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Development of a Rapid, High Throughput Quantitative Bioanalysis Method for Conjugated Payloads of Protease-Cleavable Antibody-Drug Conjugate (ADC) in Rat Serum Using Automated Immuno-Capture Based UPLC-MS/MS

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PURPOSE

Antibody-drug conjugate (ADC) is a new therapeutic modality that combines the high targeting of monoclonal antibodies and the strong cytotoxicity of small molecule toxins. Due to their precise and powerful effects, they have become an important trend in the development of anti-tumor drugs. ADC consists of three parts, including the antibody part, the small molecule payload and the linker that connects the two. ADC is more complex and challenging than other drugs. Not only the large molecule parts (total antibody and conjugated antibody) need to be analyzed, but also small molecule components such as free payload and conjugated payload. Quantitation of conjugated payload in biological samples can reflect ADC efficacy and toxicity. Quantitative bioanalysis of conjugated payloads, free payloads, and related metabolites runs through all stages of ADC drug development, including early discovery, screening, preclinical, and clinical stages.

Among the 15 ADC drugs currently on the market, there are 7 ADC drugs with protease-cleavable linkers of which 5 are MC-VC-PABC. The linker of DS-8201 is MC-GGFG which was used in many drugs' development. In this study, MC-VC-PABC and MC-GGFG, two protease-cleavable linkers with the highest proportion were used as examples to establish a rapid, high-throughput, sensitive, and reliable conjugated payloads quantification method, which was successfully applied to *in vivo* PK rat serum sample bioanalysis.

METHOD(S)

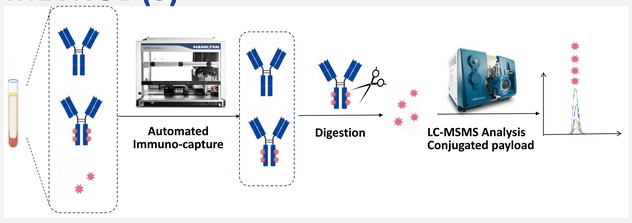

Figure1 . Sample pretreatment workflow of conjugated payload in rat serum

- Two commercial ADCs, DS-8201[1] and RC48[2] (basic information is listed in Table 1) working solutions were spiked into rat serum to prepare standards and quality controls.
- The sample pretreatment workflow is shown in Figure 1. The samples were immuno-captured by biotinylated anti-human IgG, using Hamilton with IMCS tips which were coated with streptavidin.
- Digestion was conducted with papain, and released payloads were analyzed using UPLC-MS/MS. Details of the instrumental conditions used are listed in Table 1.

RESULT(S)

- After automatic immuno-capture and enzymatic digestion, the linear range of DS-8201 was from 0.05 to 200 μg/mL with excellent linearity (r^2 > 0.999). This range corresponds to conjugated payload (DXd) concentration of 1.3 to 5045.3 ng/mL. The lower limit of quantitation (LLOQ) of DS-8201 was 50 ng/mL in rat serum, equivalent to the LLOQ of DXd was 1.3 ng/mL, as shown in Figures 2(A) and 2(B). Results showed that within-run accuracy was from 3.26% to 13.1%, and precision was from -10.8% to 7.61% (Table 2).
- The linear range of RC48 was from 0.01 to 200 μg/mL with good linearity (r^2 > 0.994). This range corresponds to a conjugated payload (MMAE) concentration of 0.2 to 3984 ng/mL. The LLOQ of RC48 was 10 ng/mL, equivalent to the LLOQ of MMAE was 200 pg/mL in rat serum, as shown in Figures 2(C) and 2(D). Results showed that the within-run accuracy was from 5.00% to 11.0%, and precision was from -4.21% to 3.26% (Table 2).
- No matrix effect or carryover was observed with this method.
- To prevent non-specific binding, 0.05% Triton was added to the working solution, and low-binding materials were used to avoid non-specific binding.
- The immuno-capture time using traditional magnetic bead is about 8 hours, which is shortened to 2 hours with automation. Compared to the magnetic beads immuno-capture method, the automated immuno-capture method has advantages, such as rapid, higher throughput and reduced human errors.
- The enzyme digestion efficiency is 92.1% for the MC-GGFG linker and 78.0% for the MC-VC-PABC linker. (Table 2)

Table 1. Basic information and UPLC-MS/MS conditions for DS-8201 and RC48

Items	Method	
ADC	Trastuzumab deruxtecan (DS-8201)	DisitamabVedotin (RC48)
Linker	MC-GGFG	MC-VC-PABC
DAR	About 8	About 4
Payload	DXd	MMAE
Instrument	Waters ACQUITY UPLC system coupled with Sciex 6500+	
Mobile Phase A	0.1% formic acid in water	
Mobile Phase B	0.1% formic acid in acetonitrile	
Column	ACQUITY UPLC HSS T3 (1.8 μm, 2.1 x 50 mm)	ACQUITY UPLC Protein BEH C4 (1.7 μm, 2.1 x 50mm)
Run Time	4.5 min	1.8 min
Flow rate	0.50 mL/min	0.65 mL/min
MS	ESI / positive ion mode	
MRM Transitions	Dxd: m/z 494.1/375.3	MMAE: m/z 718.5/686.5
	Verapamil (IS): m/z 455.2/164.9	Verapamil (IS): m/z 455.2/164.9

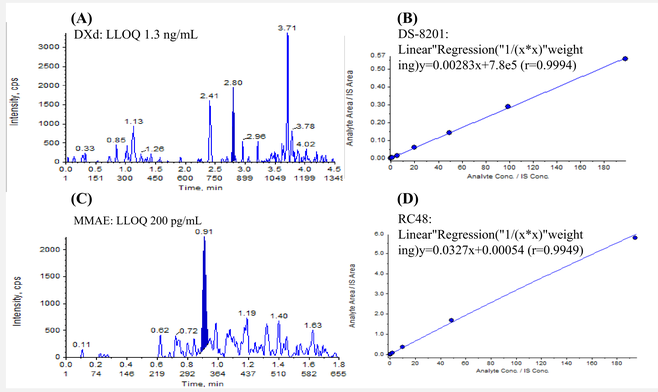

Figure 2. Chromatogram of LLOQ for DXd and MMAE in rat serum
(A) LLOQ of DS-8201 conjugated payload DXd was 1.3 ng/mL; (B) Calibration Curve of DS-8201; (C) LLOQ of RC48 conjugated payload MMAE was 200 pg/mL; (D) Calibration Curve of RC48

Table 2. Accuracy and precision of DXd and MMAE in rat serum samples and digestion efficiency Results

AP Run 1	LQC (ADC 0.3 μg/mL DXd 7.57 ng/mL)	%Bias	MQC (ADC 10 μg/mL DXd 252 ng/mL)	%Bias	HQC (ADC 160 μg/mL DXd 4036 ng/mL)	%Bias
DXd	7.34	-3.0	218	-13.5	4430	9.76
	8.63	14.0	227	-9.92	4290	6.29
	6.77	-10.6	228	-9.52	4100	1.59
	9.23	21.9	218	-13.5	4500	11.5
	6.96	-8.1	219	-13.1	4320	7.04
	8.71	15.1	238	-5.56	4420	9.51
Within-Run Mean	7.94		225		4343	
Within-Run SD	1.04	NA	7.94	NA	142	NA
Within-Run %CV*	13.1		3.53		3.26	
Within-Run %Bias**	4.89		-10.8		7.61	
AP Run 1	**LQC (ADC 0.05 μg/mL MMAE 0.996 ng/mL)**	**%Bias**	**MQC (ADC 10 μg/mL MMAE 199 ng/mL)**	**%Bias**	**HQC (ADC 160 μg/mL MMAE 3187 ng/mL)**	**%Bias**
MMAE	0.865	-13.2	174	-12.7	2790	-12.5
	1.03	3.94	184	-7.64	2780	-12.8
	1.1	10.9	195	-2.12	3050	-4.31
	1.19	19.3	192	-3.62	3250	1.96
	1.06	6.24	202	1.40	3250	1.96
	0.926	-7.07	199	-0.108	3200	0.394
Within-Run Mean	1.03		191		3053	
Within-Run SD	0.118	NA	10.4	NA	220	NA
Within-Run %CV*	11.0		5.00		7.00	
Within-Run %Bias**	3.26		-4.12		-4.21	

Name	Analyte	ADC Concentration (μg/mL)	Intensity	Average Intensity	Digestion Efficiency (%)
Sample-(DS-8201) (without immunocapture, with digestion)	DXd	2.00	1.22E+05	1.17E+05	92.1%
			1.10E+05		
			1.20E+05		
Control sample (Add theoretical concentration of DXd in blank)			1.31E+05	1.27E+05	
			1.29E+05		
			1.22E+05		
Sample-(RC48) (without immunocapture, with digestion)	MMAE	0.10	1.98E+04	2.20E+04	78.0%
			2.20E+04		
			2.41E+04		
Control sample (Add theoretical concentration of MMAE in blank)			2.63E+04	2.83E+04	
			2.95E+04		
			2.92E+04		

CONCLUSION(S)

- In this work, we developed a rapid, high throughput automated immuno-capture based UPLC-MS/MS bioanalysis method for the quantitation of conjugated payloads in rat serum.
- This method is applicable to protease-cleavable linkers such as MC-GGFG and MC-VC-PABC. The validated method showed efficient and reliable sensitivities, good linearity, intra-day precision and accuracy, as well as excellent stability. Established methods were successfully applied to the *in vivo* rat serum PK study.
- The advantages of this approach:
  - ✓ **Automated Immuno-capture**: Compared with the magnetic bead method, the pretreatment time is reduced from 8 h to 2 h.
  - ✓ **High digestion efficiency**: The enzyme digestion efficiency is 92.1% for the MC-GGFG linker and 78.0% for the MC-VC-PABC linker.
  - ✓ **High throughput**: The automated immune-capture method can operate 96 samples simultaneously.

REFERENCES

[1] Azar I, Alkassis S, Fukui J, Alsawah F, Fedak K, Al Hallak MN, Sukari A, Nagasaka M. Spotlight on Trastuzumab Deruxtecan (DS-8201,T-DXd) for HER2 Mutation Positive Non-Small Cell Lung Cancer. Lung Cancer (Auckl). 2021 Oct 7;12:103-114.

[2] Shi F, Liu Y, Zhou X, Shen P, Xue R, Zhang M. Disitamab vedotin: a novel antibody-drug conjugates for cancer therapy. Drug Deliv. 2022 Dec;29(1):1335-1344.

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