WuXi AppTec

WHITEPAPER

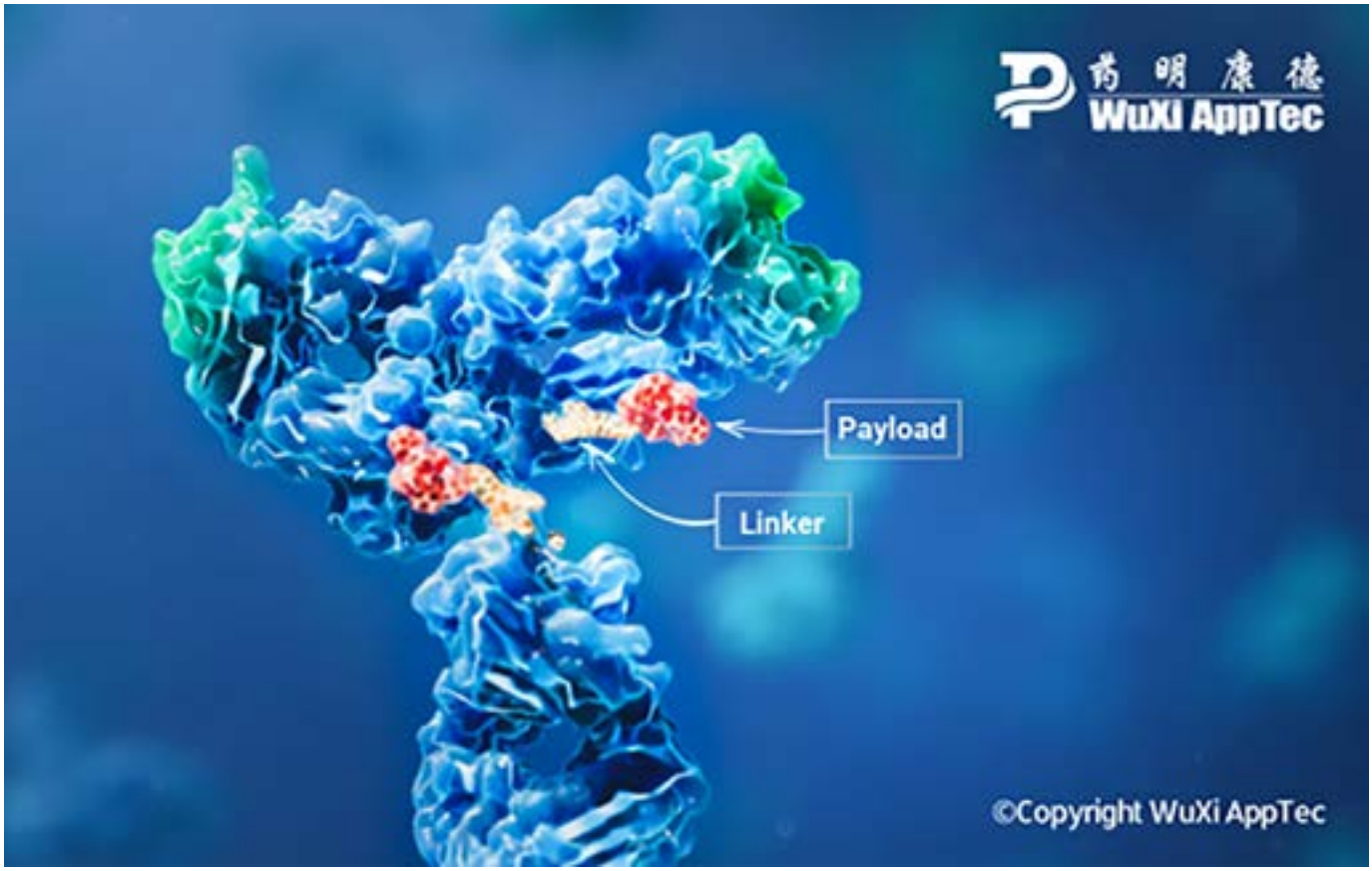
DETECT AND CHARACTERIZE *IN VITRO* PCCS OF NON-CLEAVABLE ADCS USING A NEW LC-HRMS METHOD

ADC Whitepaper 5.02.23

INDEX

WHITEPAPER

INTRODUCTION



Profiling *in vitro* PCCs of non-cleavable ADCs is vital for preclinical evaluation. However, the analytical approaches used today are insufficient to provide reliable PCC profiling results. WuXi AppTec explores a new LC-HRMS method.

Profiling *in vitro* payload-containing catabolites (PCCs) of non-cleavable antibody drug conjugates (ADCs) is important for optimizing payload design and preclinical evaluation. However, today's analytical approaches are insufficient, often failing to provide reliable PCC profiling results efficiently.

WuXi AppTec's recent work in this area introduces a new liquid chromatography-high resolution mass spectrometry (LC-HRMS) method to quickly and comprehensively detect and characterize PCCs released from a non-cleavable ADC in liver lysosomes and S9 incubations.

The complete study and methodology was published online in January 2023 in Drug Metabolism and Disposition.

Background on ADCs

ADCs are a relatively new biotherapeutic modality consisting of a monoclonal antibody, a linker, and a payload (or toxin).[1,2] The monoclonal antibody targets the antigen expressed on cancer cells and delivers the toxic payload into the cells. ADCs offer high specificity, making them a fast-growing and essential therapeutic option for various diseases.

ADC linkers include cleavable and non-cleavable types, depending on how they are cleaved when releasing the payload or payload containing catabolites (PCCs).[3,4]

- Cleavable ADCs release the payloads or a payload derivative in a predicted manner under a specific intracellular environment, such as low pH, protease, high GSH concentration etc.[5,6,7]

- Non-cleavable ADCs usually do not undergo specific cleavages of linkers to release the payload. Instead, they generate one or a few major unknown payload-containing catabolite(s) via protein hydrolysis of the antibody moiety.[8]

Payload release consisting of major PCCs in targeted cancer cells plays a dominant role in the therapeutic effect of the ADC.[9] In fact, the toxicity of an ADC is directly associated with the off-target release or accumulation of the payload or PCCs in untargeted normal cells or tissues.

In early discovery, it is necessary to identify the major pharmacologically active PCCs of a non-cleavable ADC *in vitro*. This information is required to develop a bioanalytical method for evaluating the exposure of toxic PCC in animals and humans[10] and to study its metabolism and *in vitro* DDI potential as part of the ADC's preclinical evaluation.[11,12,13,14]

However, detecting and characterizing PCCs' structure of non-cleavable ADCs and their metabolites *in vitro* and *in vivo* still pose significant analytical challenges.

PCC Challenges

Various bioanalytical methods have been developed to characterize and quantify ADCs, including[15,16] immuno-capture to enrich ADCs followed by LC-MS analysis, ligand binding assays to analyze total antibody or drug conjugated species, and intact protein characterization by LC-HRMS.

However, very few documented instances exist of applying analytical technologies to profile and identify ADCs *in vitro* or *in vivo*. Usually, concentrations of PCCs of an ADC are much lower than the number of metabolites in small molecule drugs. Further complicating the analysis, ADCs have unpredictable molecular weights, mass defect values and fragmentation of payload-containing catabolites released from a non-cleavable ADC.[17,18] Commonly used LC-HRMS data processing tools for metabolite profiling of small molecule drugs, such as mass defect filter and targeted extracted ion chromatographic analysis, are also ineffective in finding PCCs.

Historically, the most effective way to profile and characterize PCCs of ADCs would have been to use an ADC with a radiolabeled payload or linker.[19,20,21] However, preparing such a radiolabeled ADC is time-consuming, costly, and not well-suited for drug discovery. Additionally, LC-radio detection may not have sufficient sensitivity to detect low levels of radiolabeled PCCs.

The WuXi AppTec Solution

To overcome these challenges, WuXi AppTec conducted a study to develop and apply a generic and effective LC-HRMS method for comprehensive detection and characterization of *in vitro* PCCs of a non-cleavable ADC using both untargeted background subtraction processing (PATBS) and targeted product ion filtering (PIF).

PATBS effectively detect drug metabolites or xenobiotic components in test samples that are absent or have much lower concentrations in a control sample.[22,23] While targeted PIF was employed to process LC-MS/MS datasets to find payload-containing components that generated one or a few known or predictable product ions under CID.

In addition, EIC was used to confirm detected PCCs by PATBS and PIF and to search for any predictable catabolites of a testing ADC and their metabolites formed in incubations. After data mingling was used to detect payload-containing components in an incubation sample, their MS/MS spectral data were retrieved from the acquired LC-MS/MS dataset. Structures of the detected PCCs were characterized based on their spectral interpretation. If MS/MS spectrum of a PCC was not triggered in the acquisition, additional LC-HRMS analyses of the same sample using targeted product ion scanning were carried out to acquire its product ion spectrum.

The effectiveness of the LC-HRMS-based method was first evaluated by analyzing PCCs generated in incubation of a well-known non-cleavable ADC (TDM1) in human liver lysosomes. The technique was further applied to profiling PCCs formed in incubations of ADC-1, a non-cleavable ADC, with human liver S9 and a cancer cell line.

A key element in effectively using PATBS to find PCCs of a test ADC is suitable control sample(s). In the WuXi AppTec study, the same antibody of a testing ADC (without the payload and linker) was used as a control sample of the background subtraction processing. Consequently, peptides without payload from protein hydrolysis of the ADC were effectively removed by background subtraction. Applying a sequential background subtraction using an ADC-1 pre-incubation sample (0 h) further removed interference components from the ADC dosing solution, generating a very clear and complete profile of the PCCs of ADC-1.

Impact on the Industry

The WuXi AppTec study demonstrated the advantages of using a new LC-HRMS method combined with targeted PIF. Untargeted background subtraction processing of an LC-MS dataset and using the corresponding antibody as a control sample enabled selectively detecting payload-containing components of a non-cleavable ADC regardless of their molecular weights, charge states, fragmentations, and mass defect values. As a complementary tool, PIF detected specific PCCs with superior sensitivity. The combination of the *in vitro* metabolism systems and the LC-HRMS method can provide fast, sensitive, and selective profiling of *in vitro* PCCs of non-cleavable ADCs in support of drug dzzziscovery programs.

Moreover, using common product ion(s) from payload-containing derivatives to perform product ion filtering of LC-MS/MS data allows researchers to detect PCCs with better analytical sensitivity if their product ion spectra are automatically acquired.

Results from detection and structural characterization of payload-containing components generated from ADC catabolism in liver lysosomes and S9 fraction can provide valuable information for optimizing payload design, verifying toxicological species for ADC safety testing, and selecting the major payload-containing component(s) for evaluation of *in vitro* DDI potential and exposure in animals and humans. However, the effectiveness of this LC-HRMS method for profiling *in vivo* payload-containing catabolites of ADCs remains to be tested.

Talk To Our Experts Today

To understand how this data can impact one of your studies, talk with a WuXi AppTec expert

labtesting.wuxiapptec.com/contact-us/

REFERENCES:

1. Khongorzul P, Ling CJ, Khan FU, Ihsan AU and Zhang J (2020) Antibody-Drug Conjugates: A Comprehensive Review. Mol Cancer Res 18(1): 3-19.
2. Mukherjee A, Waters AK, Babic I, Nurmemmedov E, Glassy MC, Kesari S and Yenugonda VM (2019) Antibody drug conjugates: Progress, pitfalls, and promises. Human antibodies 27(1): 53-62.
3. Kommineni N, Pandi P, Chella N, Domb AJ and Khan W (2020) Antibody drug conjugates: Development, characterization, and regulatory considerations. 31(6): 1177-1193.
4. Pander G, Uhl P, Kühl N, Haberkorn U, Anderl J and Mier W (2022) Antibody–drug conjugates: What drives their progress? Drug Discovery Today.
5. Anami Y, Yamazaki CM, Xiong W, Gui X, Zhang N, An Z and Tsuchikama K (2018) Glutamic acid–valine–citrulline linkers ensure stability and efficacy of antibody–drug conjugates in mice. Nature Communications 9(1): 2512.
6. Bargh JD, Isidro-Llobet A, Parker JS and Spring DR (2019) Cleavable linkers in antibody–drug conjugates. Chemical Society Reviews 48(16): 4361-4374.
7. Bargh JD, Walsh SJ, Ashman N, Isidro-Llobet A, Carroll JS and Spring DR (2021) A dualenzyme cleavable linker for antibody–drug conjugates. Chemical Communications 57(28): 3457-3460.
8. García-Alonso S, Ocaña A and Pandiella A (2018) Resistance to Antibody-Drug Conjugates. Cancer research 78(9): 2159-2165.
9. Zhang D, Yu SF, Khojasteh SC, Ma Y, Pillow TH, Sadowsky JD and Hop C (2018) Intratumoral Payload Concentration Correlates with the Activity of Antibody-Drug Conjugates. Molecular cancer therapeutics 17(3): 677-685.
10. Khera E and Thurber GM (2018) Pharmacokinetic and Immunological Considerations for Expanding the Therapeutic Window of Next-Generation Antibody-Drug Conjugates. BioDrugs: clinical immunotherapeutics, biopharmaceuticals and gene therapy 32(5): 465-480.
11. Han TH and Zhao B (2014) Absorption, distribution, metabolism, and excretion considerations for the development of antibody-drug conjugates. Drug metabolism and disposition: the biological fate of chemicals 42(11): 1914-1920.
12. Hinrichs MJ and Dixit R (2015) Antibody Drug Conjugates: Nonclinical Safety Considerations. The AAPS journal 17(5): 1055-1064.
13. Kraynov E, Kamath AV, Walles M, Tarcsa E, Deslandes A, Iyer RA and Moore DJ (2016) Current Approaches for Absorption, Distribution, Metabolism, and Excretion Characterization of Antibody-Drug Conjugates: An Industry White Paper. Drug metabolism and disposition: the biological fate of chemicals 44(5): 617-623.
14. Widdison W, Wilhelm S, Veale K, Costoplus J, Jones G, Audette C and Chari R (2015) Metabolites of antibody-maytansinoid conjugates: characteristics and *in vitro* potencies. Molecular pharmaceutics 12(6): 1762-1773.
15. Wei C, Su D, Wang J, Jian W and Zhang D CPR (2017) LC–MS Challenges in Characterizing and Quantifying Monoclonal Antibodies (mAb) and Antibody-Drug Conjugates (ADC) in Biological Samples. Current Pharma. Reports 4: 45-63.
16. Zhu X, Huo S, Xue C, An B and Qu J (2020) Current LC-MS-based strategies for characterization and quantification of antibody-drug conjugates. Journal of pharmaceutical analysis 10(3): 209-220.
17. Rangan VS, Myler H, Kozhich A, Wang J, Randazzo R and Deshpande S (2015) Biotransformation and stability of antibody-drug conjugates: payload metabolism and linker cleavage delineation. Bioanalysis 7(11): 1319-1323.
18. Su D and Zhang D (2021) Linker Design Impacts Antibody-Drug Conjugate Pharmacokinetics and Efficacy via Modulating the Stability and Payload Release Efficiency. Frontiers in pharmacology 12: 687926.
19. Baron JM, Boster Bl and Barnett CM (2015) Ado-trastuzumab emtansine (T-DM1): a novel antibody-drug conjugate for the treatment of HER2-positive metastatic breast cancer. Journal of oncology pharmacy practice: official publication of the International Society of Oncology Pharmacy Practitioners 21(2): 132-142.
20. Bolleddula J, Shah A, Shadid M, Kamali A, Smith MD and Chowdhury SK (2020) Pharmacokinetics and Catabolism of [(3)H]TAK-164, a Guanylyl Cyclase C Targeted Antibody-Drug Conjugate. Drug metabolism and disposition: the biological fate of chemicals 48(11): 1239-1245.
21. Erickson HK, Lewis Phillips GD, Leipold DD, Provenzano CA, Mai E, Johnson HA and Tibbitts J (2012) The effect of different linkers on target cell catabolism and pharmacokinetics/pharmacodynamics of trastuzumab maytansinoid conjugates. Molecular cancer therapeutics 11(5): 1133-1142.
22. Xing J, Zang M, Zhang H and Zhu M (2015) The application of high-resolution mass spectrometry-based data-mining tools in tandem to metabolite profiling of a triple drug combination in humans. Analytica chimica acta 897: 34-44.
23. Zhu P, Ding W, Tong W, Ghosal A, Alton K and Chowdhury S (2009) A retention-time-shift tolerant background subtraction and noise reduction algorithm (BgS-NoRA) for extraction of drug metabolites in liquid chromatography/mass spectrometry data from biological matrices. Rapid communications in mass spectrometry: RCM 23(11): 15631572.