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LIFE SCIENCES

HOW DO YOU DEVELOP A METHOD TO DETERMINE THE SIZE COMPONENTS AND ELECTROPHORETIC PROFILES OF AN ANTIBODY DRUG CONJUGATE?

APPLICATION NOTE

Method Development and Validation of a Denaturing Capillary Electrophoresis (CE-SDS) for the Determination of Molecular Size Components in Antibody Drug Conjugate (ADC)

By R. Tobias[1], P. Shetty[1], B. Glowinski[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 current study is to develop and validate a denaturing capillary electrophoresis (CE-SDS) for the determination of the size components and electrophoretic profiles of an Antibody Drug Conjugate (ADC) and its monoclonal antibody intermediate.

OBJECTIVE

To develop and validate a CE-SDS for the determination of the size components and electrophoretic profiles of an Antibody Drug Conjugate (ADC) and its monoclonal antibody intermediate.

METHOD

The CE-SDS analysis was conducted under reducing and non-reducing conditions. For the reducing condition, the samples were mixed with Tris buffer and DTT, and heated at 90 °C before testing. For the non-reducing condition, the samples were mixed with Tris buffer and iodoacetamide, and incubated at 70 °C prior to analysis. The size components were resolved in a bare-fused silica capillary containing the SDS gel mixture with 0.2% SDS at pH 8. A UV electropherogram was obtained using a PDA detector.

RESULT(S)

Under reducing condition, both ADC and antibody intermediate exhibited two main peaks corresponding to light chain and heavy chain. Under non-reducing condition, the electrophoretic profile of ADC is consist of a light chain, heavy chain, one light and one heavy chain (1L1H), two heavy chains (2H), two heavy chains and one light chain (2H1L), and two heavy chains and two light chains (2H2L). The electropherogram of the antibody intermediate demonstrated a predominant molecular size corresponding to 2H2L and a minor component corresponding to 2H1L under non-reducing condition. Analytical validation of the CE-SDS method showed assay linearity (r) of ≥ 0.99 for the all the major components observed in both ADC and antibody intermediate under reducing and non-reducing conditions. Assay precision was < 10% RSD for the major components observed in both ADC and its antibody intermediate. For assay accuracy, recovery in the spiked samples ranged from 99 to 104% for the various size components in both ADC and antibody under reducing condition. Accuracy by recovery under non-reducing condition ranged from 82 to 106% for the various size components in the ADC and antibody intermediate. No assay interference was observed from the blank control and formulation buffers.

TABLE 1: PRECISION AND ACCURACY OF 2H2L FOR ANTIBODY DRUG CONJUGATE

	Conc (mg/mL)	MT	Area	% Area	% Recovery
Rep1	0.90	29.43	33696	19.9	108
Rep2		29.47	31697	19.7	107
Rep3		29.52	32740	18.3	99
Avg.		29.47	32691	19.3	105
SD		0.04	1030	0.9	
%RSD		0	3	5	
Rep1	1.20	29.55	42924	19.2	104
Rep2		29.55	41240	18.4	100
Rep3		29.28	38546	17.5	95
Avg.		29.36	40903	18.4	100
SD		0.16	2208	0.8	
%RSD		1	5	5	
Rep1	1.50	29.31	58372	20.6	112
Rep2		29.35	55391	18.5	100
Rep3		29.43	57477	19.2	104
Avg.		29.36	57080	19.4	105
SD		0.06	1530	1.1	
%RSD		0	3	6	

CONCLUSION

The molecular size components and electrophoretic profiles of the ADC and antibody intermediate were determined using CE-SDS. The current CE-SDS method was deemed validated for use in release and stability testing of ADC and its corresponding antibody intermediate.