

# Supporting Information

## A Purification Strategy Utilizing Hydrophobic Interaction Chromatography to Obtain Homogeneous Species from a Site-Specific Antibody Drug Conjugate Produced by AJICAP™ First Generation

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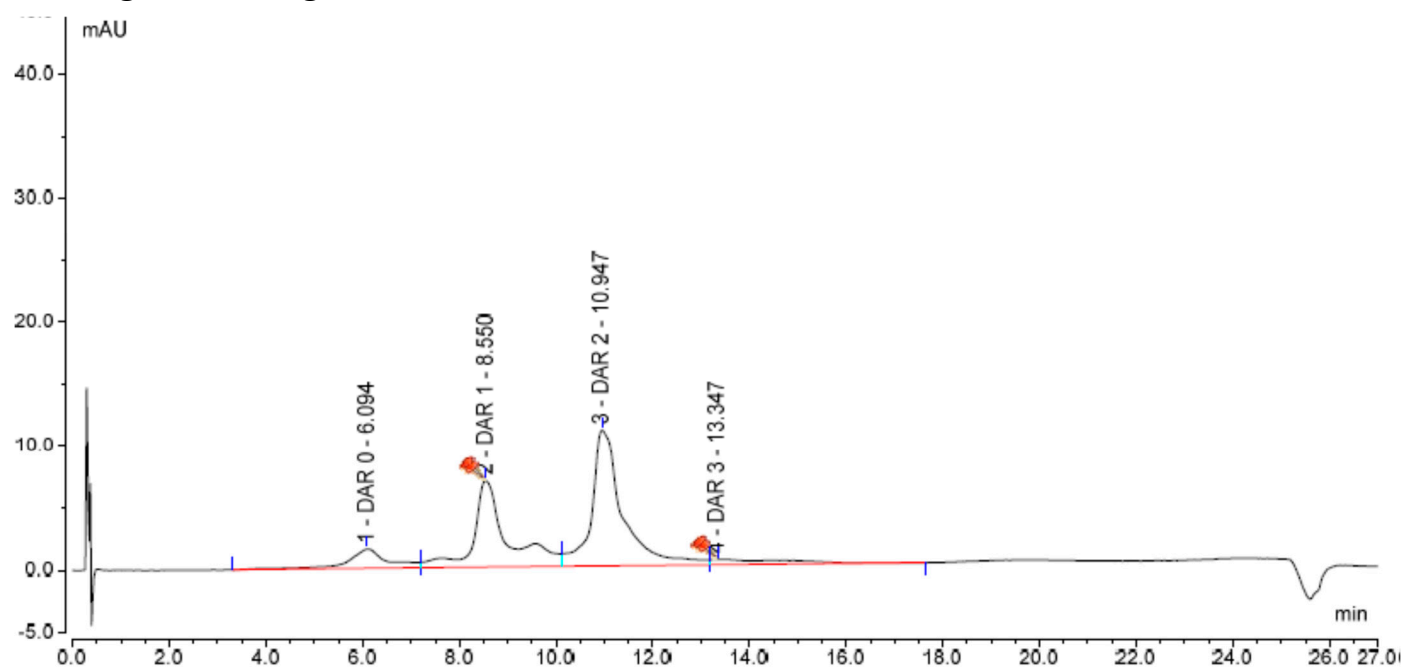
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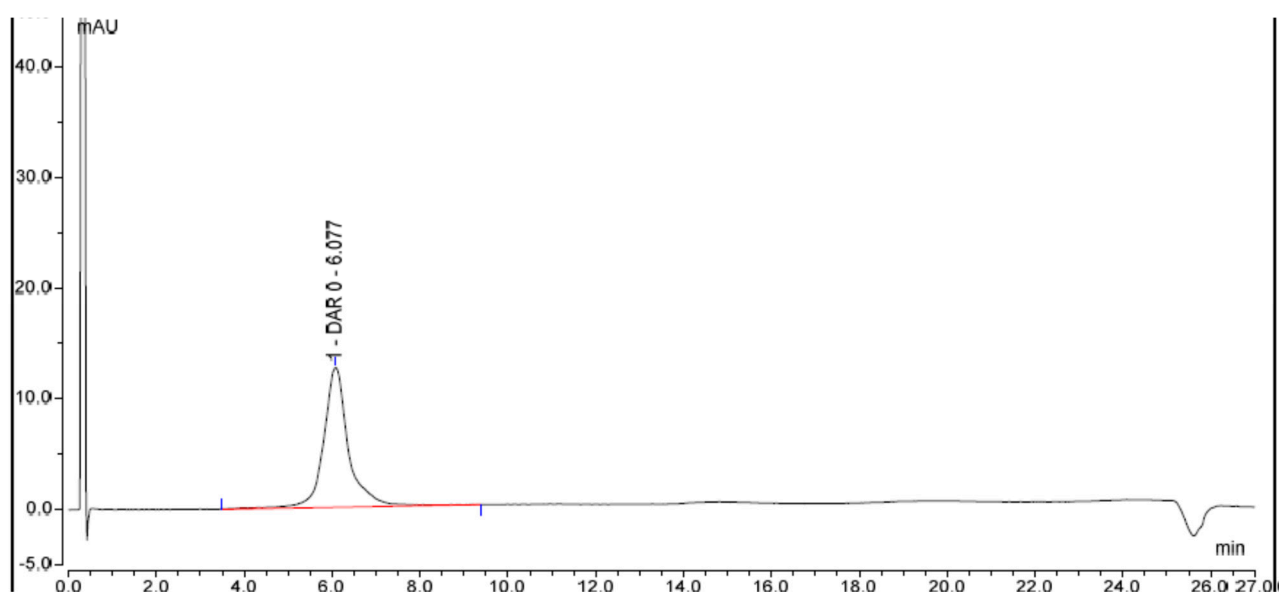
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## Full length chromatograms HIC-HPLC



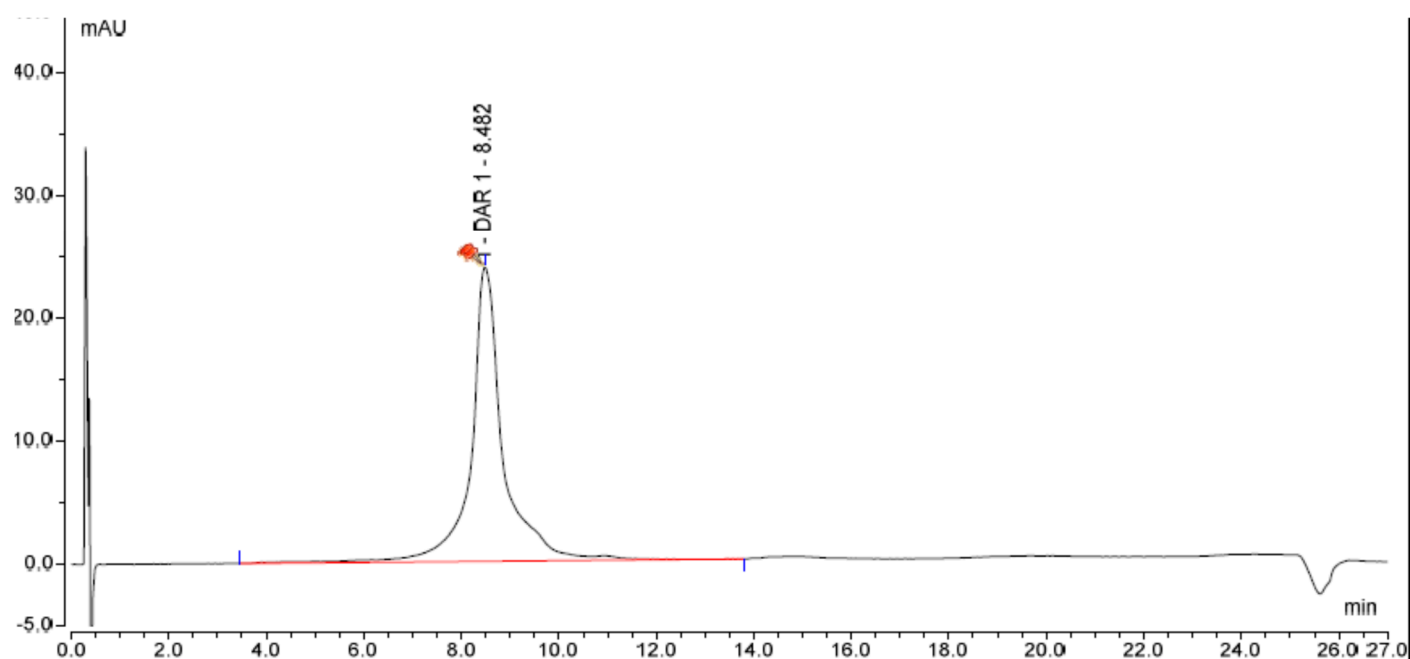
No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	DAR = 0	6.09	0	8.28	0
2	DAR = 1	8.55	1	34.01	34.01
3	DAR = 2	10.95	2	51.03	102.06
4	DAR = 3	13.35	3	6.68	20.04
Average DAR					1.6

Figure S1. Full length results of unpurified trastuzumab-AJICAP™-MMAE (4)



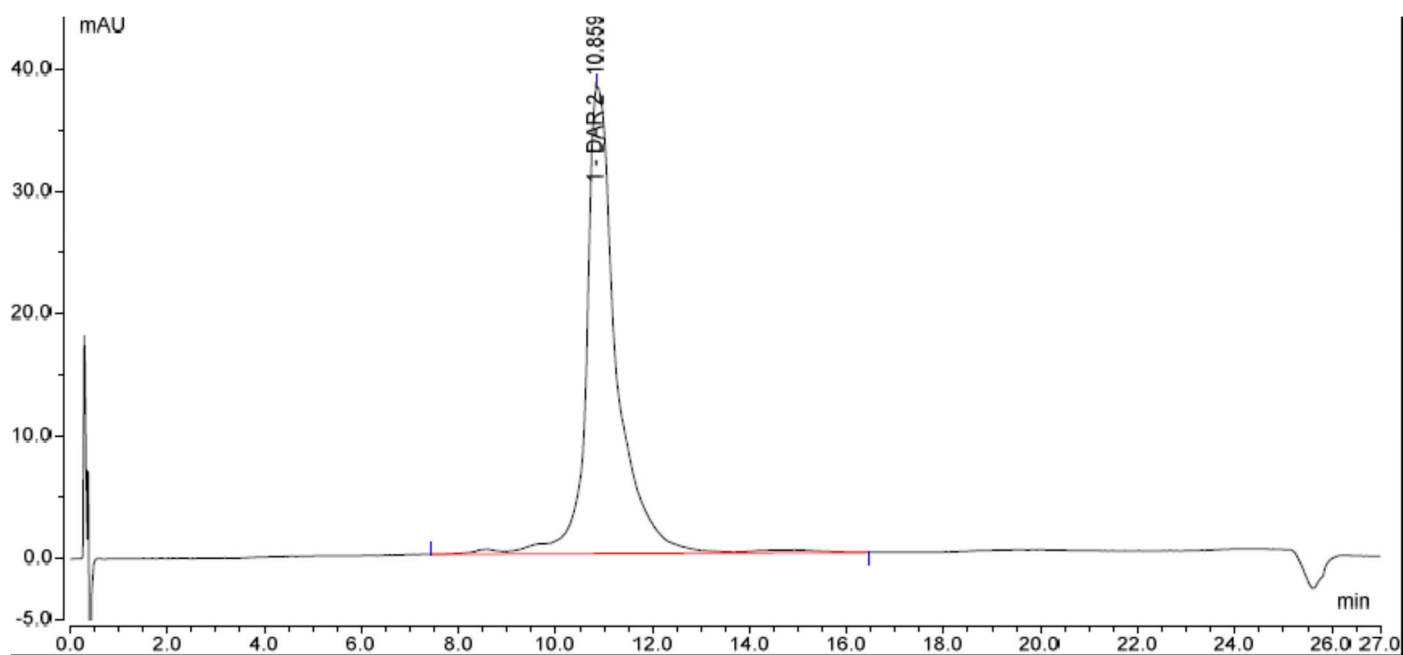
No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	DAR = 0	6.08	0	100	0
Average DAR					0.0

Figure S2. Full length results of unpurified trastuzumab-AJICAP™-MMAE (**DAR = 0**)



No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	DAR = 1	8.48	1	100	1
Average DAR					1.0

Figure S3. Full length results of unpurified trastuzumab-AJICAP™-MMAE (**DAR = 1**)



No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	DAR = 2	10.86	2	100	2
Average DAR					2.0

Figure S4. Full length results of unpurified trastuzumab-AJICAP™-MMAE (**DAR = 2**)

DAR calculation results from Q-TOF MS

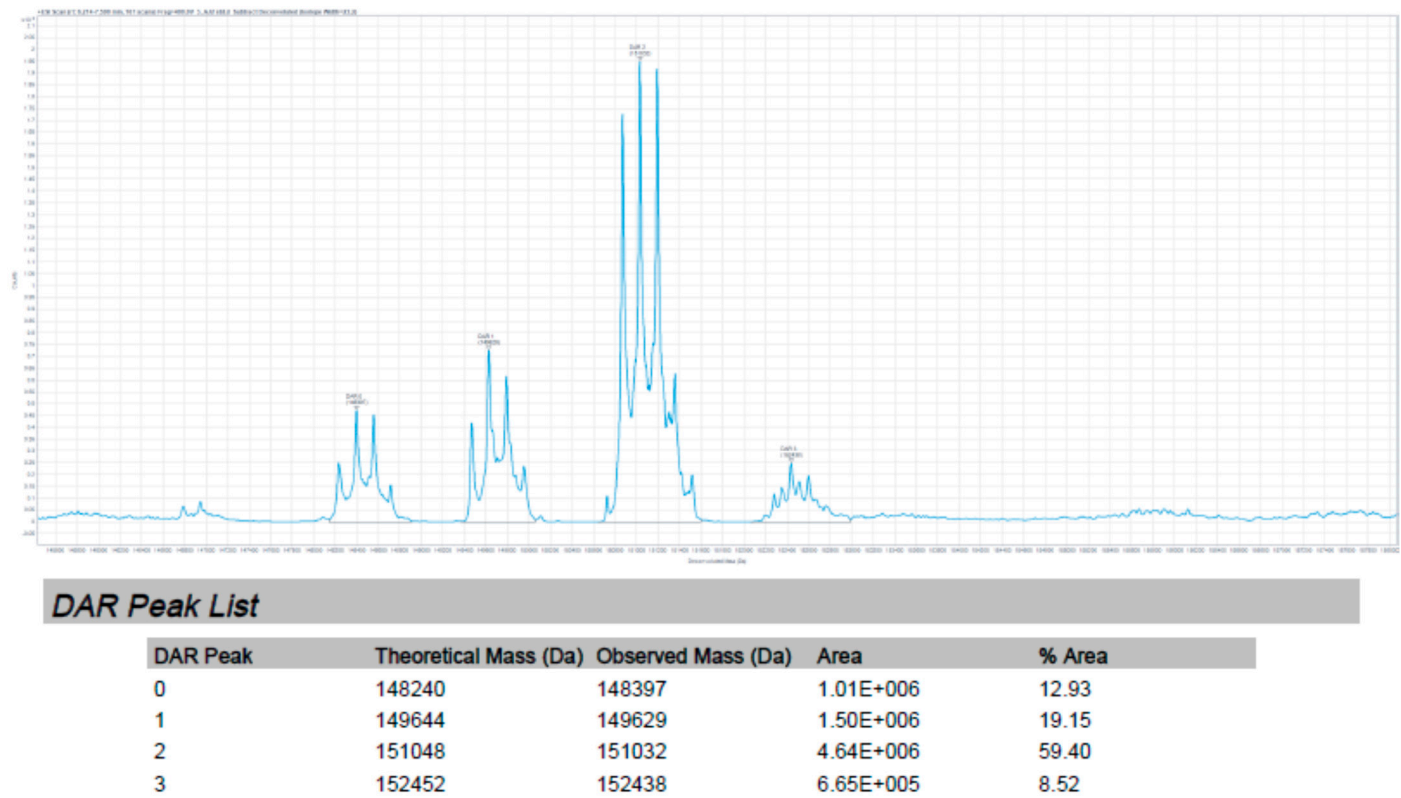


Figure S5 Deconvoluted spectra of unpurified trastuzumab-AJICAP™-MMAE (4)

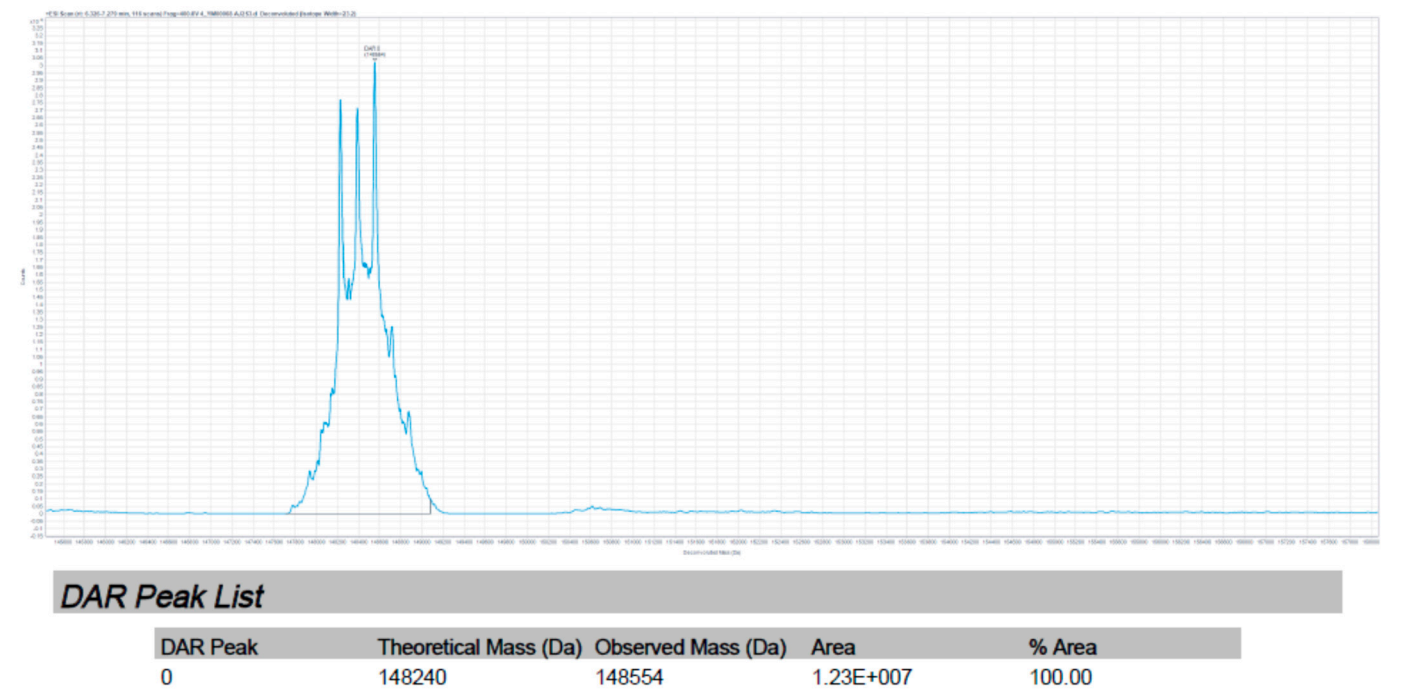
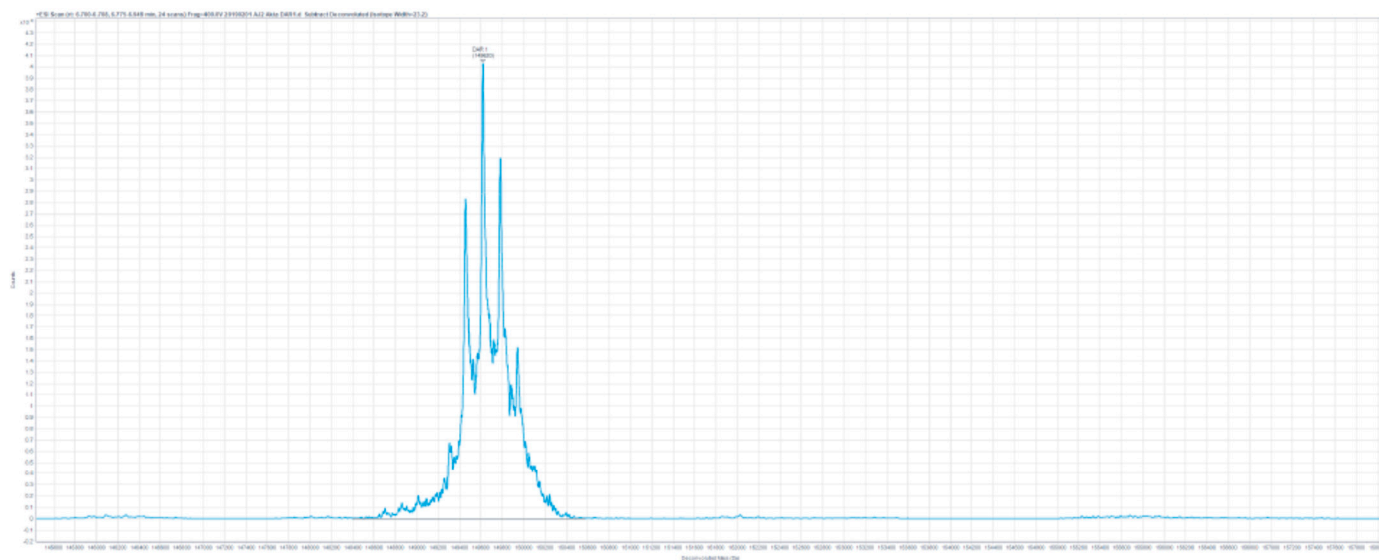


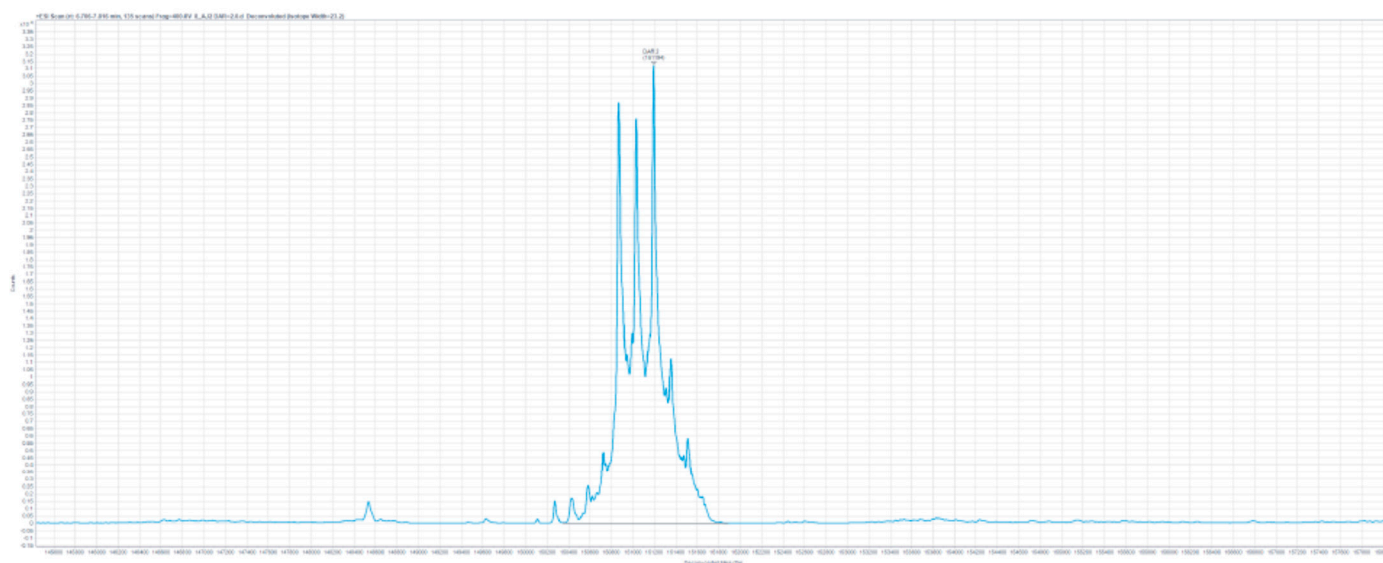
Figure S6 Deconvoluted spectra of trastuzumab-AJICAP™-MMAE (DAR = 0)



### DAR Peak List

DAR Peak	Theoretical Mass (Da)	Observed Mass (Da)	Area	% Area
1	149644	149620	1.22E+007	100.00

Figure S7 Deconvoluted spectra of trastuzumab-AJICAP™-MMAE (**DAR = 1**)

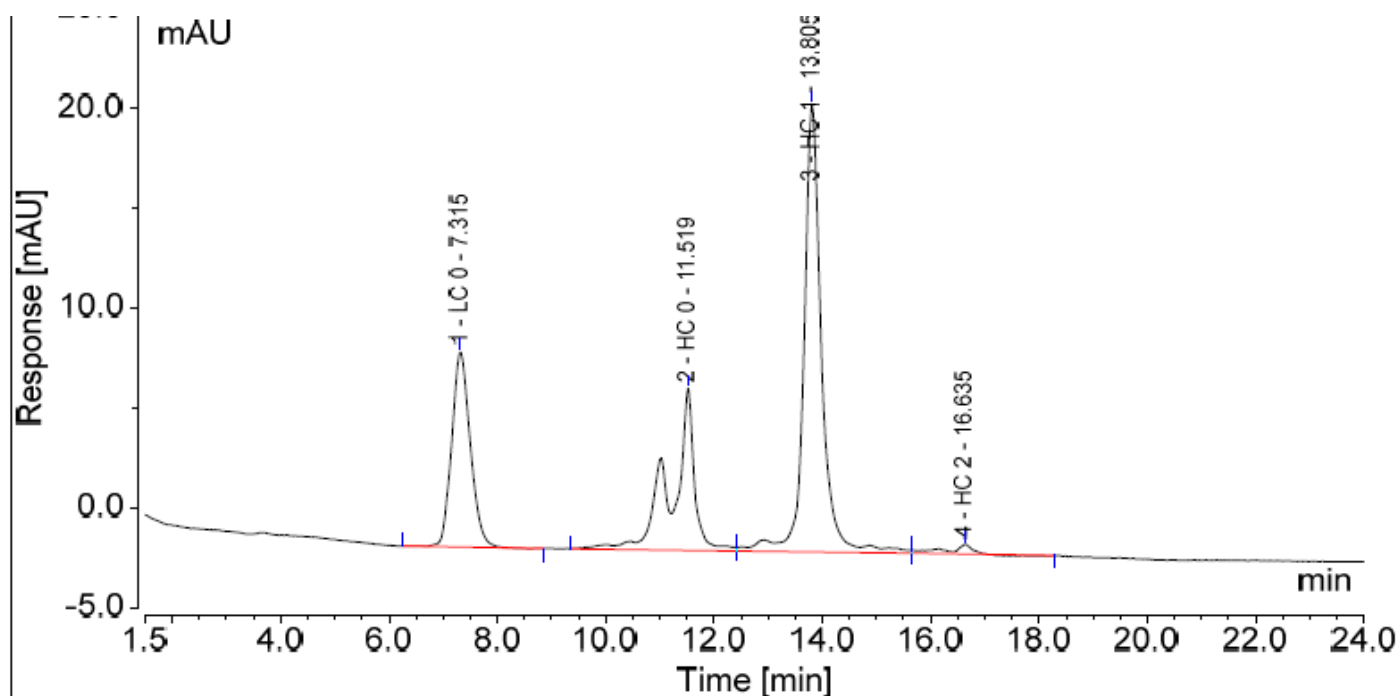


### DAR Peak List

DAR Peak	Theoretical Mass (Da)	Observed Mass (Da)	Area	% Area
2	151048	151194	9.99E+006	100.00

Figure S8 Deconvoluted spectra of trastuzumab-AJICAP™-MMAE (**DAR = 2**)

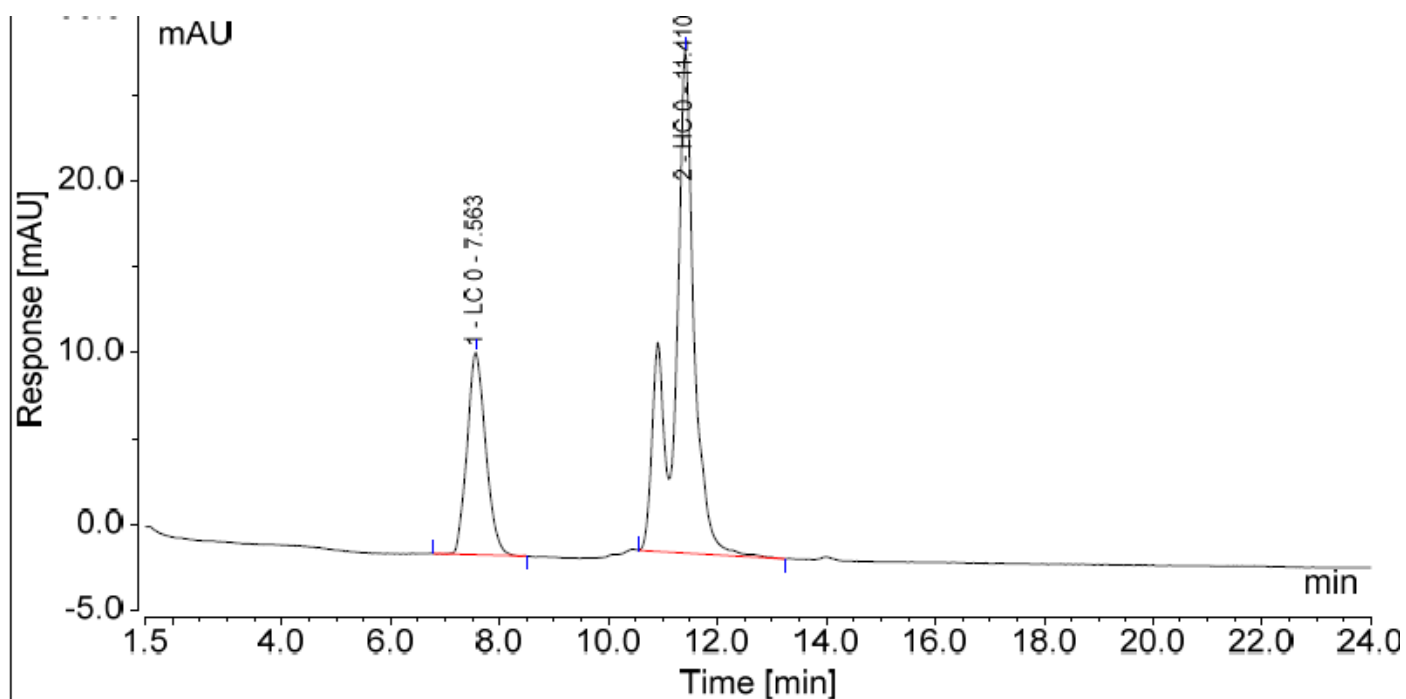
## Full length chromatograms RP-HPLC



No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	LC 0	7.32	0	100	0
2	HC 0	11.52	0	28.24	0
3	HC 1	13.81	1	70.1	70.1
4	HC 2	16.64	2	2.66	5.32
				DAR	1.5

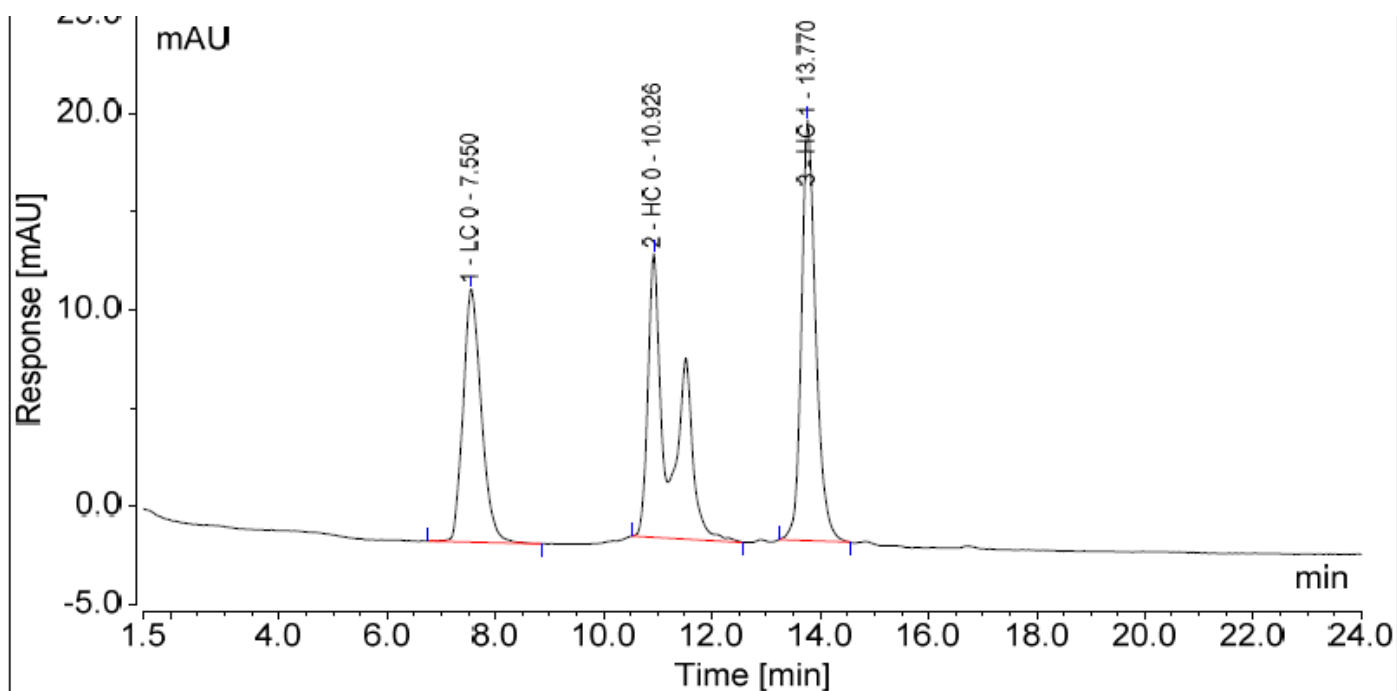
Figure S9 Full length results of unpurified trastuzumab-AJICAP™-MMAE (4); LC 0: light chain, HC 0: heavy chain, HC 1: heavy chain conjugated with one MMAE, HC 2: heavy chain conjugated with two MMAEs





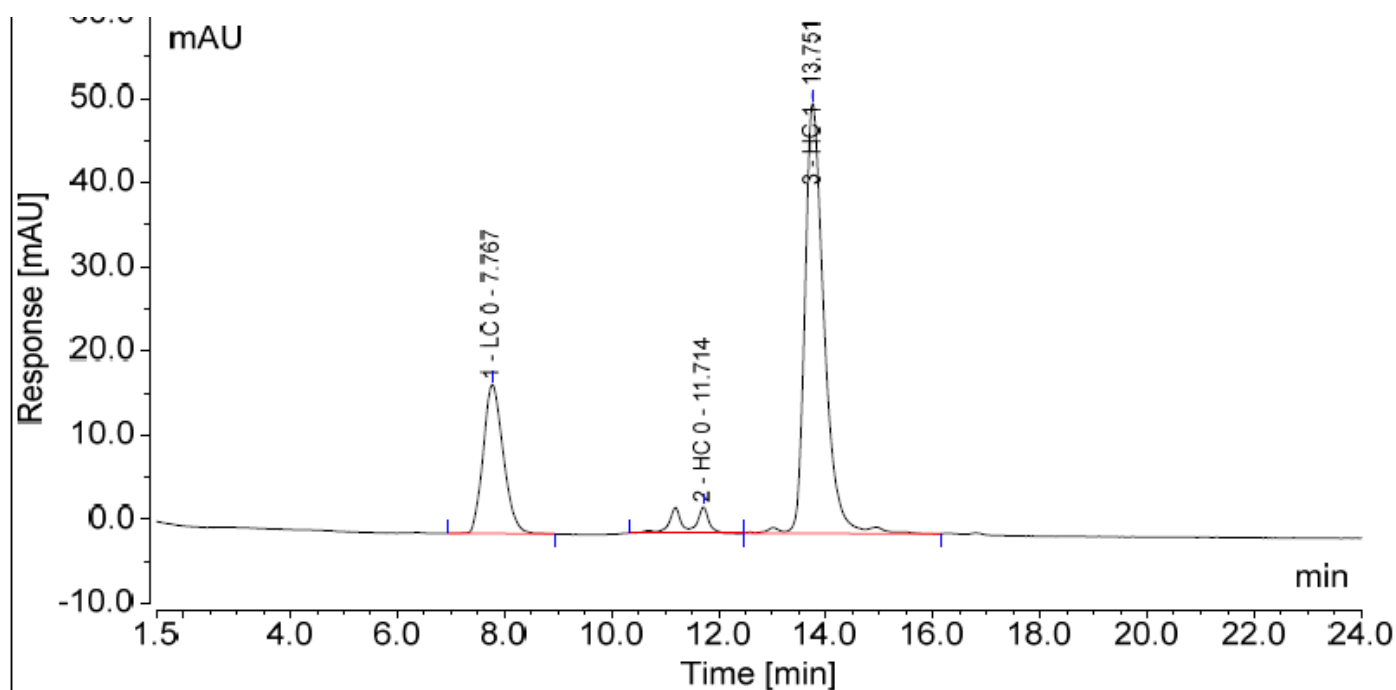
No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	LC 0	7.57	0	100	0
2	HC 0	11.41	0	100	0
DAR					0.0

Figure S10 Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 0**), LC 0: light chain, HC 0: heavy chain



No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	LC 0	7.55	0	100	0
2	HC 0	10.93	0	51.14	0
3	HC 1	13.77	1	48.86	48.86
DAR					0.98

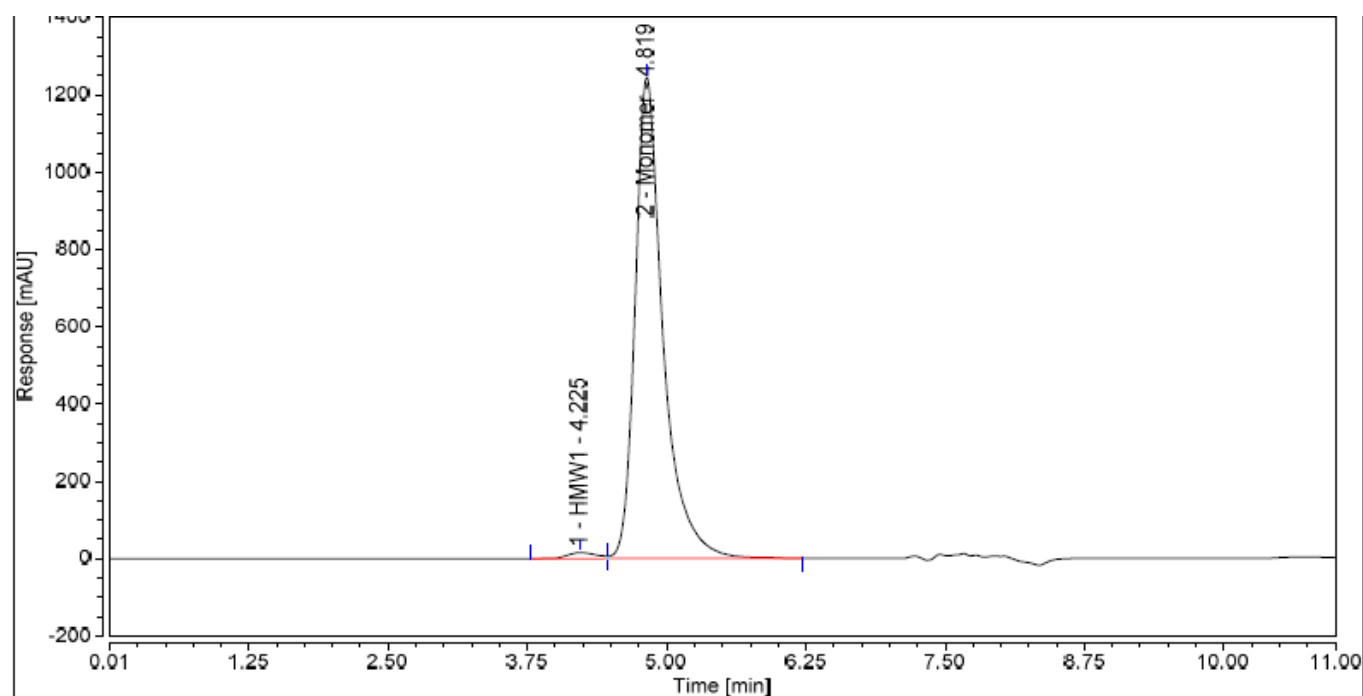
Figure S11 Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 1**) LC 0: light chain, HC 0: heavy chain, HC 1: heavy chain conjugated with one MMAE



No	Peak time	retention time	Drug load	Relative area %	Weighted peak area
1	LC 0	7.76	0	100	0
2	HC 0	11.71	0	6.79	0
3	HC 1	13.75	1	93.21	93.21
DAR					1.9

Figure S12 Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 2**); LC 0: light chain, HC 0: heavy chain, HC 1: heavy chain conjugated with one MMAE

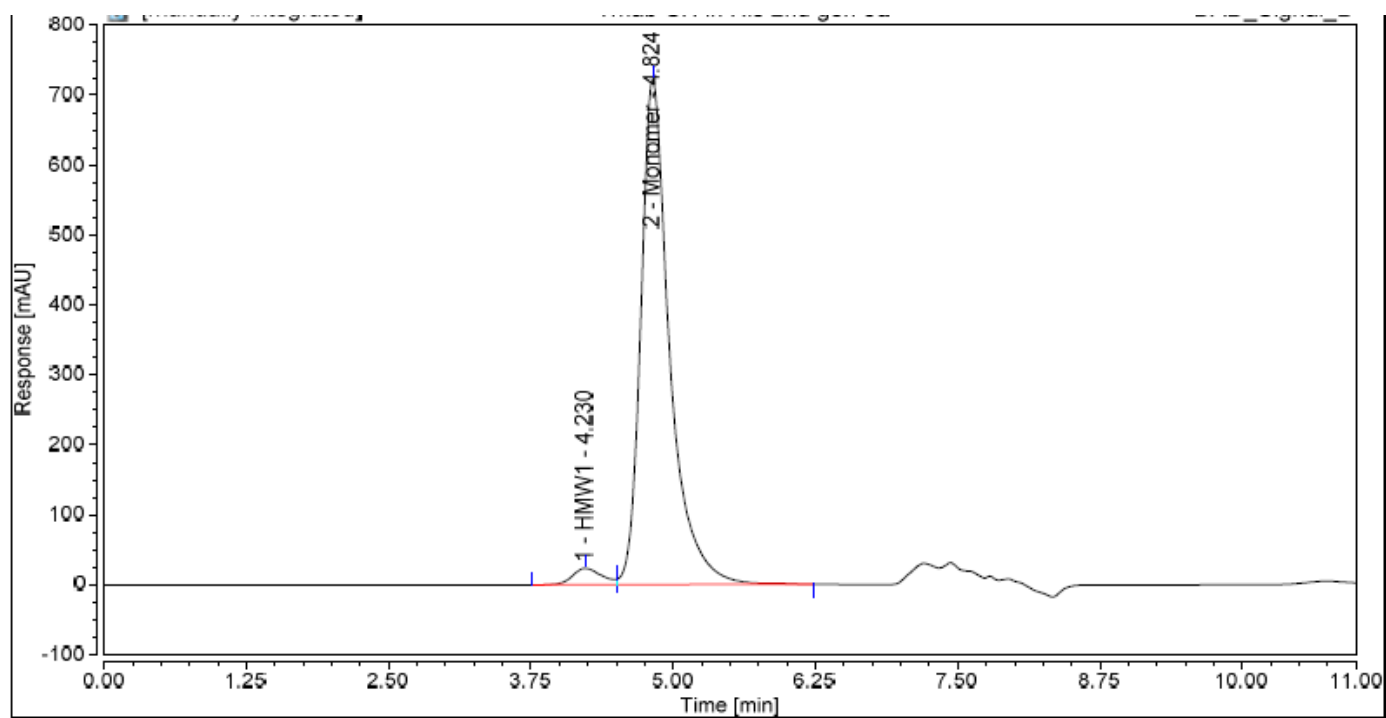
## Full length chromatograms SEC-HPLC



No	Peak time	retention time	Relative area %
1	HWE	4.22	1.79
2	Monomer	4.82	98.2

No	Peak time	retention time	Relative area %
1	HWE	4.22	1.79
2	Monomer	4.82	98.2

Figure S13. Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 1**)



No	Peak time	retention time	Relative area %
1	HWE	4.22	3.40
2	Monomer	4.82	96.6

Figure S14. Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 2**)