Mitigation of Challenges in Impurity Specifications for Linker-Payloads

Li Jianqiang, Qu Haijun, Xu Feng, Shen Jianhua, Gu Wei, Sudhir Patil, Wei Xudong
WuXi XDC

Abstract

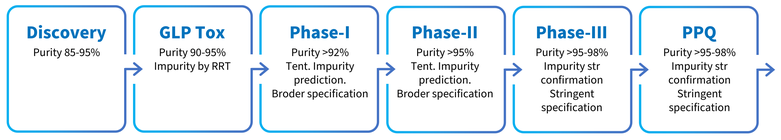
Establishing specifications for impurities in linker-payloads during the early stages of development presents significant challenges, particularly considering impurities' conjugatable nature and feedback from regulatory agencies. The presence of multiple chiral centers, various homologues of polymeric spacers, and the propensity for epimerization, along with other reaction-related impurities in linkers and payloads, complicates the formulation of impurity specifications in the initial phases of an antibody-drug conjugate (ADC) project. Adopting a fit-for-purpose development strategy allows preliminary identification of impurity structures using liquid chromatography-mass spectrometry (LCMS) for reaction impurities. However, accurately assigning structures to polymeric homologues and chiral diastereomers and enantiomers remains difficult, creating further obstacles in impurity resolution and identification. The impurities in question might be conjugatable or non-conjugatable, both of which can be present in the final ADC drug substance (DS) and drug product (DP). To address these challenges, WuXi XDC has developed specialized analytical methods to assess the content and conjugatable characteristics of these impurities. WuXi XDC verifies the conjugatable nature of impurities through mock-conjugation experiments and, through analytical techniques, controls homologue impurities in starting materials to reduce their presence in the final linker-payload.

Discussion

Fit-for-purpose approach

Setting impurity limits for the linker-payload in ADCs is essential to ensure safety and efficacy. While specific guidelines for ADC impurities are not fully established, general principles from ICH guidelines (Q3A, Q3B, and Q6B) can be applied in a phase-appropriate manner. Due to the complex structure, multiple chiral centers, polymeric segments, and unstable reaction conditions, several reaction and product-related impurities are generated, which are difficult to control. impurity limits might initially be broader in phase I based on the availability of toxicology data and the ADC's therapeutic index. Based on further phase transition and study results, the specification can be further tightened (Fig. 1).

Figure 1



Three categories of impurities are frequently encountered, including product-related impurities, reaction impurities, and impurities arising from the epimerization of chiral centers. In initial phases, the structure of impurities can be tentatively identified using LCMS. However, diastereomeric impurities resulting from the epimerization of chiral centers pose a challenge for assignment due to the extensive number of potential diastereomers (2^n, where n represents the number of chiral centers) and the absence of chiral markers in the early stages of the project. Furthermore, polymeric homologues such as PSAR/GLYSAR often elute alongside the main peak or at the inflection point of the main peak, complicating the identification of their content. Additionally, a significant concern regarding impurities in ADC DS is the presence of conjugatable impurities carried over through the linker-payload. We will explore strategies to address the challenges defining specifications for these impurities. We will focus on detecting conjugatable and non-conjugatable impurities, resolution method for PEG, PSAR, and GLYSAR.

Equipment used for impurity analysis and structure determination (Fig 2)

Figure 2

Identification of Conjugatable and Non-Conjugatable Impurities

To determine the conjugatable characteristics of impurities, a mock conjugation with the relevant counter chemical moiety will be performed under bioconjugation conditions. The resulting conjugation products will then be analyzed using high-performance liquid chromatography (HPLC) to identify the presence of any conjugatable impurities. Understanding the distinction between conjugatable and non-conjugatable impurities will enable the establishment of more stringent specifications for conjugatable impurities, thereby minimizing potential interference in toxicology studies. Some common linkers are below.

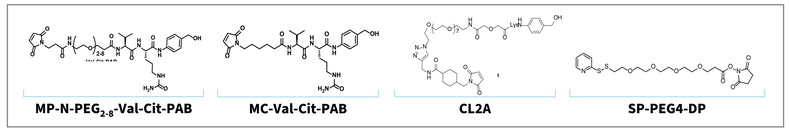


Figure 3

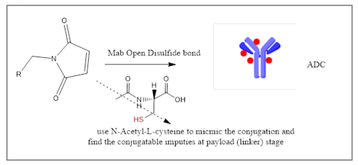
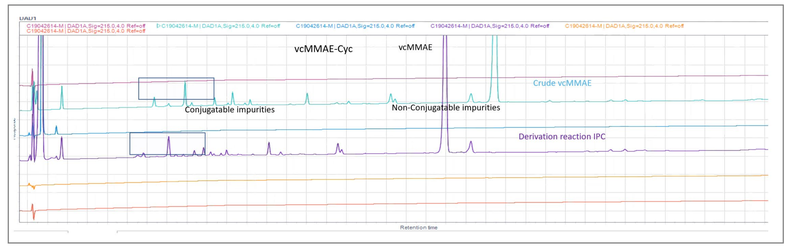


Fig 3 and 4: Cystine conjugation

A typical example of determination of the conjugatable using crude vcMMAE, as shown in Fig. 3. Using a crude linker-payload to conduct a mock conjugation is preferable. The reaction can be analyzed by using LCMS, focusing on peak shifts and the corresponding change in peak mass (Fig 4).

Figure 4



Analytical Method Development of Polymeric Sarcosine Homologue

Polysarcosine (PSAR) and GLYSAR are crucial components of the linker-payload for ADCs because they significantly enhance the hydrophilicity of the drug-linker complex. This improvement effectively masks the inherent hydrophobicity of the cytotoxic payload, which could otherwise compromise the pharmacokinetics and overall efficacy of the ADC. Discovery research requires a diverse array of custom-designed research materials, including antigens, antibody and protein reagents, cell lines, and detection antibodies for screening assays, among others. Typically, polysarcosine units are synthesized from smaller sarcosine units, leading to the formation of corresponding homologues. The linker-payload produced through this method might also contain these impurities, which generally elute alongside the main peak. The WuXi XDC analytical team has optimized analytical methods to separate these impurities from the main peak, enabling the quantification of their content. While these impurities can be resolved for analytical purposes, their separation on reparative (prep HPLC) remains challenging, necessitating reliance on the starting materials for impurity control.

Figure 5: PSARn linker-payload and its homologue impurities

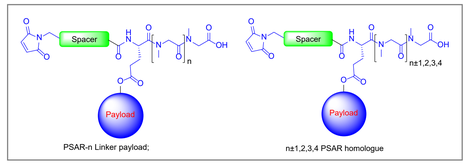
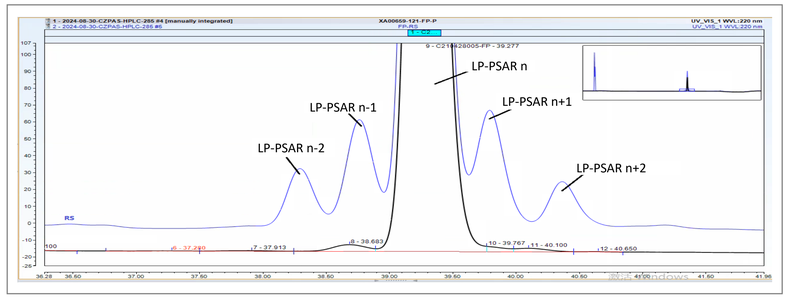
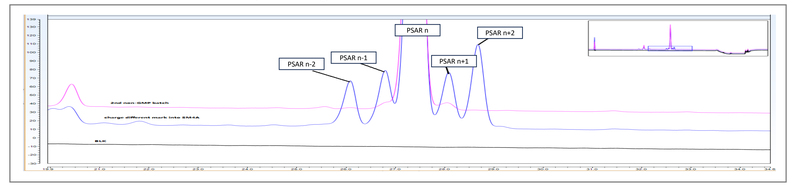


Figure 6: HPLC chromatogram for a PSARn linker-payload and its homologue impurities



Additionally, we have developed an analytical method to address these impurities in the starting material PSARn, which serves as the current control point. We are also optimizing a prep HPLC method to further resolve and separate these impurities during bulk separation.

Figure 7: Resolution of PSAR (Starting material)



Analytical Method Development of Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) is commonly used in the synthesis of ADC as a linker between antibody and cytotoxic payload or at the branch terminal of the linker segment. The main advantage of PEG is to shield the hydrophobic payload, enhancing the solubility and stability of the ADC in bloodstream. Another is the prevention of ADC aggregation, which is crucial for maintaining efficacy and safety. Larger PEG molecules are synthesized by smaller units of polyethylene glycol, which results in obvious presence of its close homologues. The linker-payload prepared using these PEG molecules carries these impurities being difficult to separate from main peak. At WuXi XDC, the analytical team separated these impurities very well.

Figure 8: PEG24 linker payload and its homologue impurities

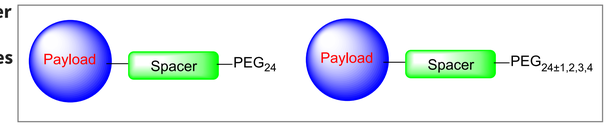
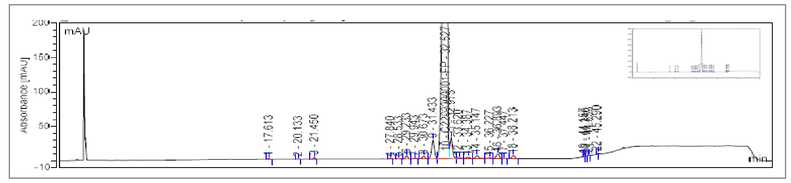
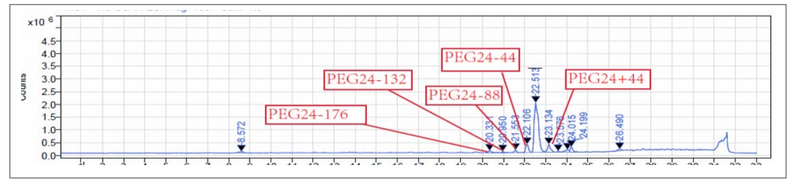


Figure 9: HPLC chromatogram for PEG24 linker payload and its homologue impurities



Additionally, we developed an analytical method to address the impurities in the starting material PEG24, which serves as the control point to minimize these impurities in PEG24 (starting material). We are also optimizing a prep HPLC method to further resolve and separate these impurities during bulk separation.

Figure 10: Resolution in PEG (Starting material)



Conclusions

An analytical method for a complex linker payload-structure offers several key benefits, particularly in the context of ADCs. These methods he WuXi XDC analytical team can develop complex analytical methods to identify most impurities present in the linker-payload, even those with closely eluting peaks. Analytical methods identify most impurities present in the linker-payload, including those with closely eluting peaks. This approach enables effective control of conjugatable impurities from both the reaction and starting materials, providing support for regulatory compliance.

About WuXi XDC

WuXi XDC (2268.HK) is a leading global CRDMO focused on ADCs and the broader bioconjugate market. It provides end-to-end contract research, development and manufacturing services for bioconjugates, including ADCs. Its services cover antibody intermediates and other biologics intermediates, chemical payloads and linkers, as well as bioconjugate drug substances and drug products.

wuxixdc.com
wuxixdc_info@wuxibiologics.com

Poster modified on 10/10/2024

To discuss this poster, email patilsudhir@wuxibiologics.com