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SIMULTANEOUS QUANTIFICATION OF MULTIPLE ADC COMPONENT CONCENTRATIONS AND DAR PROFILING WITH ONE ALIQUOT OF PLASMA IN NHP TOXICOLOGY STUDY BY HYBRID LC-MS

Pei Zhang[a], Yifan Wang[a], Wenqin Xu[a], Jing Huang[a], Xiaoying Jin[a], Lan Li[b]

[a]WuXi AppTec, (Suzhou) Co., Ltd., 1318 WuZhong Avenue, Suzhou, Jiangsu, 215104, China
[b]WuXi AppTec, (Shanghai) Co., Ltd., 288 FuTe Zhong Road, Shanghai, 200131, China

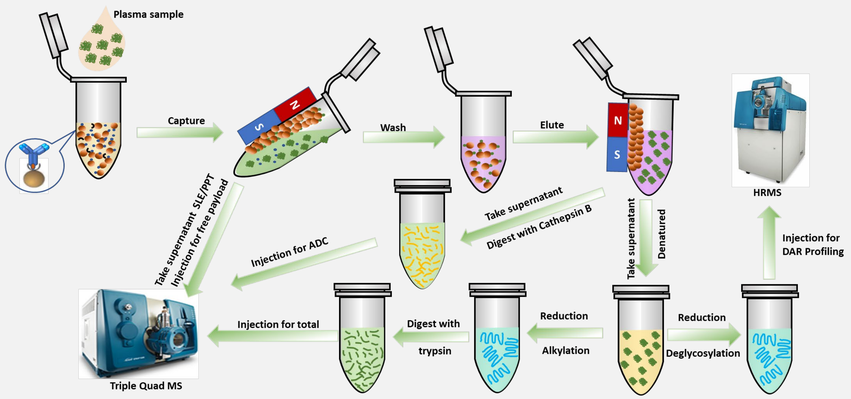
PURPOSE

The field of Antibody-Drug Conjugates (ADCs) has been experiencing a remarkable surge in development and research, making it one of the most exciting and rapidly evolving areas in oncology in recent years. ADCs represent a revolutionary approach to cancer treatment by combining the specificity of monoclonal antibodies with the potent cytotoxic effects of chemotherapeutic drugs, leading to enhanced efficacy and reduced side effects.

However, the complexity and heterogeneity of ADC molecules bring unique challenges to bioanalysis. One of the main challenges is the characterization and quantification of the various components that make up the ADC molecule. ADCs consist of a monoclonal antibody, a cytotoxic drug payload, and a linker molecule. Each component may undergo modifications, such as drug-to-antibody ratio (DAR) variability and degradation. Therefore, it is essential to develop analytical methods capable of accurately distinguishing and quantifying these different forms of ADCs.

A liquid chromatography coupled with mass spectrometry (LC-MS) method has been developed in this paper, allowing for simultaneous quantification of free payload, conjugated ADC, total antibody and DAR profiling with only one aliquot of sample in non-human primate (NHP) plasma. By combining the analysis of multiple components in a single treatment, the method significantly reduces the time and resources required for bioanalysis, making it highly efficient and enabling meaningful comparisons between different components. The reduction in sample volume is particularly advantageous when working with limited sample volumes or precious samples, conserving valuable resources and allowing for broader analysis.

Figure 1. *Sample treatment workflow for quantification of free payload, ADC, total antibody concentration and DAR profiling*



METHOD

Aliquot 30uL of plasma sample for immunocapture, and then take the supernatant to protein precipitation or supported liquid extraction (SLE) to detect free payload. After eluting the ADC from the beads, divide the elution into two parts for enzymatic digestion. Use one part for digestion with Cathepsin B or papain to break the linker and release the conjugated payload for detection, which provides information on ADC concentration. The other part undergoes high-temperature denaturation and is further divided into two portions. One portion is enzymatically digested to obtain a surrogate peptide for total antibody analysis, while the other portion undergoes deglycosylation after reduction and is injected into a high-resolution mass spectrometer for DAR profiling.

Figure 2. *Calibration curve and 5 QC levels from 3 accuracy and precision runs by LC-MS/MS method to quantify total antibody and conjugated payload of ADC.*

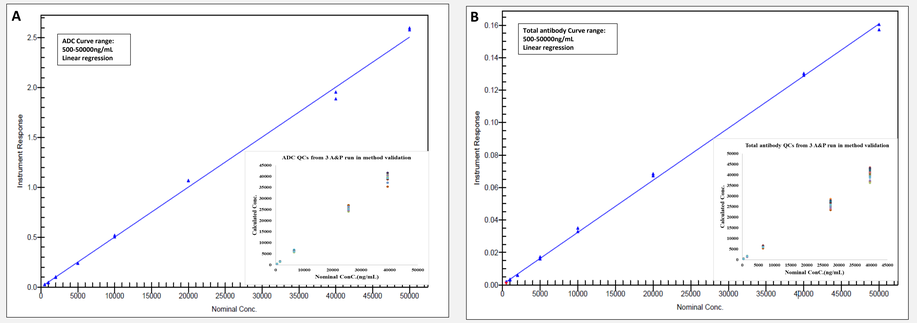


Figure 3. *Typical chromatogram of free payload, conjugated payload of ADC and surrogate peptide for total antibody quantification from LC-MS/MS*

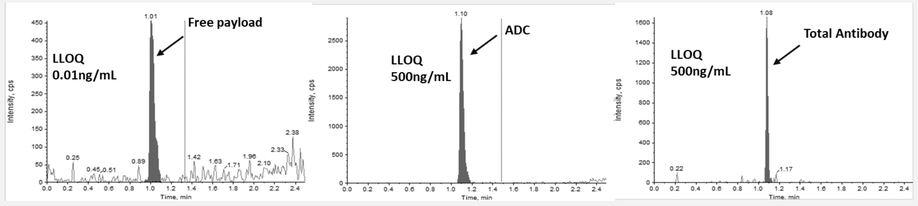


Figure 4. *Typical mass spectrum of ADC subunit with LC-TOF-MS method*

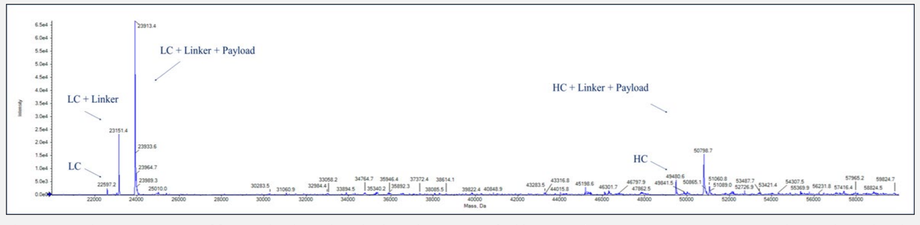
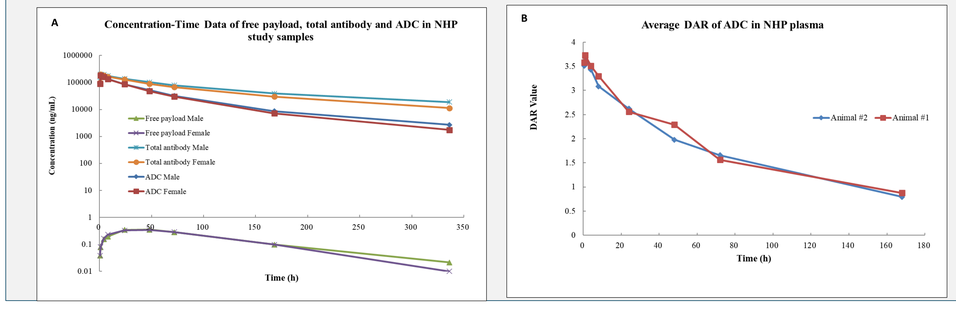


Figure 5. *A: The concentration of free payload, total antibody and ADC quantified by LC-MS/MS. B: Average DAR in NHP study sample analyzed by LC-TOF-MS in different time points*



RESULT

Method performance:

A robust method has been developed for the simultaneous quantification of multiple ADC components and DAR profiling. The calibration curve ranges for free payload, total antibody, and ADC were determined to be 0.01 ng/mL to 10 ng/mL, 500 ng/mL to 50,000 ng/mL, and 500 ng/mL to 50,000 ng/mL, respectively. Rigorous validation was performed to assess the performance of the method, including accuracy and precision, linearity, recovery, stability, selectivity and matrix effect. The method has been successfully applied to the analysis of samples from NHP toxicology studies.

Sample analysis:

This study analyzed 600 NHP plasma samples collected at different times and from various dosing levels to determine the concentrations of free payload, ADC, and total antibody. Additionally, DAR profiling was conducted for 18 samples.

After intravenous administration of the ADC, the metabolism of the ADC in vivo leads to linker cleavage and payload release. At 48 hours post-administration, the plasma concentration of free payload reaches its maximum concentration (Cmax) at approximately 0.3 ng/mL, while at 336 hours post-administration, it gradually decreases to around the lower limit of quantification, 0.01 ng/mL. At 1-hour post-administration, ADC and total antibody concentrations reach their Cmax at approximately 200,000 ng/mL. After 336 hours post-administration, the ADC concentration decreases to around 2,000 ng/mL, while the total antibody concentration decreases to approximately 10,000 ng/mL.

The average DAR value decreases over time. At 1-hour post-administration, the average DAR value is about 3.7, close to the theoretical Dar value of 4, indicating a higher number of drug molecules attached to each antibody molecule. By 168 hours post-administration, the average DAR value decreases to 0.8, suggesting a lower number of conjugated drug molecules per antibody molecule, which is consistent with the metabolic profile.

CONCLUSION

A reliable and robust LC-MS method was successfully developed to simultaneously quantify free payload, total antibody, ADC and DAR profiling in NHP plasma using only 30 µL of plasma, with the LLOQ being 0.01ng/mL, 500ng/mL and 500ng/mL, respectively. The method was validated and applied to acquire toxicokinetic data, highlighting the dynamic nature of the ADC in vivo, including the release of payload, clearance of free payload, and changes in ADC and total antibody concentrations over time. Understanding these pharmacokinetic characteristics is crucial for optimizing dosing regimens, evaluating therapeutic responses, and evaluating the safety and efficacy of ADC-based therapies.

REFERENCES

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