

# 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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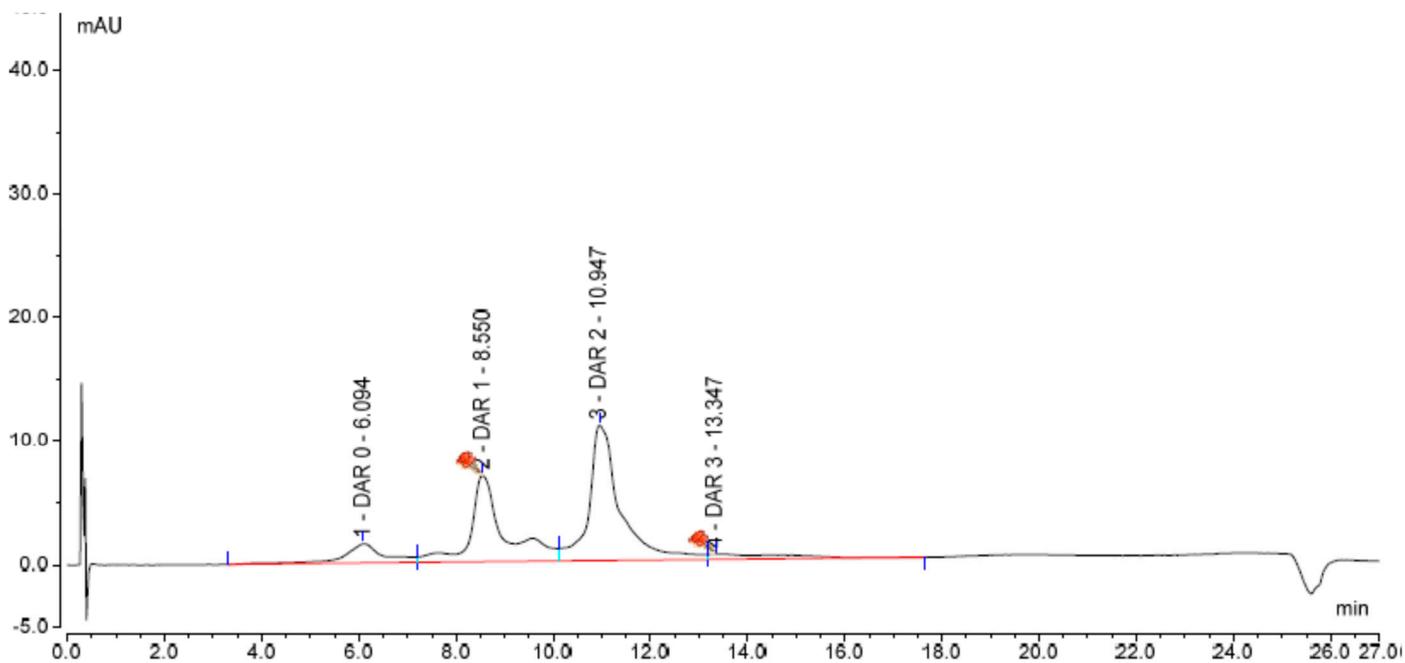
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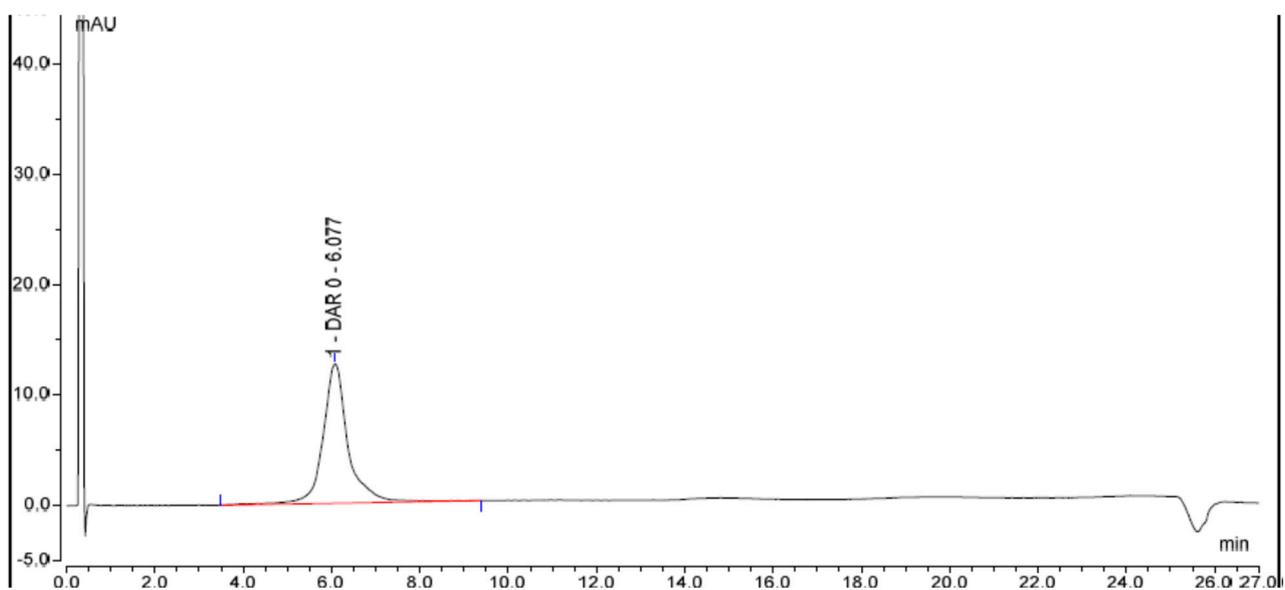
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### Full length chromatograms HIC-HPLC



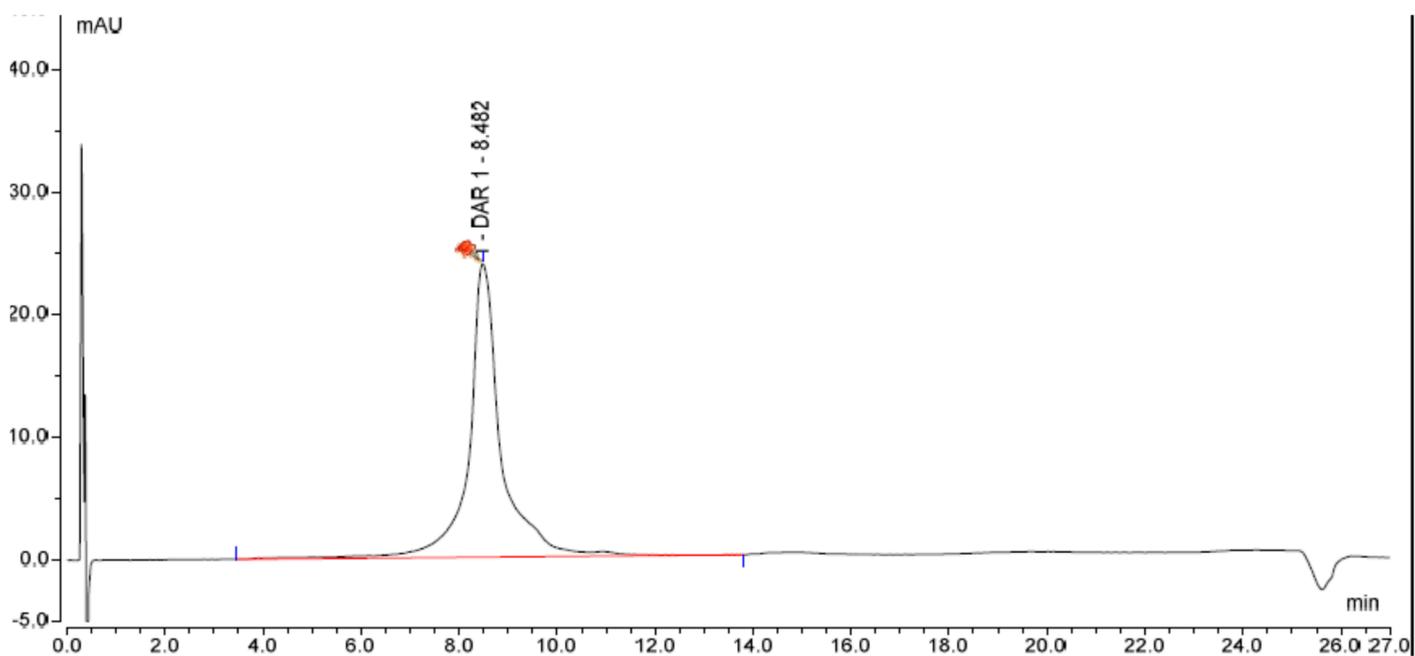
| No                 | Peak time | retention time | Drug load | Relative area % | Weighted peak area |
|--------------------|-----------|----------------|-----------|-----------------|--------------------|
| 1                  | DAR = 0   | 6.09           | 0         | 8.28            | 0                  |
| 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              |
| <b>Average DAR</b> |           |                |           |                 | <b>1.6</b>         |

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



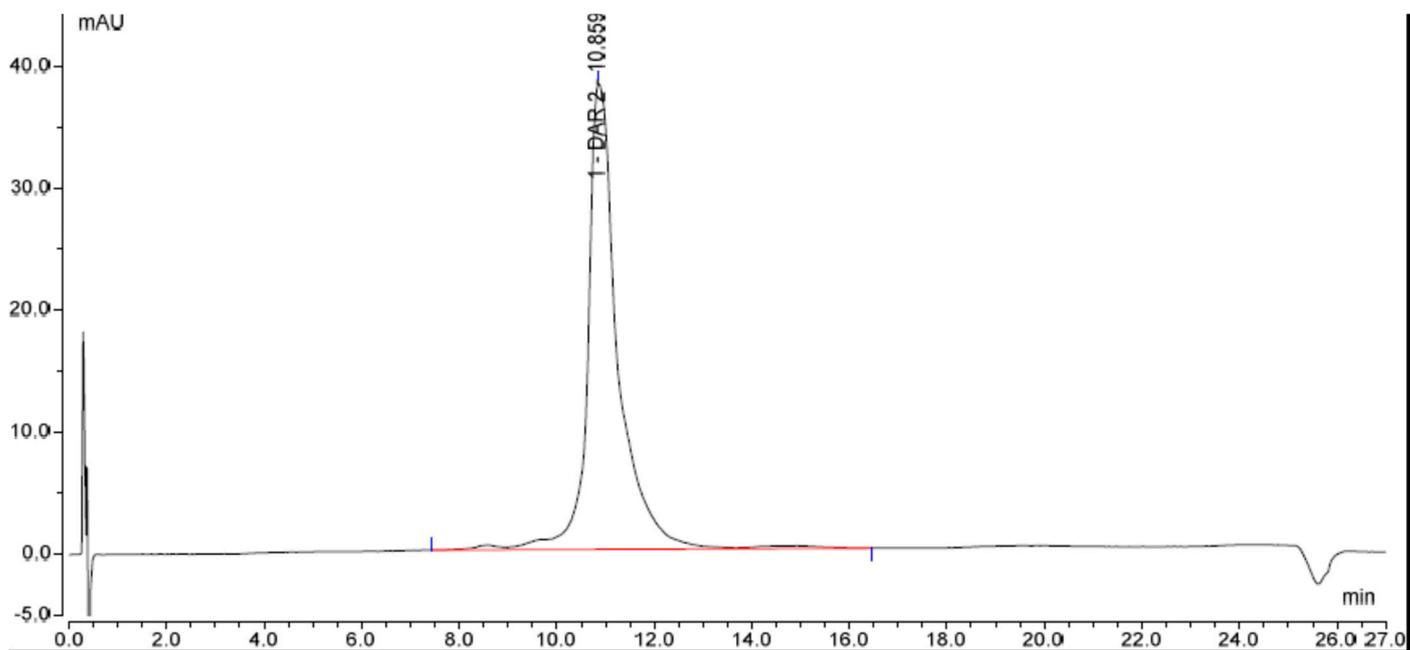
| No                 | Peak time | retention time | Drug load | Relative area % | Weighted peak area |
|--------------------|-----------|----------------|-----------|-----------------|--------------------|
| 1                  | DAR = 0   | 6.08           | 0         | 100             | 0                  |
| <b>Average DAR</b> |           |                |           |                 | <b>0.0</b>         |

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                  |
| <b>Average DAR</b> |           |                |           |                 | <b>1.0</b>         |

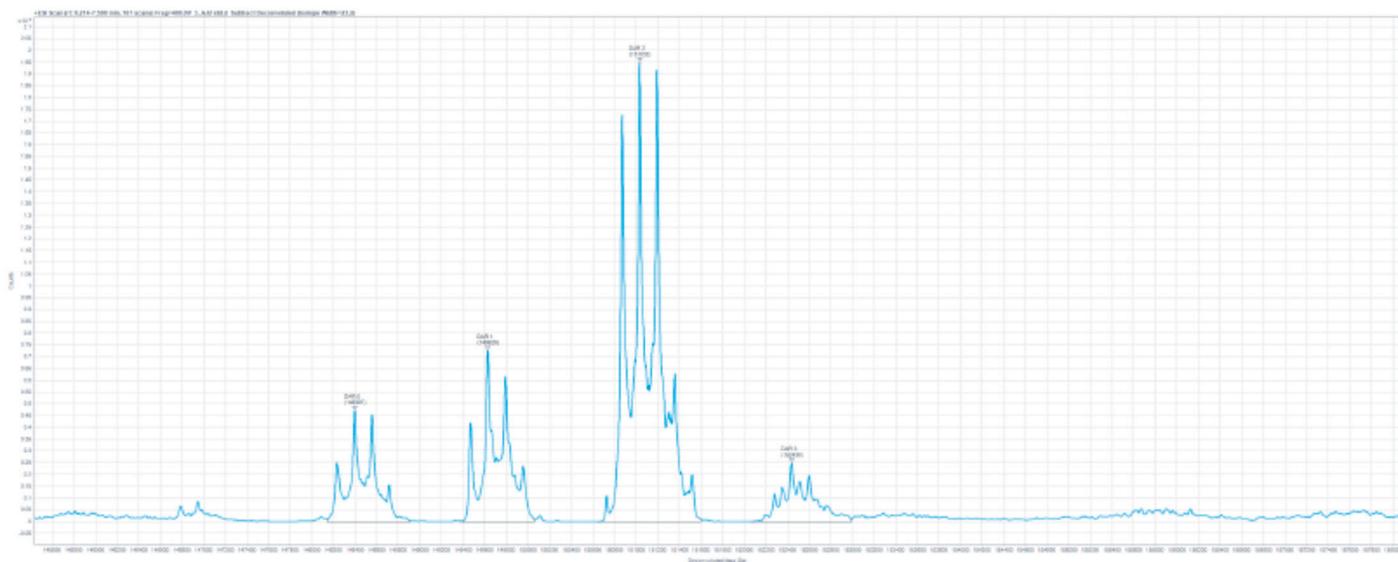
Figure S3. Full length results of unpurified trastuzumab-AJICAP™-MMAE (**DAR = 1**)



| No                 | Peak time | retention time | Drug load | Relative area % | Weighted peak area |
|--------------------|-----------|----------------|-----------|-----------------|--------------------|
| 1                  | DAR = 2   | 10.86          | 2         | 100             | 2                  |
| <b>Average DAR</b> |           |                |           |                 | <b>2.0</b>         |

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

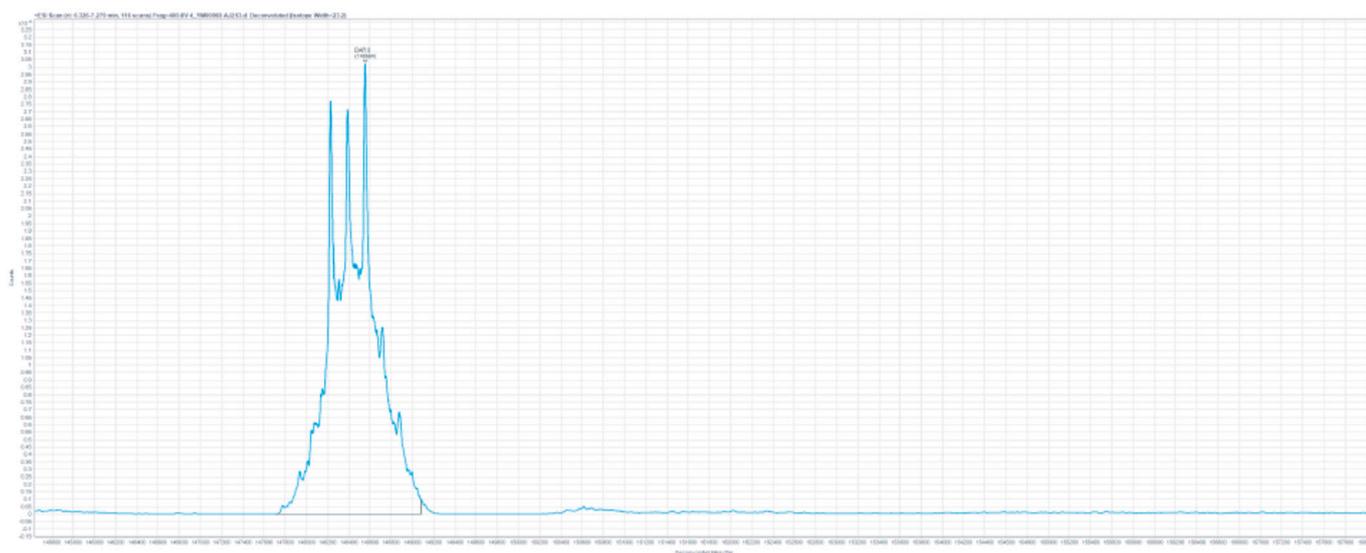
## DAR calculation results from Q-TOF MS



### DAR Peak List

| DAR Peak | Theoretical Mass (Da) | Observed Mass (Da) | Area      | % Area |
|----------|-----------------------|--------------------|-----------|--------|
| 0        | 148240                | 148397             | 1.01E+006 | 12.93  |
| 1        | 149644                | 149629             | 1.50E+006 | 19.15  |
| 2        | 151048                | 151032             | 4.64E+006 | 59.40  |
| 3        | 152452                | 152438             | 6.65E+005 | 8.52   |

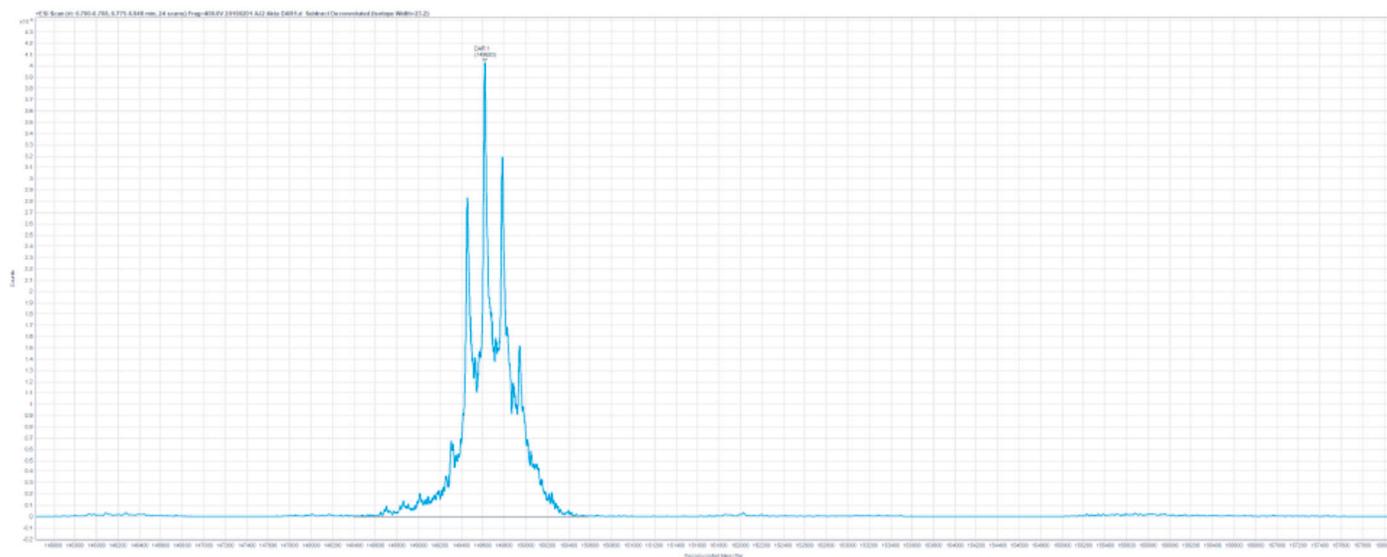
Figure S5 Deconvoluted spectra of unpurified trastuzumab-AJICAP™-MMAE (4)



### DAR Peak List

| DAR Peak | Theoretical Mass (Da) | Observed Mass (Da) | Area      | % Area |
|----------|-----------------------|--------------------|-----------|--------|
| 0        | 148240                | 148554             | 1.23E+007 | 100.00 |

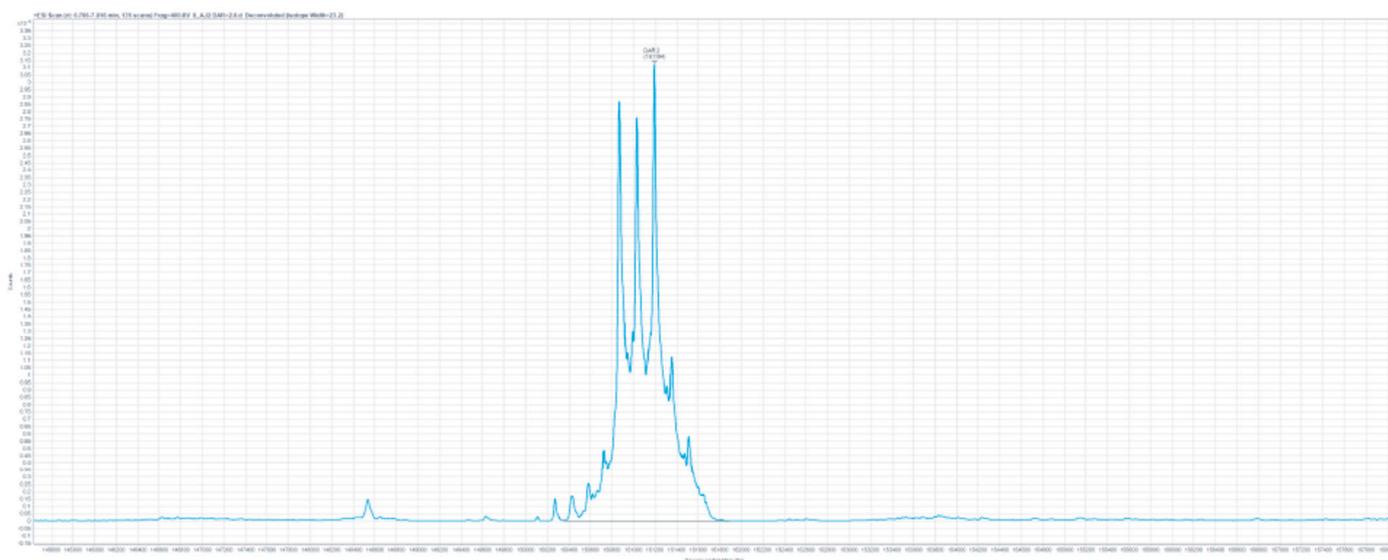
Figure S6 Deconvoluted spectra of trastuzumab-AJICAP™-MMAE (DAR = 0)



**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)

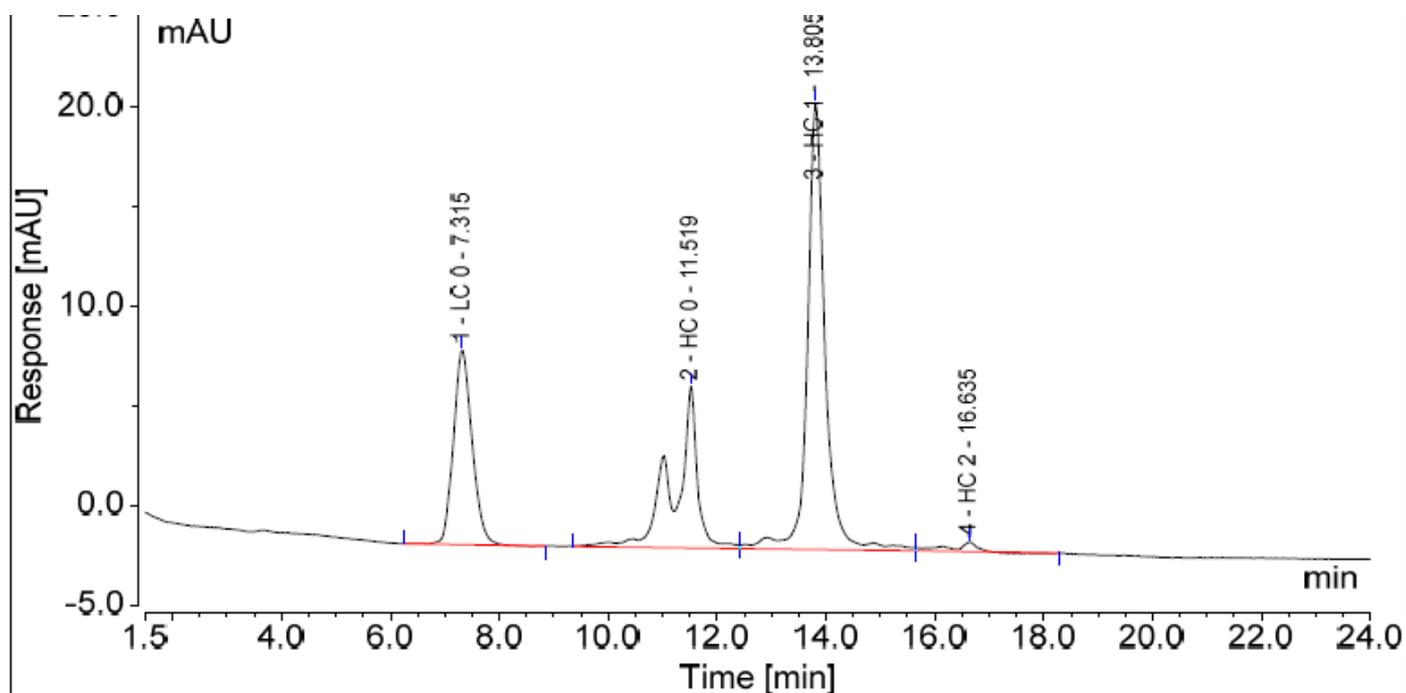


**DAR Peak List**

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

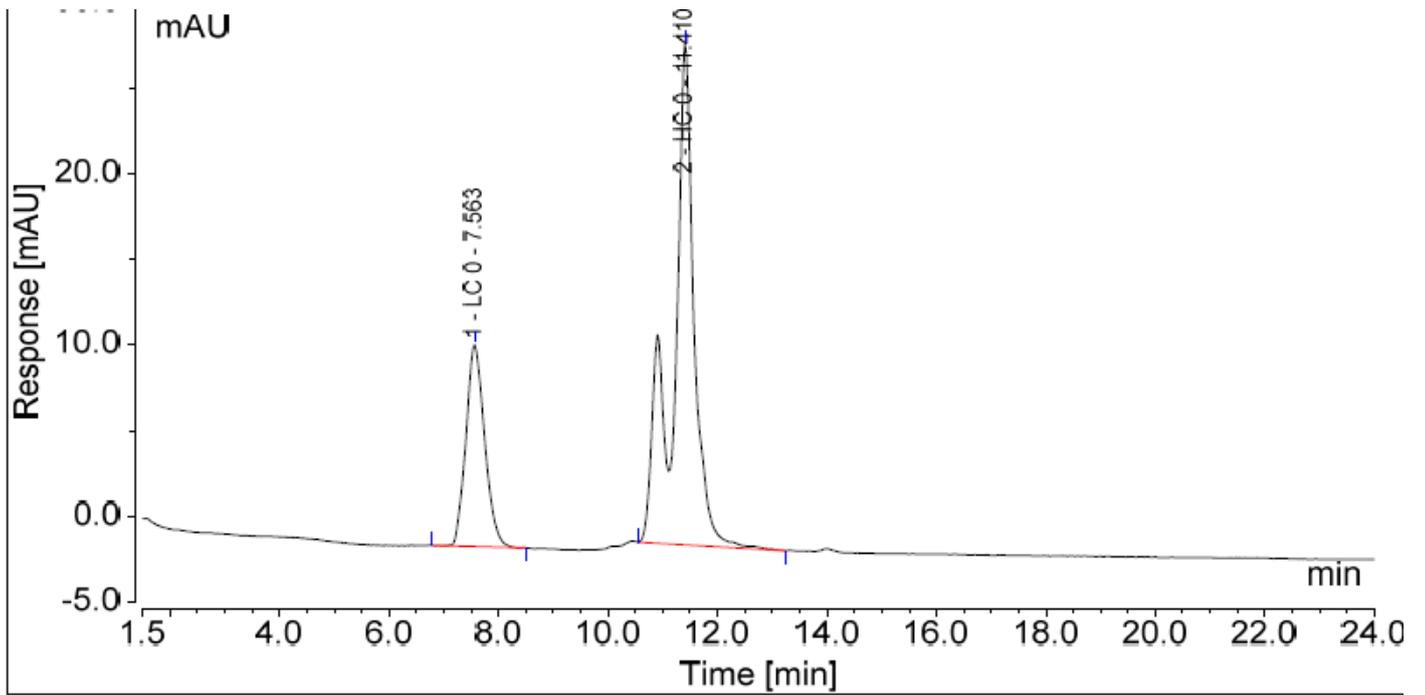
Figure S8 Deconvoluted spectra of trastuzumab-AJICAP™-MMAE (DAR = 2)

## Full length chromatograms RP-HPLC



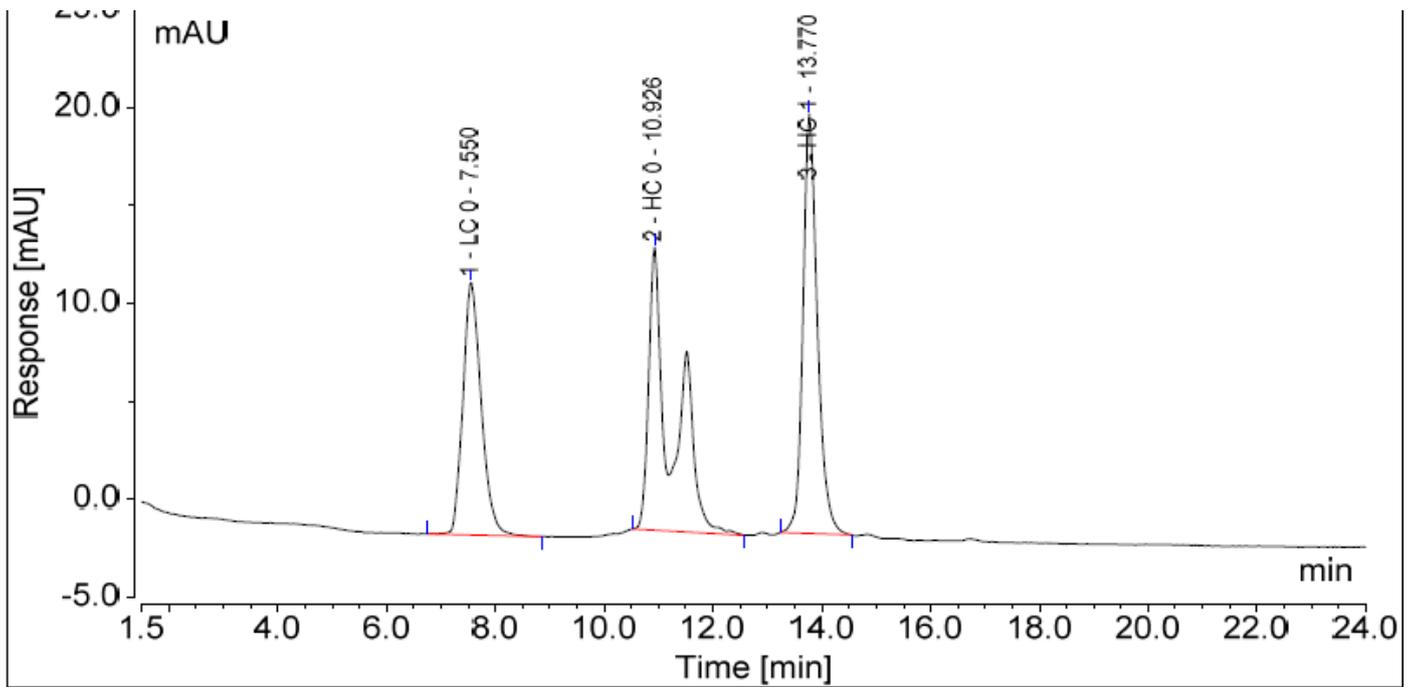
| No | Peak time | retention time | Drug load | Relative area % | Weighted peak area |
|----|-----------|----------------|-----------|-----------------|--------------------|
| 1  | LC 0      | 7.32           | 0         | 100             | 0                  |
| 2  | HC 0      | 11.52          | 0  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

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



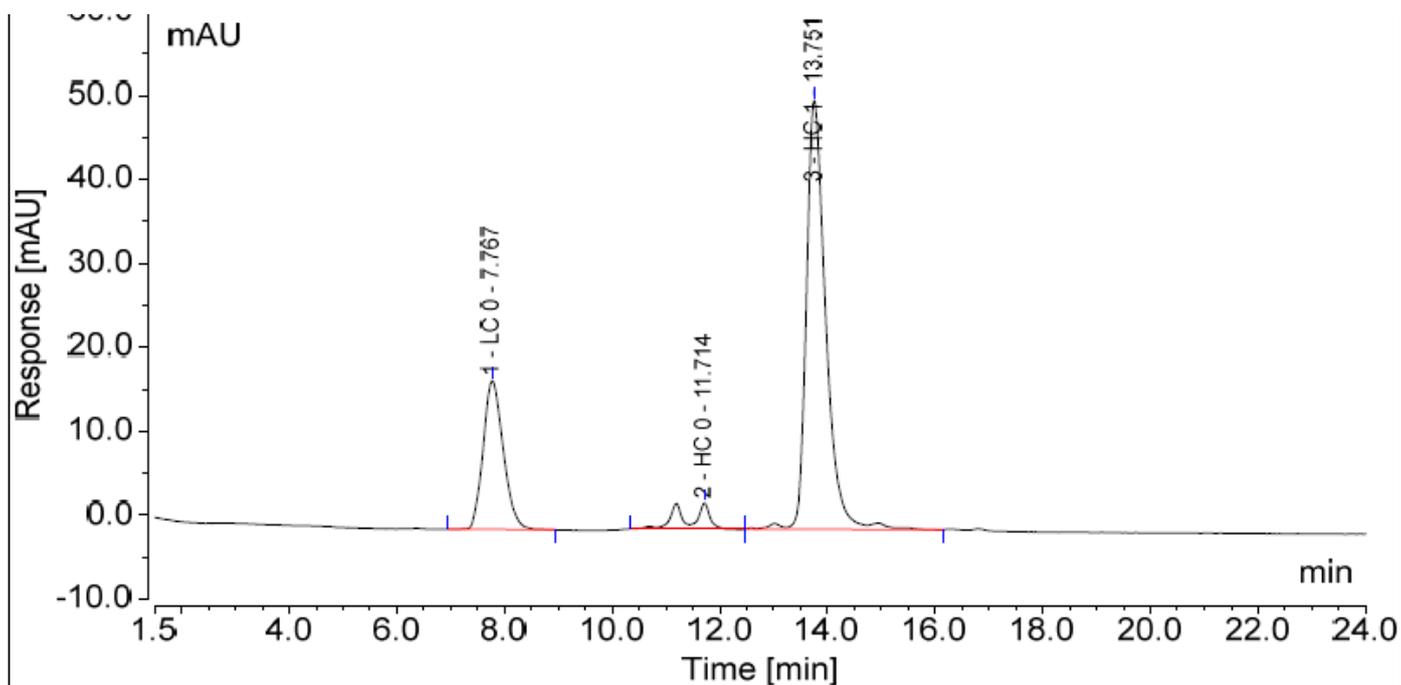
| No | Peak time | retention time | Drug load | Relative area % | Weighted peak area |
|----|-----------|----------------|-----------|-----------------|--------------------|
| 1  | LC 0      | 7.57           | 0         | 100             | 0                  |
| 2  | HC 0      | 11.41          | 0         | 100             | 0                  |
|    |           |                |           | DAR             | <b>0.0</b>         |

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 |           |                |           |                 | <b>0.98</b>        |

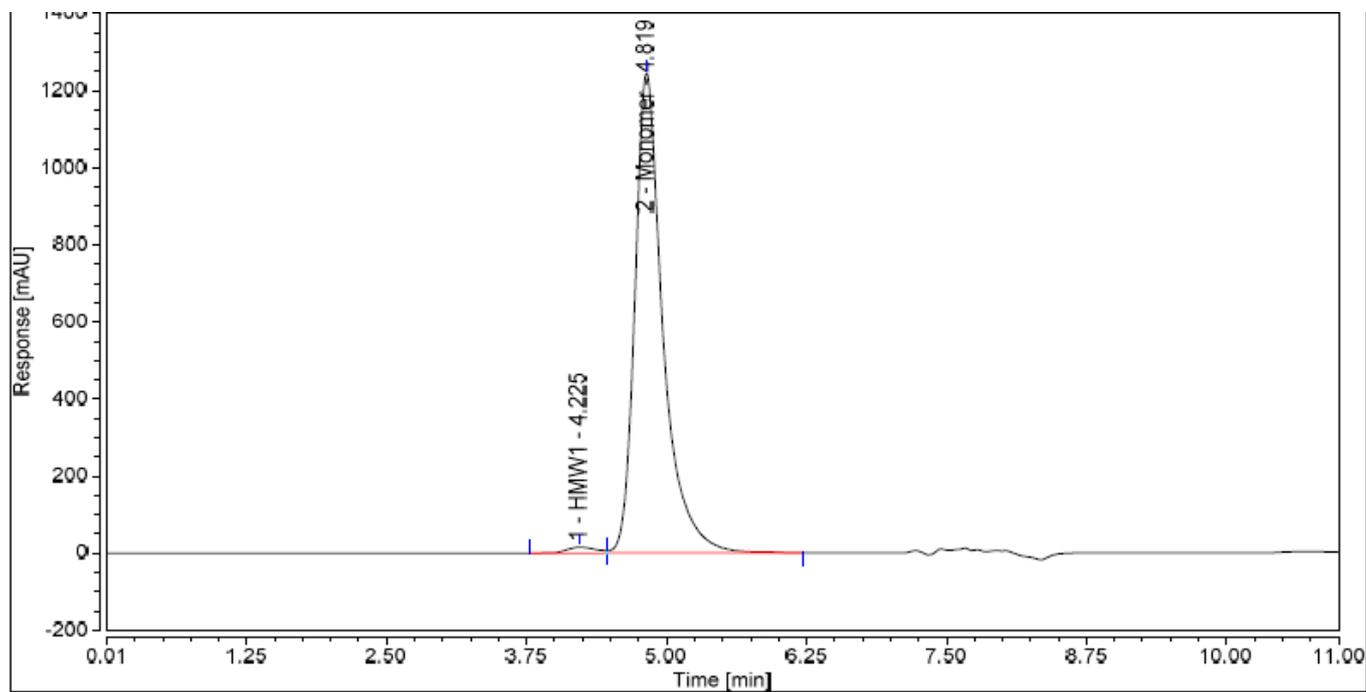
Figure S11 Full length results of trastuzumab-AJICAP™-MMAE (**DAR = 1**) LC 0: light chain, HC 0: heavy chain, HC 1: heavy chain conjugated with one MMAE



| 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

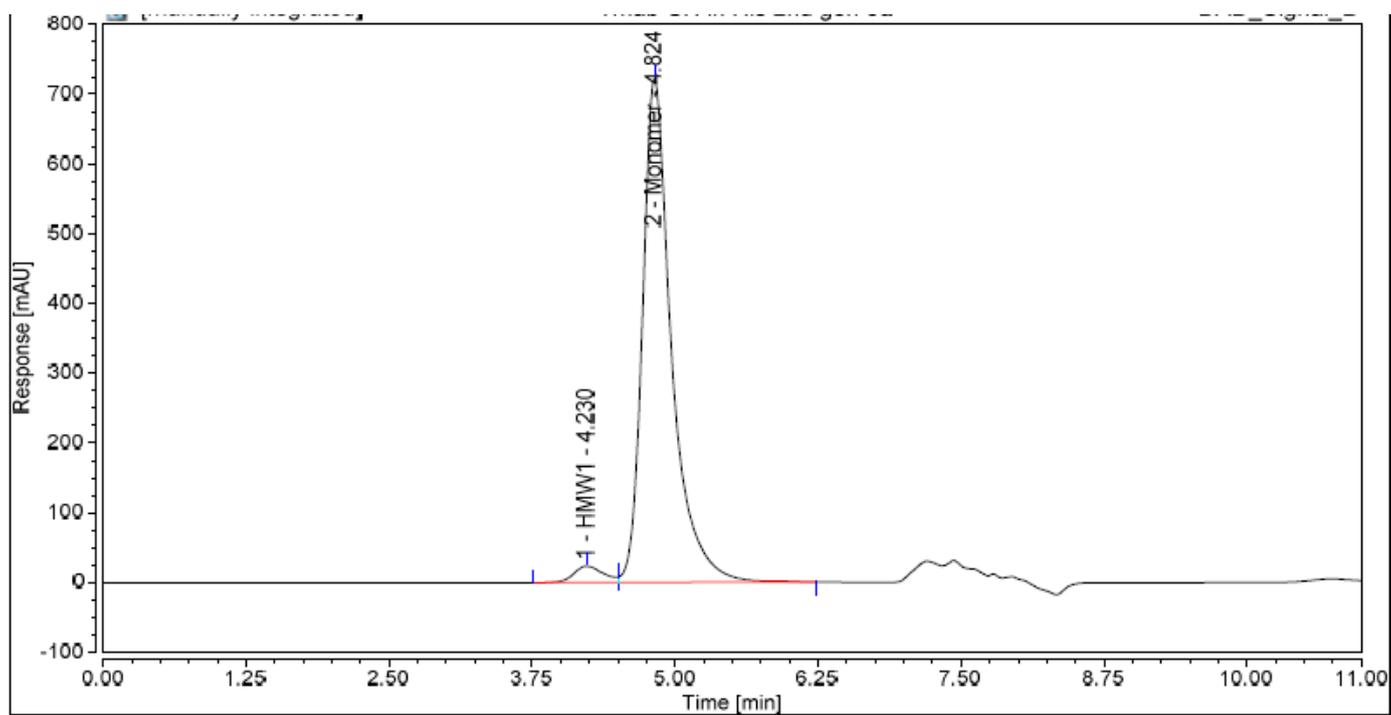
### Full length chromatograms SEC-HPLC



| No | Peak time | retention time | Relative area % |
|----|-----------|----------------|-----------------|
| 1  | HWE       | 4.22           | 1.79            |
| 2  | Monomer   | 4.82           | <b>98.2</b>     |

| No | Peak time | retention time | Relative area % |
|----|-----------|----------------|-----------------|
| 1  | HWE       | 4.22           | 1.79            |
| 2  | Monomer   | 4.82           | <b>98.2</b>     |

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           | <b>96.6</b>     |

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