. https://www.europeanpharmaceuticalreview.com/article/1263/lyophilization-cycle-robustness-and-process-tolerances-transfer-and-scale-up/. Accessed 5 Jan 2026
112. Jameel F, Alexeenko A, Bhambhani A, Sacha G, Zhu T, Tchessalov S, et al. Recommended best practices for lyophilization validation—2021 Part I: process design and modeling. AAPS PharmSciTech. 2021;22:221. https://doi.org/10.1208/s12249-021-02086-8.
113. Scutellà B, Bourlès E. Development of freeze-drying cycle via design space approach: a case study on vaccines. Pharm Dev Technol. 2020;25:1302–13. https://doi.org/10.1080/10837450.2020.1806298.
114. Koci M, Martinez M, O'Brien RL, Molteni L, McGarvey O. Freeze drying cycle optimisation by means of statistical models and process analytical tools. Presented at: ISLFD 2024 - 10th International Symposium on Lyophilization of Pharmaceuticals; 8–10 May 2024; Turin, Italy.
115. Arsiccio A, Pisano R. Application of the Quality by Design approach to the freezing step of freeze-drying: building the design space. J Pharm Sci. 2018;107:1586–96. https://doi.org/10.1016/j.xphs.2018.02.003.
116. Das TK, Sreedhara A, Colandene JD, Chou DK, Filipe V, Grapentin C, et al. Stress factors in protein drug product manufacturing and their impact on product quality. J Pharm Sci. 2022;111:868–86. https://doi.org/10.1016/j.xphs.2021.09.030.
117. Tchessalov S, Shalaev E, Bhatnagar B, Nail S, Alexeenko A, Jameel F, et al. Best practices and guidelines (2022) for scale-up and tech transfer in freeze-drying based on case studies. Part 1: challenges during scale up and transfer. AAPS PharmSciTech. 2022;24:11. https://doi.org/10.1208/s12249-022-02463-x.
118. Kaur N, More A, McKeeby J, Vanipenta R, Patel F, Kish M, et al. Demystifying fogging in lyophilized products: impact of pharmaceutical processing. J Pharm Sci. 2024;113:2305–13. https://doi.org/10.1016/j.xphs.2024.03.021.
119. Huang M, Childs E, Roffi K, Karim F, Juneau J, Bhatnagar B, et al. Investigation of fogging behavior in a lyophilized drug product. J Pharm Sci. 2019;108:1101–9. https://doi.org/10.1016/j.xphs.2018.10.015.

120. Shu S, Yuan Z, Chen J, Yang Z, Zou L, Gong Y, et al. Impact of surface energy and surface tension on vial fogging within lyophilized drug products. Eur J Pharm Sci. 2025;207:107040. https://doi.org/10.1016/j.ejps.2025.107040.
121. Abdul-Fattah AM, Oeschger R, Roehl H, Dauphin IB, Worgull M, Kallmeyer G, et al. Investigating factors leading to fogging of glass vials in lyophilized drug products. Eur J Pharm Biopharm. 2013;85:314–26. https://doi.org/10.1016/j.ejpb.2013.06.007.
122. Patel SM, Nail SL, Pikal MJ, Geidobler R, Winter G, Hawe A, et al. Lyophilized drug product cake appearance: what is acceptable? J Pharm Sci. 2017;106:1706–21. https://doi.org/10.1016/j.xphs.2017.03.014.
123. Murphy MI, Patel SM, Rahman M, Hong B, Lee SP, Allmendinger A, et al. Does fogging impact container closure integrity for a lyophilized drug product? Int J Pharm. 2026;688:126452. https://doi.org/10.1016/j.ijpharm.2025.126452.
124. U.S. Food and Drug Administration. Guidance for industry process validation: general principles and practices. 2011. https://www.fda.gov/media/71021/download. Accessed 5 Jan 2026.
125. International Council for Harmonisation. ICH Q8(R2): Pharmaceutical development. ICH Harmonised Guideline. 2009. https://database.ich.org/sites/default/files/Q8%28R2%29%20Guideline.pdf. Accessed 5 Jan 2026.
126. International Council for Harmonisation. ICH Q9(R1): Quality risk management. ICH Harmonised Guideline. 2023. https://database.ich.org/sites/default/files/ICH_Q9%28R1%29_Guideline_Step4_2025_0115_0.pdf. Accessed 5 Jan 2026.
127. International Council for Harmonisation. ICH Q10: Pharmaceutical quality system. ICH Harmonised Guideline. 2008. https://database.ich.org/sites/default/files/Q10%20Guideline.pdf. Accessed 5 Jan 2026.
128. Aoyama M, Tada M, Yokoo H, Demizu Y, Ishii-Watabe A. Fcγ receptor-dependent internalization and off-target cytotoxicity of antibody–drug conjugate aggregates. Pharm Res. 2022;39:89–103. https://doi.org/10.1007/s11095-021-03158-x.
129. Alsaidalani R, Elmadhoun B. Quality risk management in pharmaceutical manufacturing operations: case study for sterile product filling and final product handling stage. Sustainability. 2022;14:9618. https://doi.org/10.3390/su14159618.
130. U.S. Food and Drug Administration. Clinical pharmacology considerations for antibody–drug conjugates guidance for industry. 2024. https://www.fda.gov/media/155997/download. Accessed 5 Jan 2026.
131. Bechtold-Peters K. CMC regulatory considerations for ADCs. Presented at: CASSS European Strategy Forum; 11–13 May 2020; virtual meeting. https://www.casss.org/docs/default-source/cmc-strategy-forum-europe/2020-cmc-europe-speaker-presentations/speaker-presentation-bechtold-peters-karoline-novartis-2020.pdf?sfvrsn=2066115f_8&utm_source=chatgpt.com. Accessed 5 Jan 2026.