HOW DO YOU DEVELOP AND VALIDATE AN icIEF METHOD
FOR USE IN RELEASE AND STABILITY TESTING OF ADC?

APPLICATION NOTE

Analytical Development and Validation of Imaging Capillary Isoelectric Focusing (icIEF) for the Analysis of Charged Isoforms in Antibody Drug Conjugate (ADC)

R. Tobias[1], P. Shetty[1], C. Busano[1], G. Petrie[1], M. Whalon[1], E. Idusogie[2], M. Mulkerrin[2]
[1]EAG –Life Sciences, [2]ADC Therapeutics

PURPOSE

The purpose of the study was to develop and validate an imaging capillary isoelectric focusing (icIEF) for charge heterogeneity analysis of an Antibody Drug Conjugate (ADC) and its corresponding antibody intermediate.

OBJECTIVE

To develop and validate an icIEF for the determination of the charged isoforms of an Antibody Drug Conjugate (ADC) and its monoclonal antibody intermediate.

METHOD

The product related charged variants of an ADC molecule and its antibody intermediate were analyzed according to their isoelectric point (pI) in a capillary containing ampholytes.

The samples were desalted and buffer-exchanged with phosphate buffer in a microcentrifuge spin column prior to analysis.

The charge isoforms were resolved in a FC-coated capillary with a pH 3 – 10 ampholyte gradient containing urea.

Table 1: Antibody Intermediate: Accuracy And Precision

Replicates	Sample Conc (mg/mL)	Acidic A4			Acidic A3			Acidic A2			Acidic A1			Main (M)		
		pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery
Rep1		7.91	2.7	85	8.04	7.8	97	8.18	12.8	101	8.36	24.4	100	8.51	52.5	101
Rep2		7.93	3.0	96	8.03	8.1	100	8.18	12.4	99	8.36	24.9	102	8.51	51.6	99
Rep3	0.20	7.90	2.9	92	8.04	8.1	100	8.18	12.4	99	8.36	24.3	100	8.51	52.3	101
Avg.		7.91	2.9	91	8.04	8.0	99	8.18	12.5	100	8.36	24.5	101	8.51	52.1	100
%RSD		0	7	7	0	2	2	0	1	1	0	1	1	0	1	1
Rep1		7.91	3.0	95	8.03	8.0	99	8.18	12.6	101	8.36	24.8	102	8.51	51.6	99
Rep2		7.92	3.1	98	8.04	8.2	101	8.19	12.7	101	8.36	24.2	99	8.51	51.9	100
Rep3	0.25	7.91	3.3	104	8.04	8.2	101	8.18	12.6	101	8.36	24.3	100	8.51	51.6	99
Avg.		7.91	3.1	99	8.04	8.2	100	8.18	12.6	101	8.36	24.4	100	8.51	51.7	100
%RSD		0	5	5	0	1	1	0	0	0	0	1	1	0	0	0
Rep1		7.94	3.5	113	8.02	8.7	107	8.18	12.0	96	8.36	25.0	103	8.51	50.7	98
Rep2		7.91	3.4	110	8.03	8.5	104	8.18	12.6	101	8.36	24.6	101	8.51	50.9	98
Rep3	0.30	7.94	3.6	116	8.03	8.6	106	8.18	12.5	100	8.36	24.0	99	8.51	51.3	99
Avg.		7.93	3.5	113	8.03	8.6	106	8.18	12.4	99	8.36	24.6	101	8.51	51.0	98
%RSD		0	3	3	0	1	1	0	2	2	0	2	2	0	1	1
Pass/Fail	PASS															

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The samples were desalted and buffer-exchanged with phosphate buffer on a microcentrifuge spin column prior to analysis. The charge isoforms were resolved in a FC-coated capillary with a pH 3 – 10 ampholyte gradient containing urea.

RESULTS

The UV electropherogram of the ADC molecule exhibited comparable charge isoform profile with its corresponding antibody intermediate.

The main charge isoform has a pI of 8.5 while the pI of the minor isoform components ranged from of 7.9 to 8.3 in both ADC and antibody intermediate.

Validation of the icIEF method demonstrated linearity of r2 ≥ 0.99 and assay range of 0.1 to 0.4 mg/mL for both ADC and antibody intermediate.

The precision of the method was ≤ 7% RSD for percent composition and ≤ 1% RSD for pI determination for all charge isoform components.

Accuracy by spiked recovery ranged from 99% to 110% in all the charged isoform components.

Specificity of the assay showed no interference from reagent controls, blanks and their respective formulation buffers.

CONCLUSION

The charged heterogeneity electrophoretic profiles of the ADC and antibody intermediate were determined using icIEF method. The current icIEF method was deemed validated for use in release and stability testing of ADC and its corresponding antibody intermediate.

Table 2: Antibody Drug Conjugate: Accuracy And Precision

Replicates	Final Conc (mg/mL)	Acidic A4			Acidic A3			Acidic A2			Acidic A1			Main (M)		
		pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery	pI	%Area (Precision)	% Recovery
Rep1		7.94	3.4	108	8.12	8.5	103	8.18	11.4	96	8.34	26.6	100	8.48	50.2	100
Rep2		7.94	3.7	120	8.12	8.6	105	8.17	11.7	100	8.34	26.1	98	8.48	49.8	99
Rep3	0.20	7.93	3.3	106	8.12	8.4	103	8.18	11.6	99	8.34	26.7	100	8.49	49.9	99
Avg.		7.94	3.5	111	8.12	8.5	104	8.17	11.6	98	8.34	26.5	99	8.48	50.0	99
RSD		0	7	7	0	1	1	0	2	2	0	1	1	0	0	0
Rep1		7.94	3.2	101	8.12	8.3	101	8.18	11.4	97	8.34	27.1	102	8.49	50.1	100
Rep2		7.94	3.3	105	8.12	8.4	102	8.18	11.7	99	8.34	27.2	102	8.49	49.5	98
Rep3	0.25	7.94	3.4	107	8.12	8.5	104	8.17	11.4	97	8.34	27.2	102	8.49	49.5	98
Avg.		7.94	3.3	104	8.12	8.4	102	8.17	11.5	98	8.34	27.2	102	8.49	49.7	99
RSD		0	3	3	0	2	2	0	1	1	0	0	1	0	1	1
Rep1		7.94	3.1	98	8.12	8.2	100	8.18	11.6	99	8.35	26.6	100	8.49	50.5	100
Rep2		7.94	3.3	104	8.12	8.4	102	8.18	11.9	101	8.34	26.7	100	8.49	49.7	99
Rep3	0.30	7.94	3.2	104	8.12	8.3	101	8.18	11.6	98	8.35	27.3	102	8.49	49.7	99
Avg.		7.94	3.2	102	8.12	8.3	101	8.18	11.7	99	8.34	26.9	101	8.49	50.0	99
RSD		0	3	3	0	1	1	0	2	2	0	1	2	0	1	1
Pass/Fail	PASS															