

Synthesis and Preclinical Evaluation of Bifunctional Ligands for Improved Chelation Chemistry of ^{90}Y and ^{177}Lu for Targeted Radioimmunotherapy

Chi Soo Kang,¹ Xiang Sun,¹ Fang Jia,² Hyun A Song,¹ Yunwei Chen,¹
Michael Lewis,² Hyun-Soon Chong^{1,*}

Supporting Information

A. TLC and HPLC chromatograms for radiolabeling kinetics studies

1. Radiolabeling of ligand-trastuzumab conjugate with ^{90}Y

- i) 3p-C-NETA-trastuzumab (HPLC method 1), pages 2-4
- ii) 3p-C-DEPA-trastuzumab (HPLC method 1), pages 5-7
- iii) C-DTPA-trastuzumab (HPLC method 1), pages 8-10
- iv) C-DOTA-trastuzumab (HPLC method 2), pages 11-13

2. Radiolabeling of ligand-trastuzumab conjugate with ^{177}Lu

- i) 3p-C-NETA-trastuzumab (HPLC method 2), pages 14-16
- ii) 3p-C-DEPA-trastuzumab (HPLC methods 2 and 3), pages 17-19
- iii) C-DTPA-trastuzumab (HPLC method 2), pages 20-22
- iv) C-DOTA-trastuzumab (HPLC method 2), pages 23-25

B. HPLC chromatograms for serum stability studies (37 °C, pH 7, human serum).

1. ^{90}Y -radiolabeled trastuzumab conjugates

- i) ^{90}Y -3p-C-NETA-trastuzumab (HPLC method 2), pages 26-29
- ii) ^{90}Y -3p-C-DEPA-trastuzumab (HPLC methods 1 and 3), page 30
- iii) ^{90}Y -C-DOTA-trastuzumab (HPLC method 2), pages 31-34

2. ^{177}Lu -radiolabeled trastuzumab conjugates

- i) ^{177}Lu -3p-C-NETA-trastuzumab (HPLC method 2), pages 35-39
- ii) ^{177}Lu -3p-C-DEPA-trastuzumab (HPLC method 2), page 40
- iii) ^{177}Lu -C-DOTA-trastuzumab (HPLC methods 2 and 3), pages 41-44

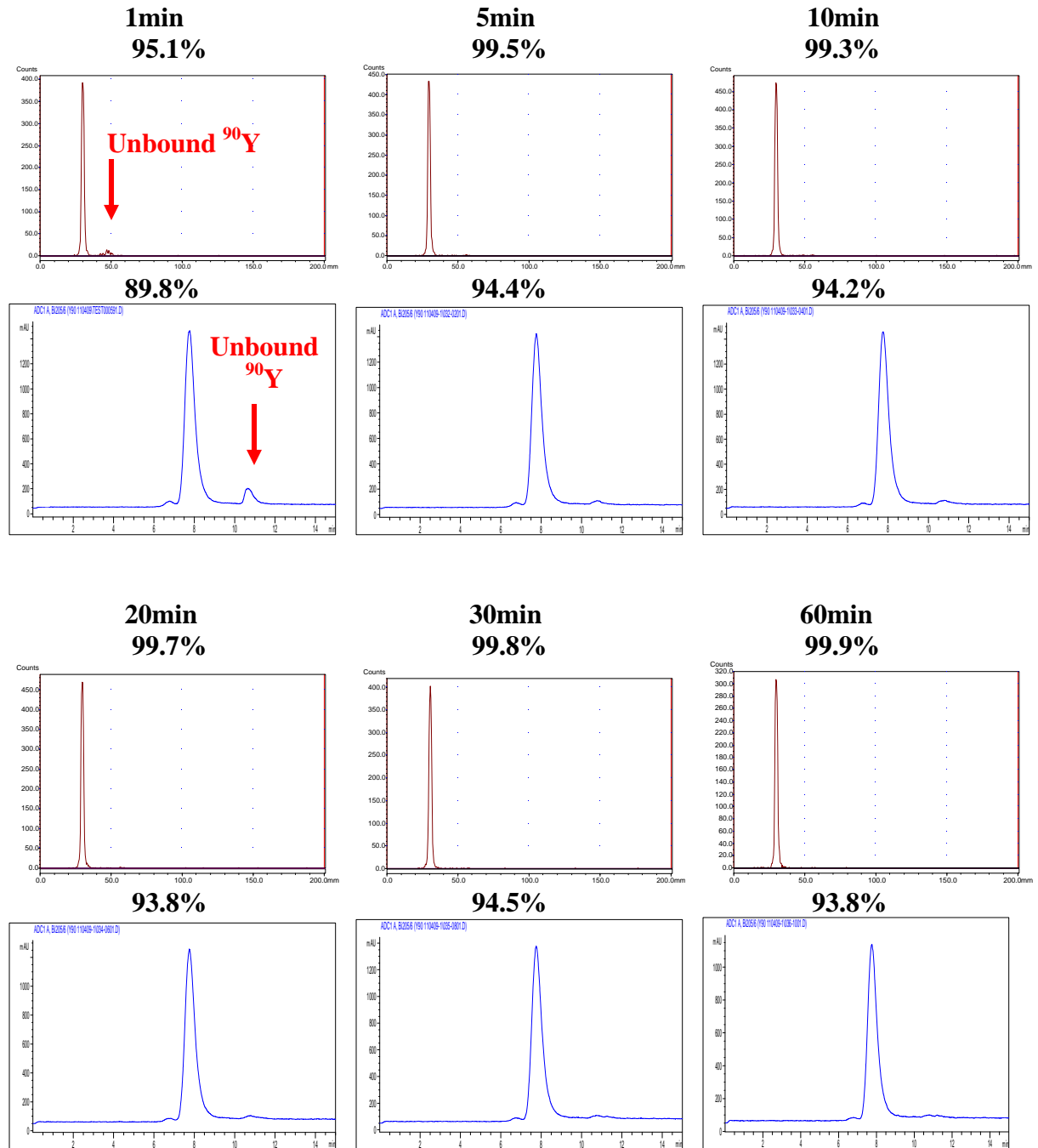
C. *In vivo* biodistribution and tumor uptake data, page 45

A. ITLC and HPLC chromatograms for radiolabeling kinetics studies

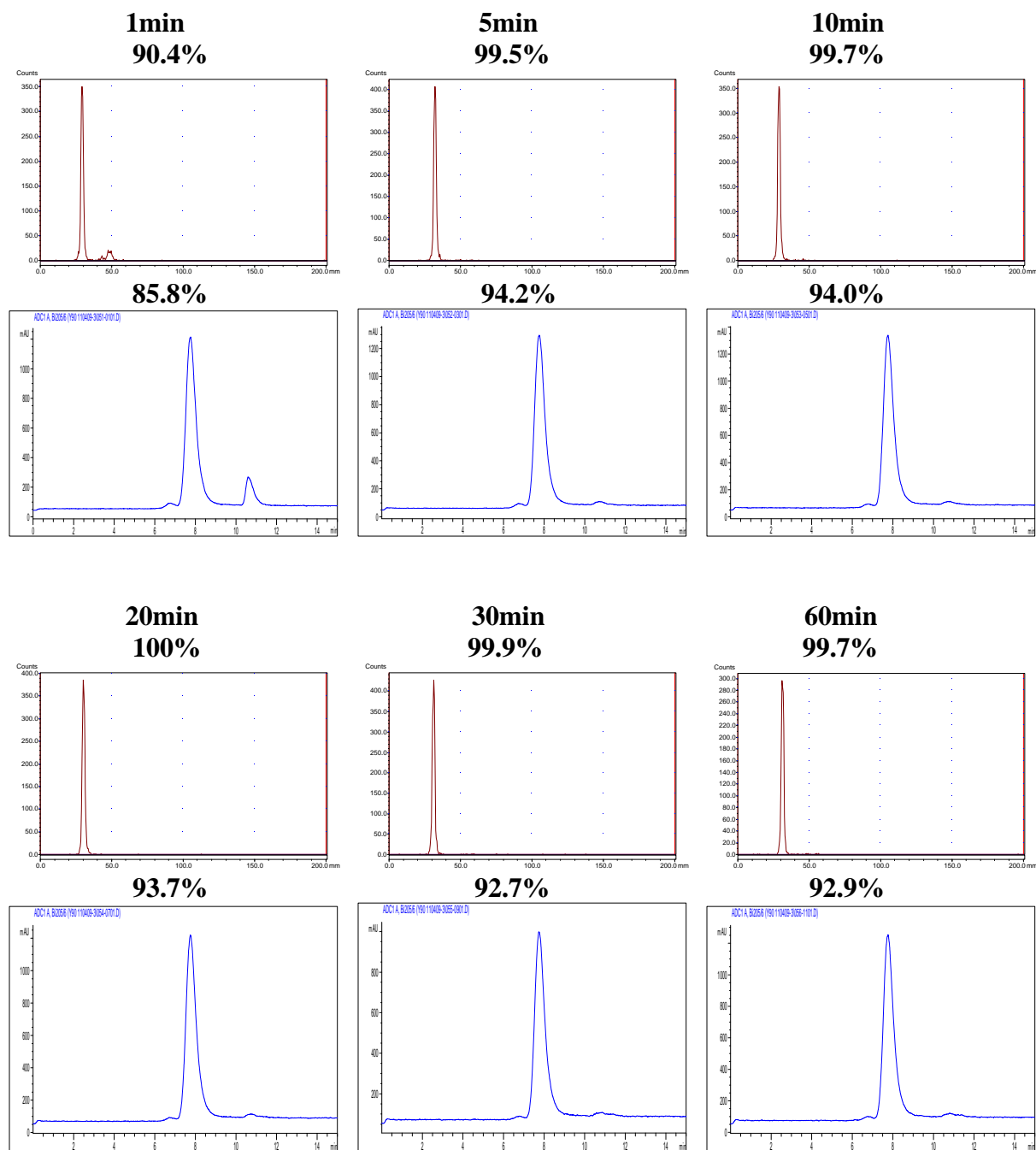
1. Radiolabeling of Ligand-Trastuzumab Conjugate with ^{90}Y

i) 3p-C-NETA-trastuzumab

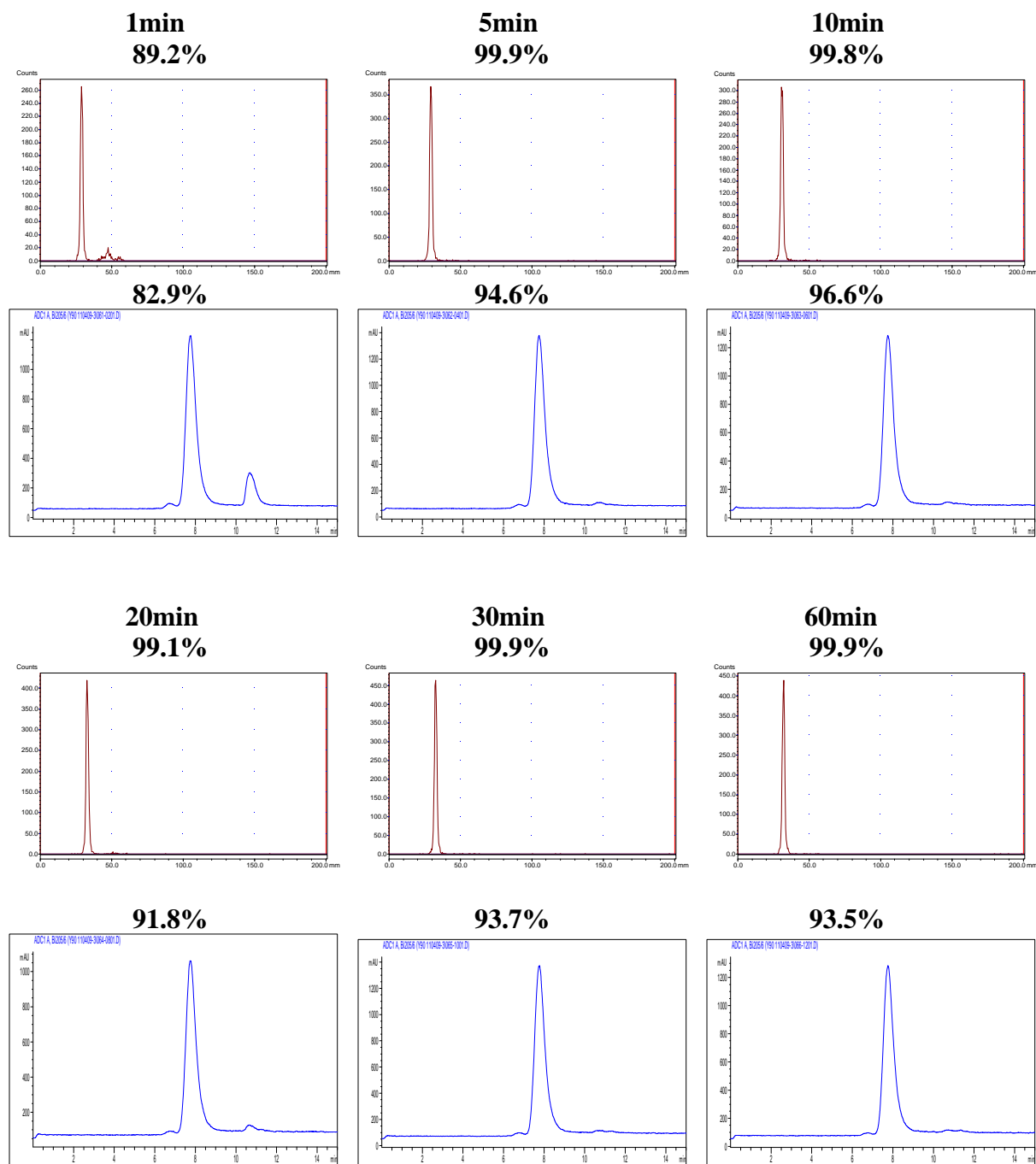
Sample 1



Sample 2

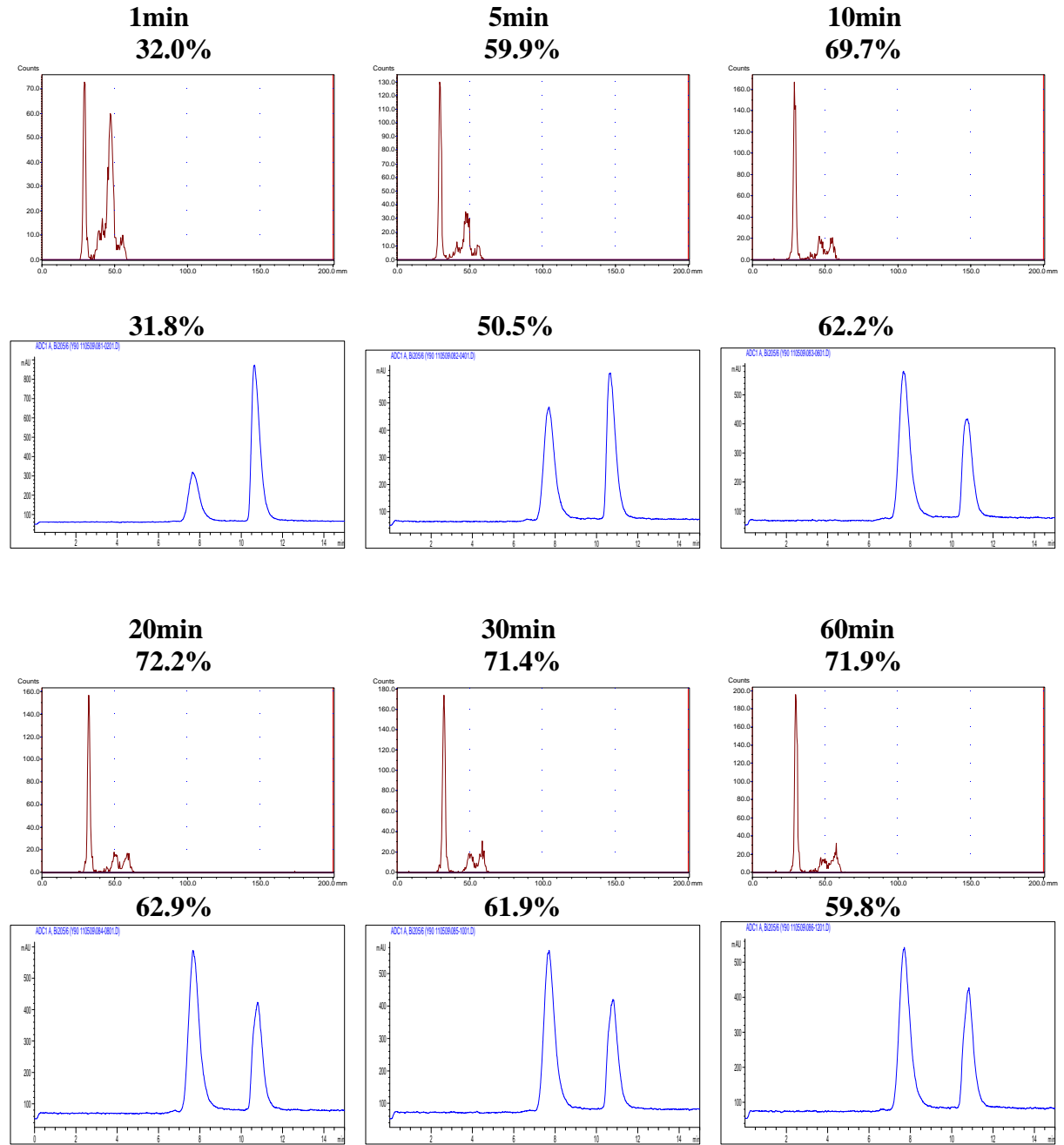


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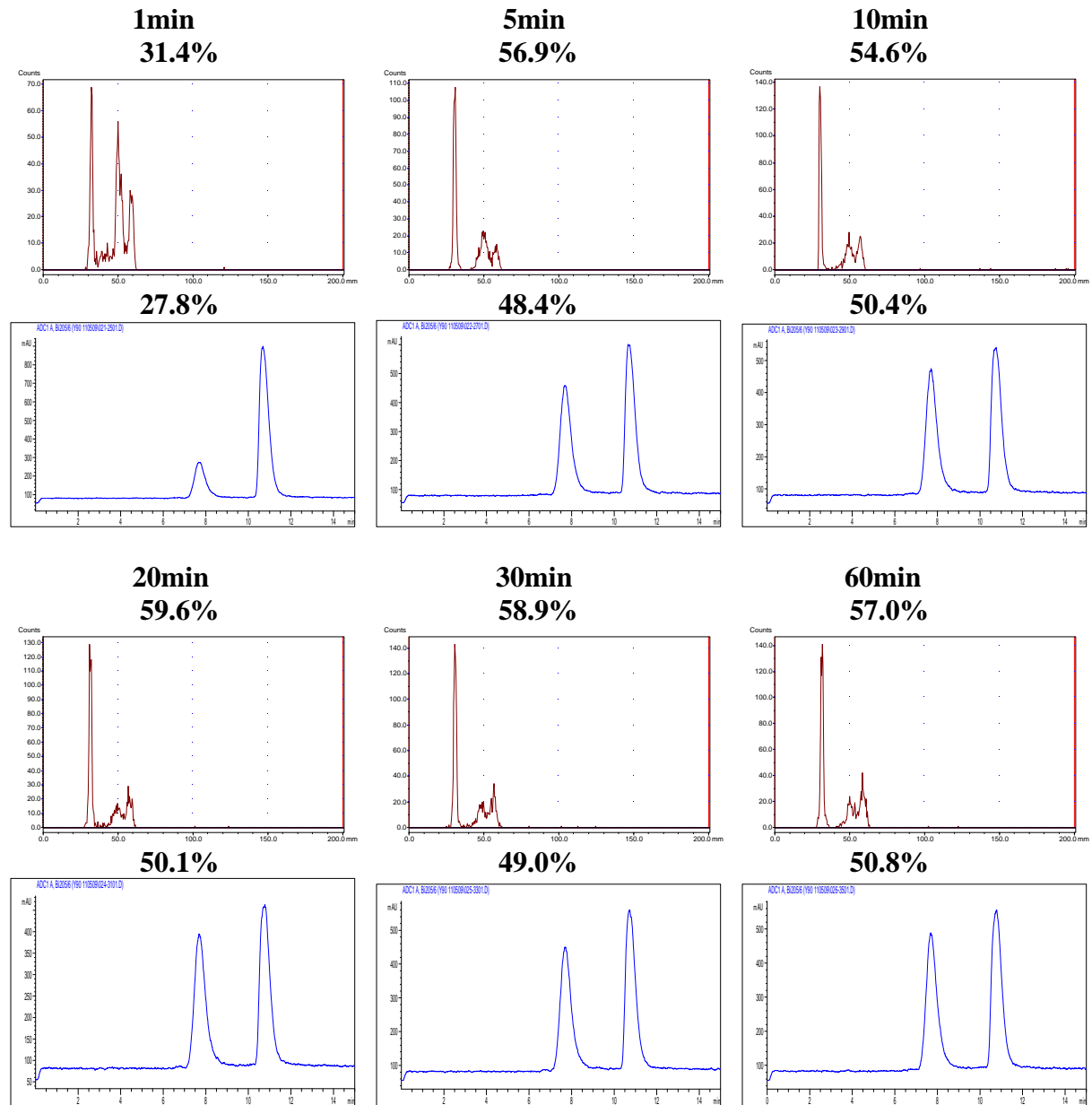


ii) 3p-C-DEPA-trastuzumab

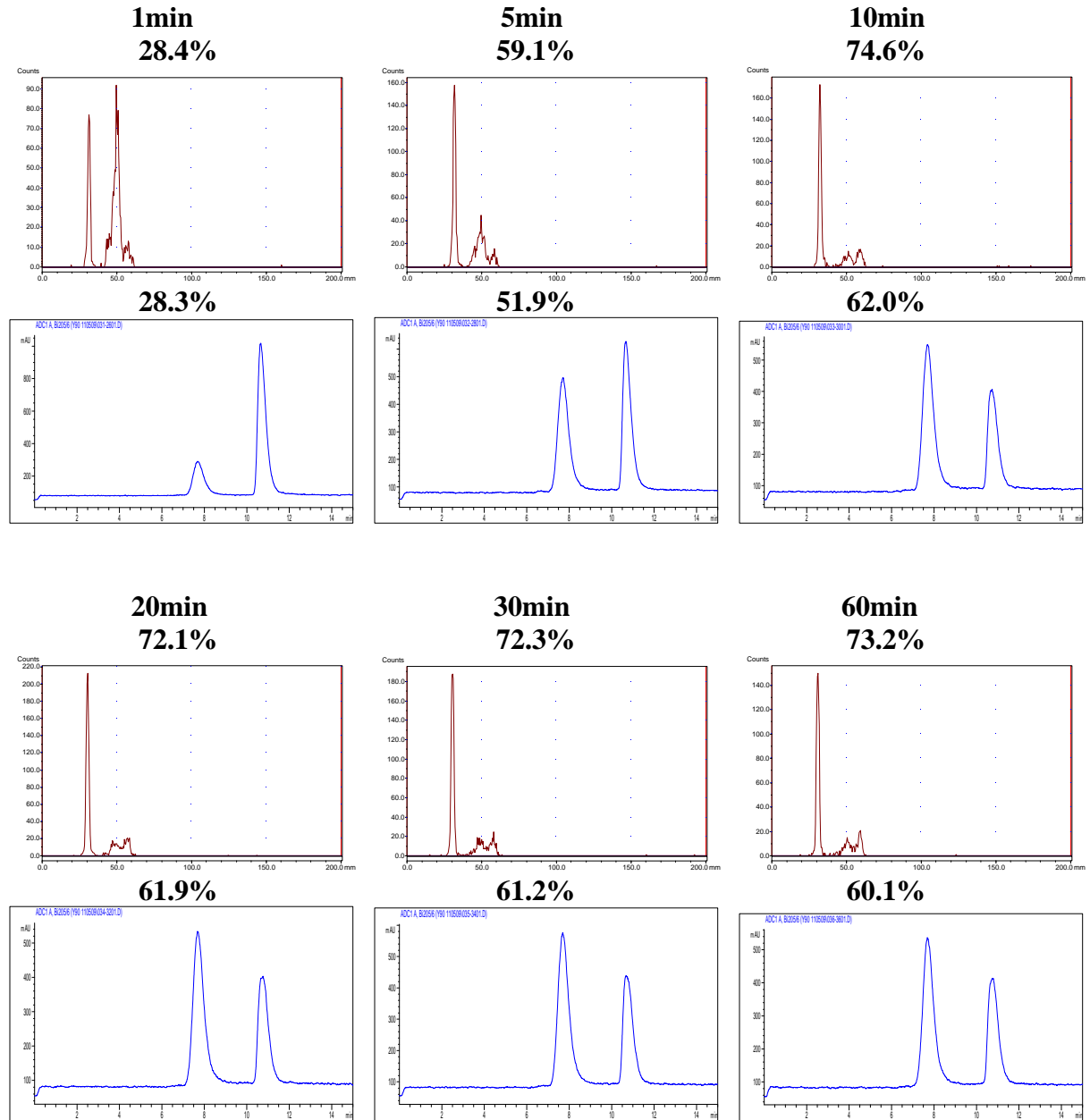
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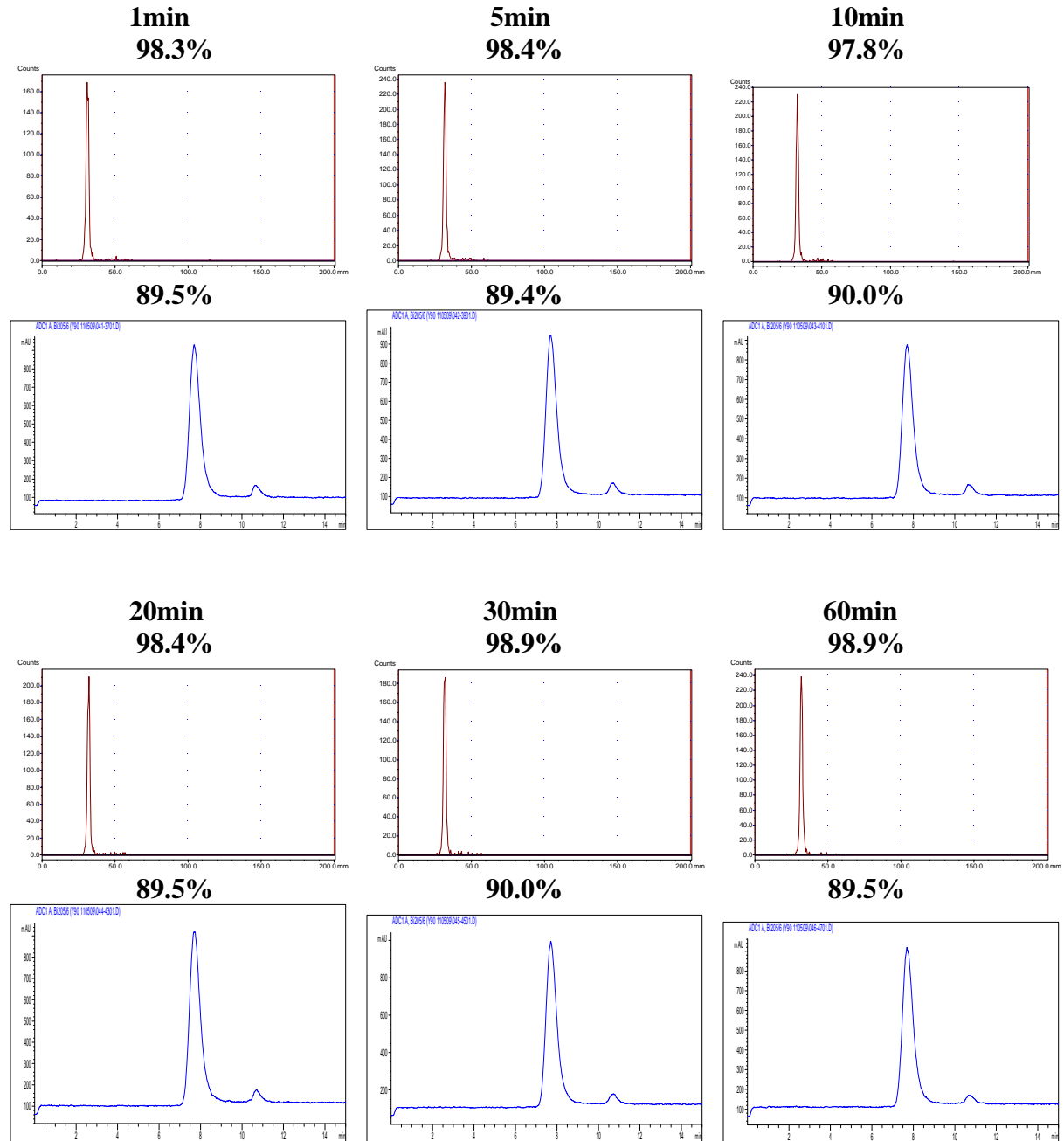


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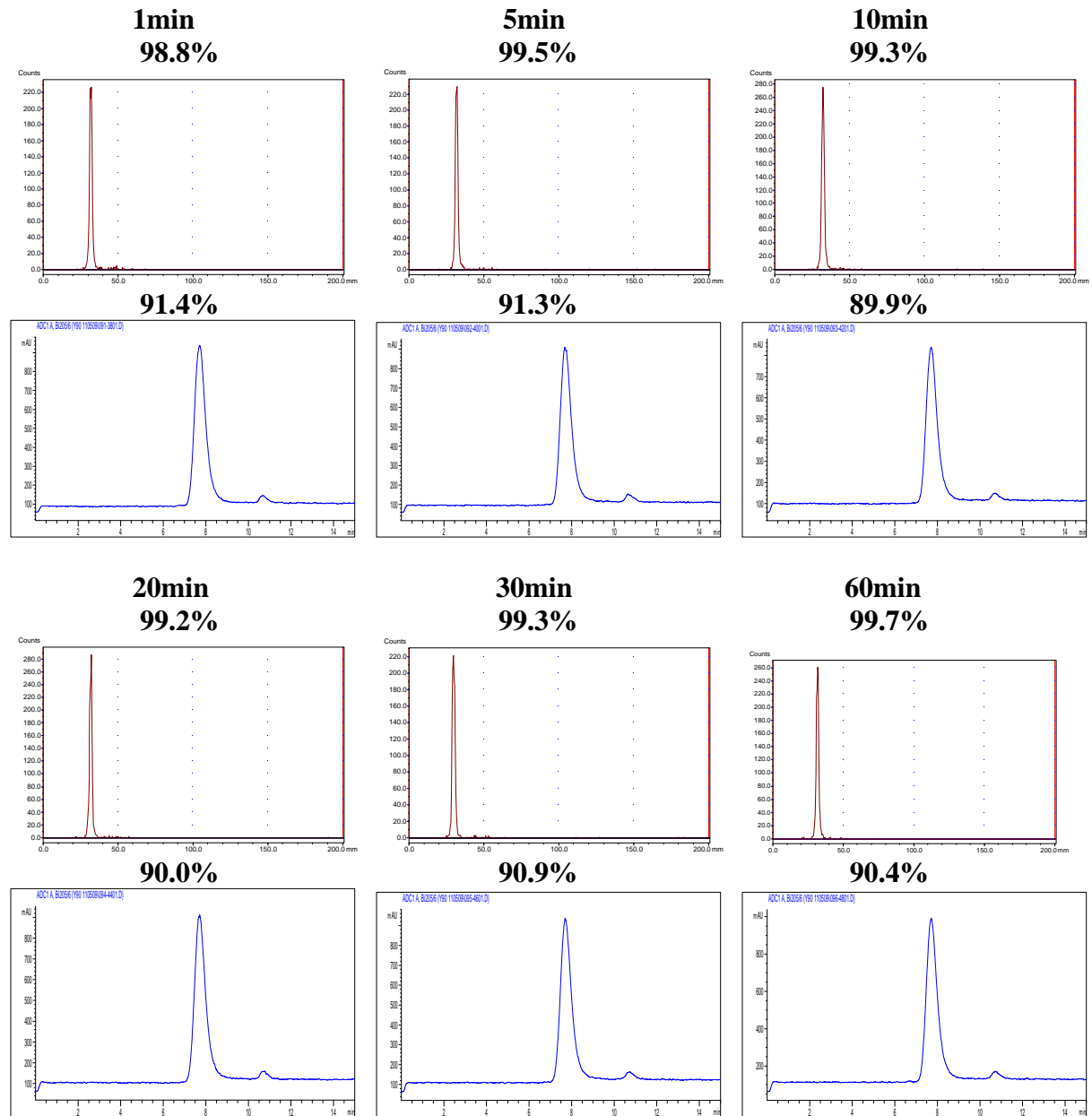


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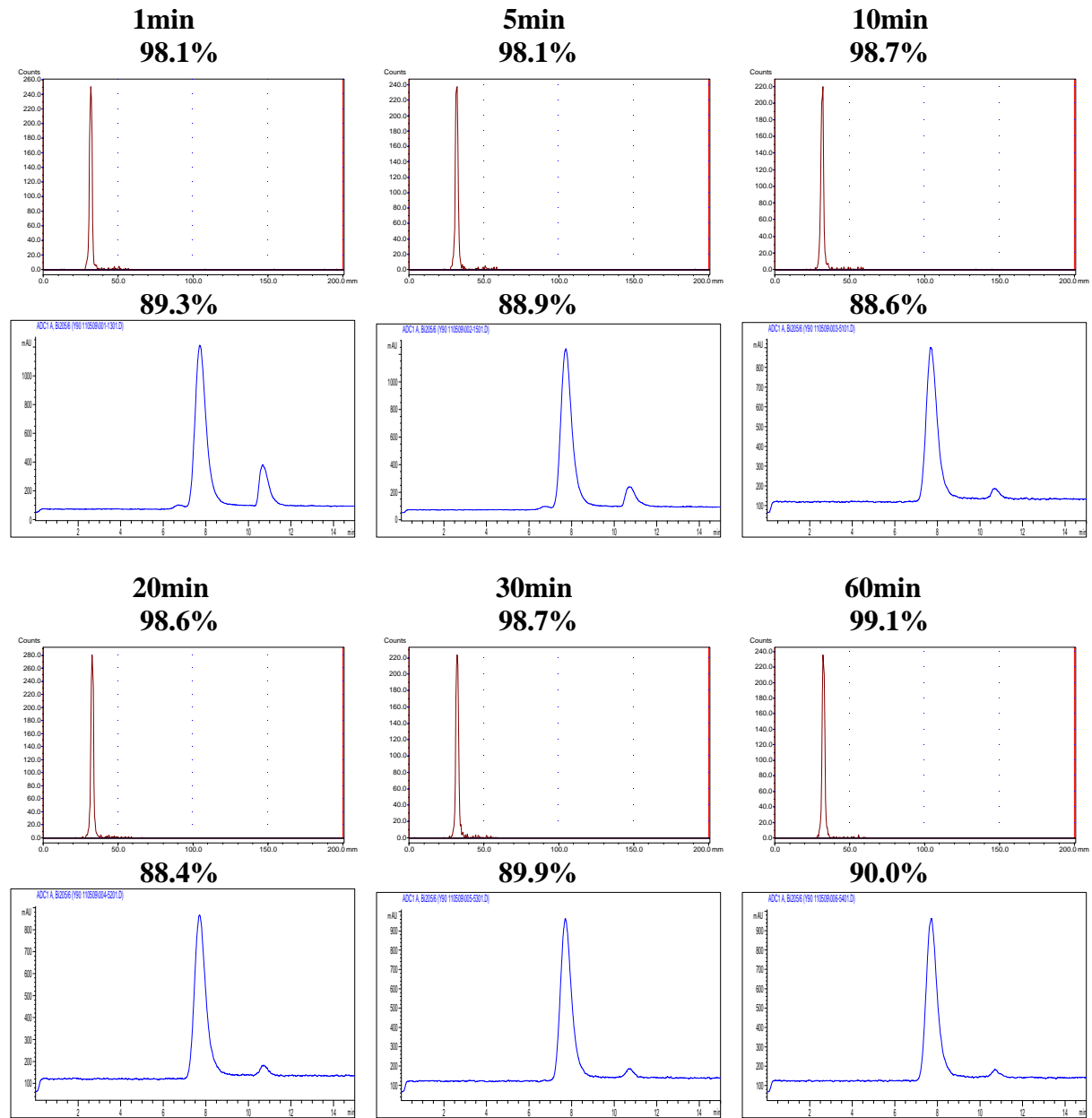
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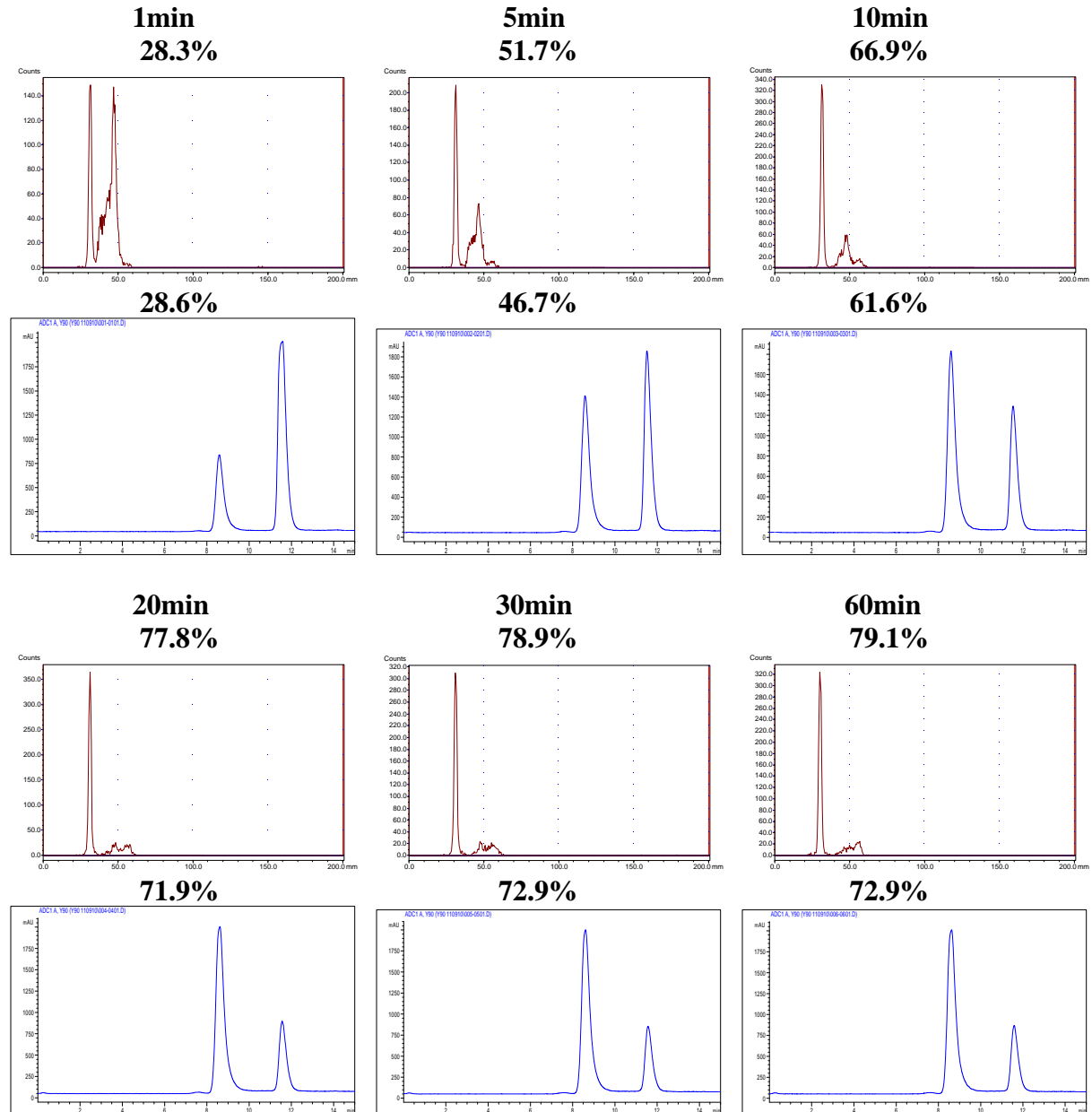


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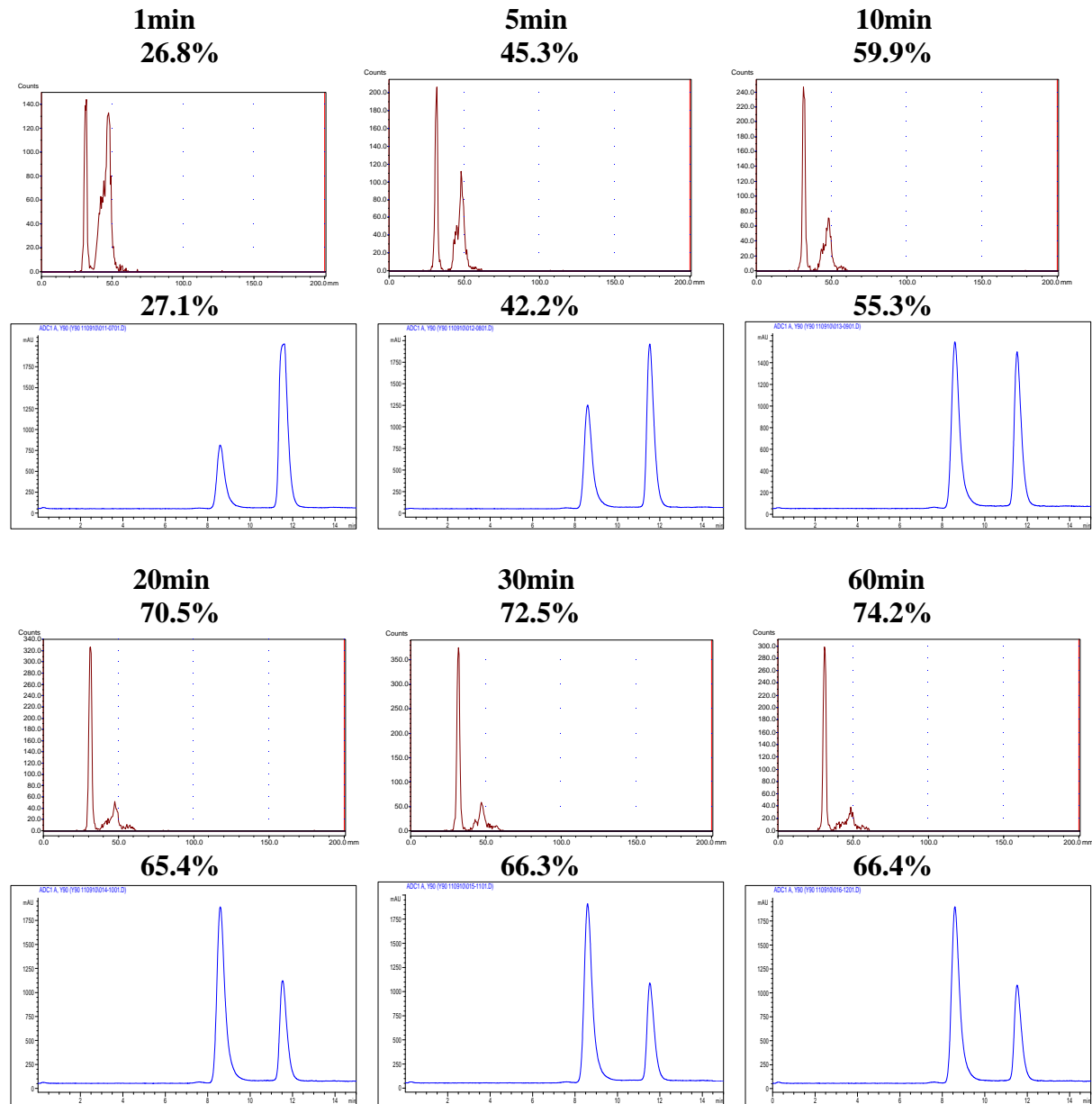


iv) C-DOTA-trastuzumab

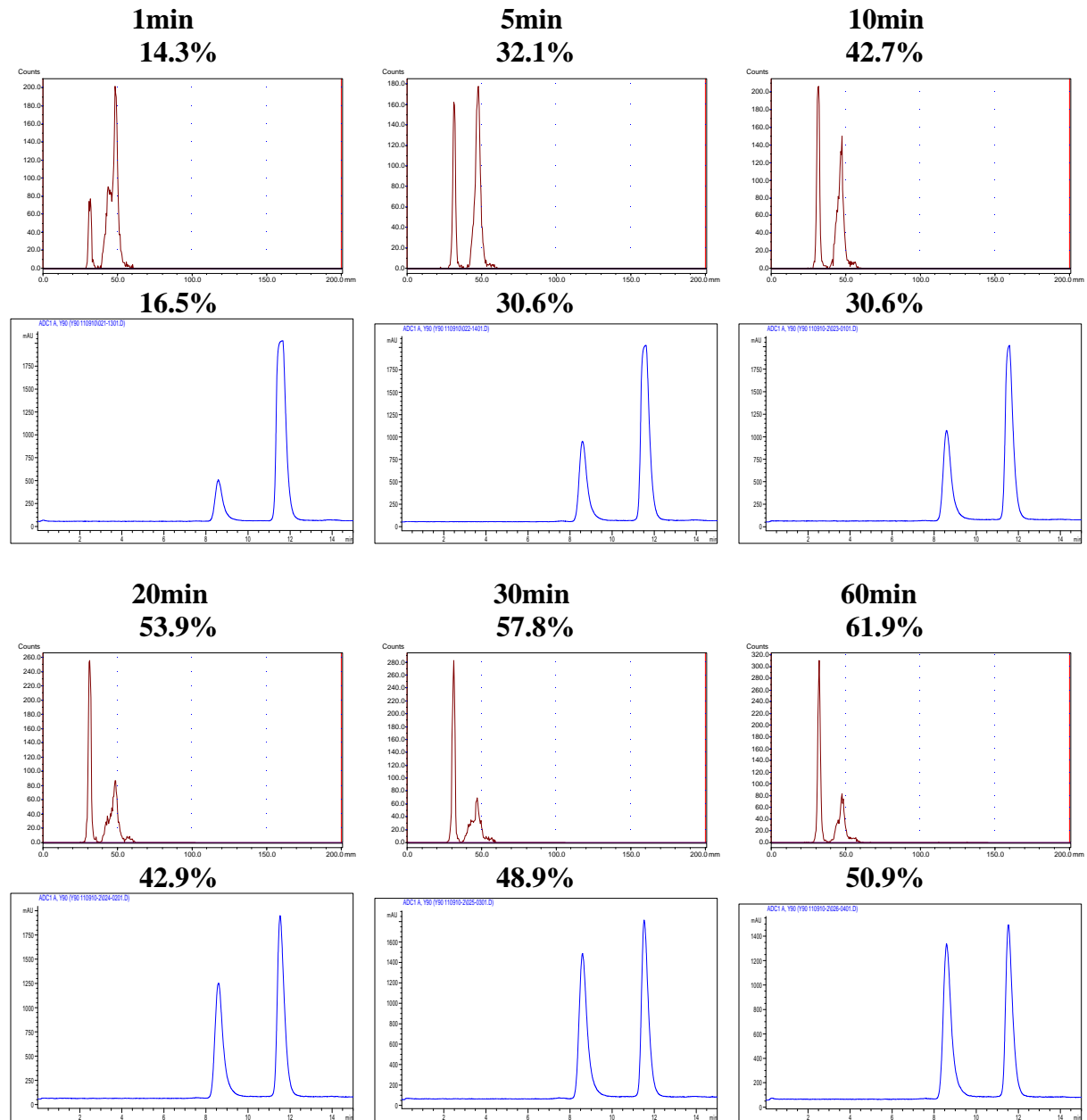
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Sample 2



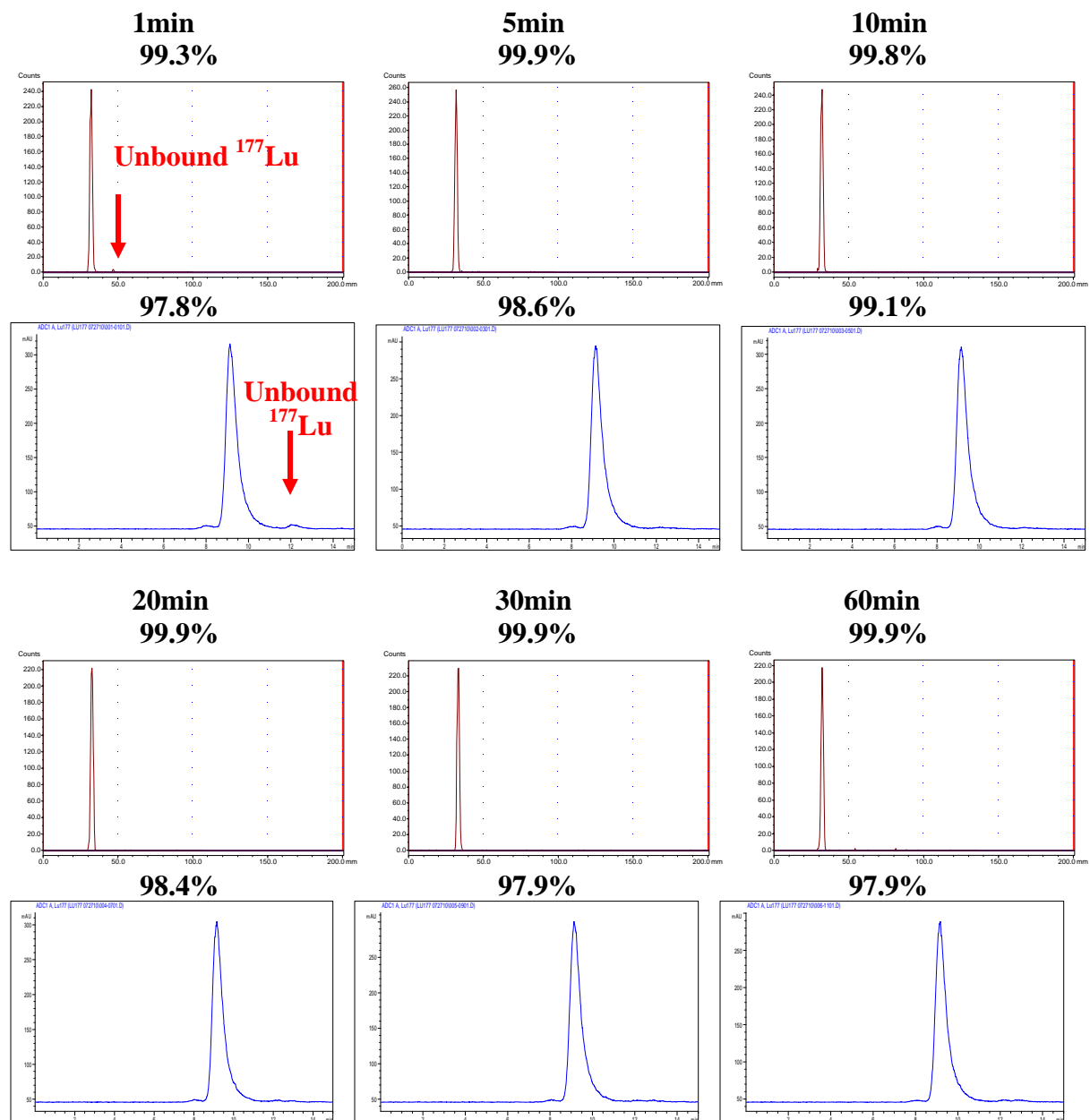
Sample 3



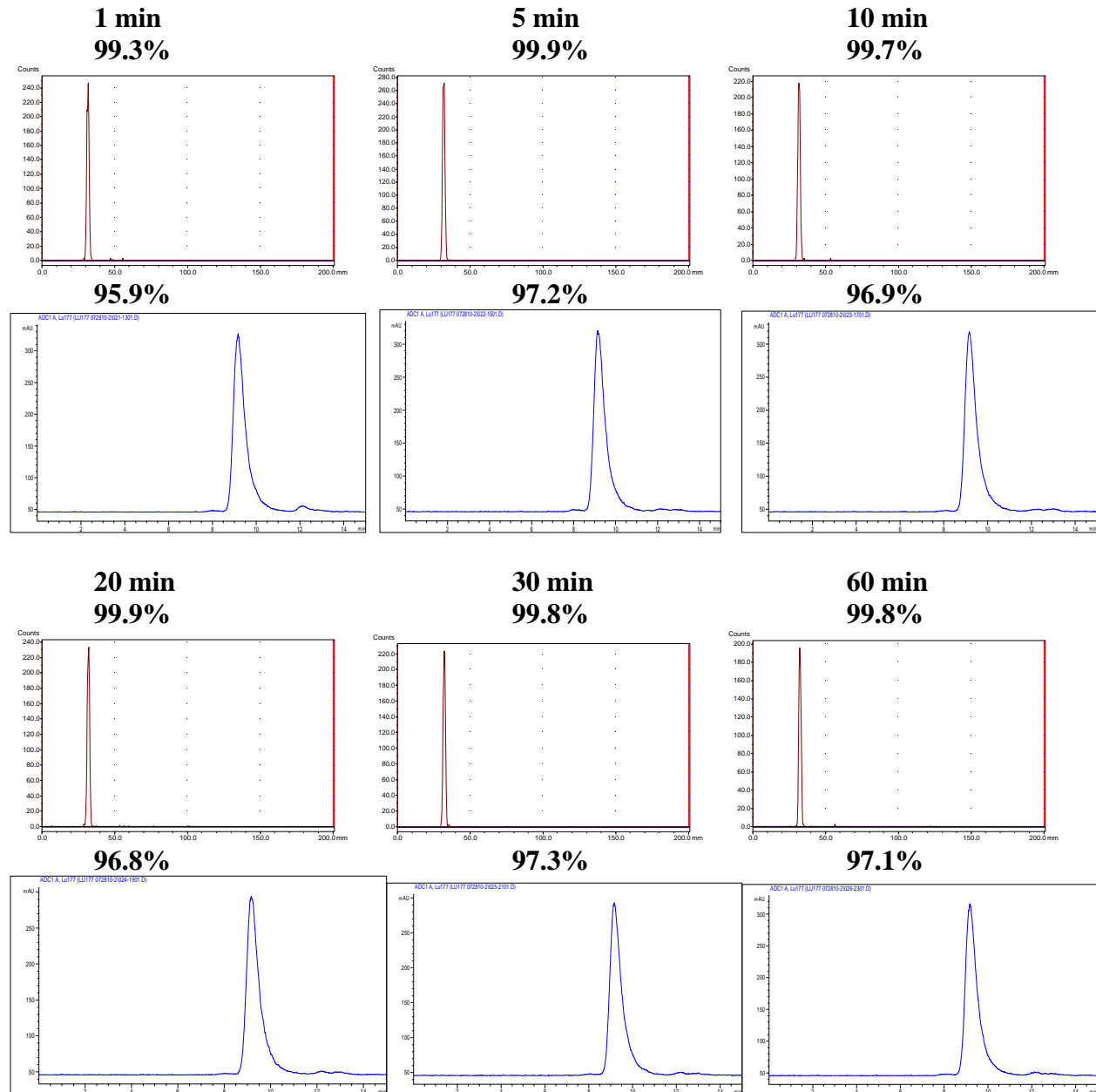
2. Radiolabeling of ligand-Trastuzumab conjugate with ^{177}Lu

i) 3p-C-NETA-trastuzumab

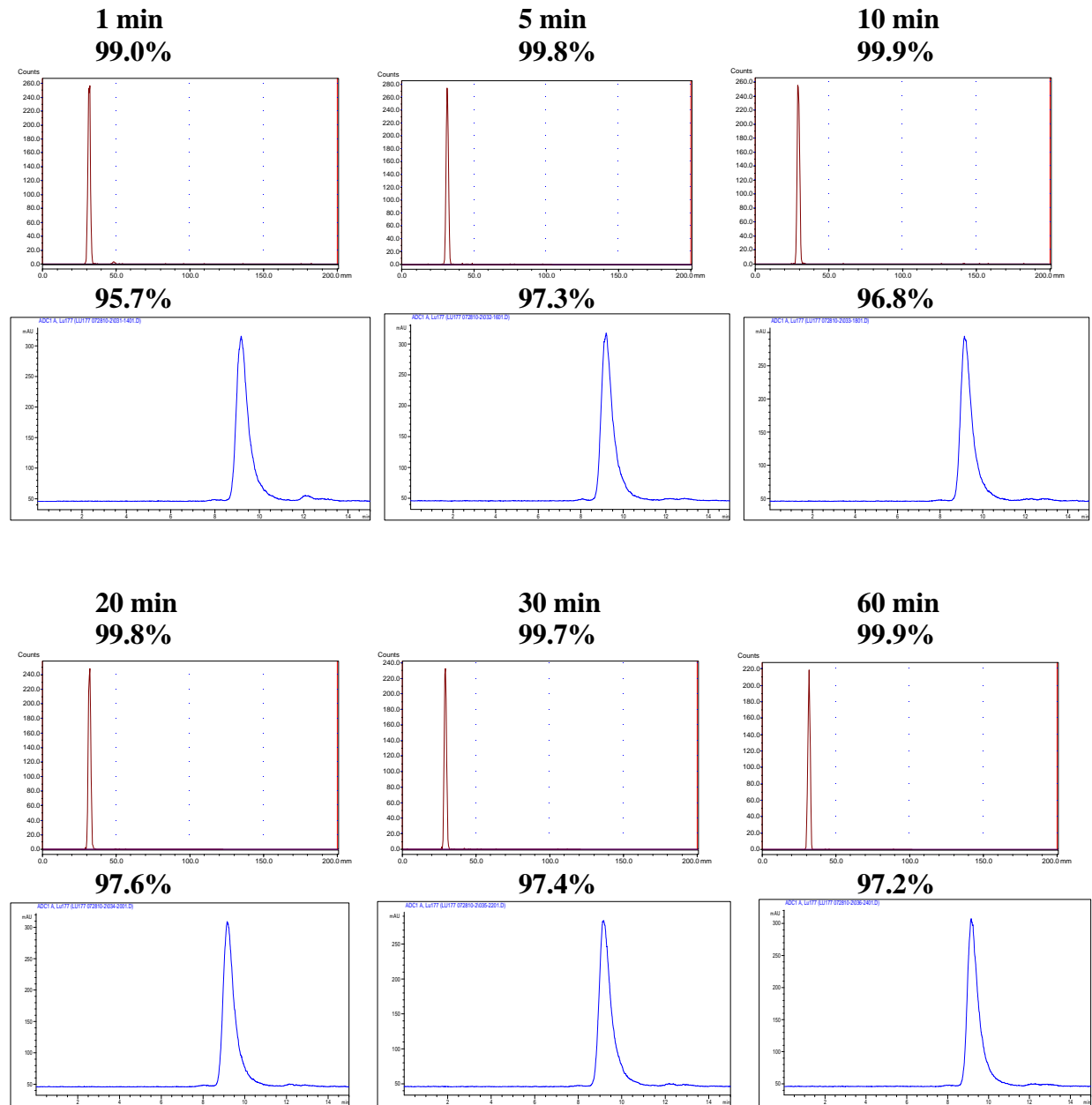
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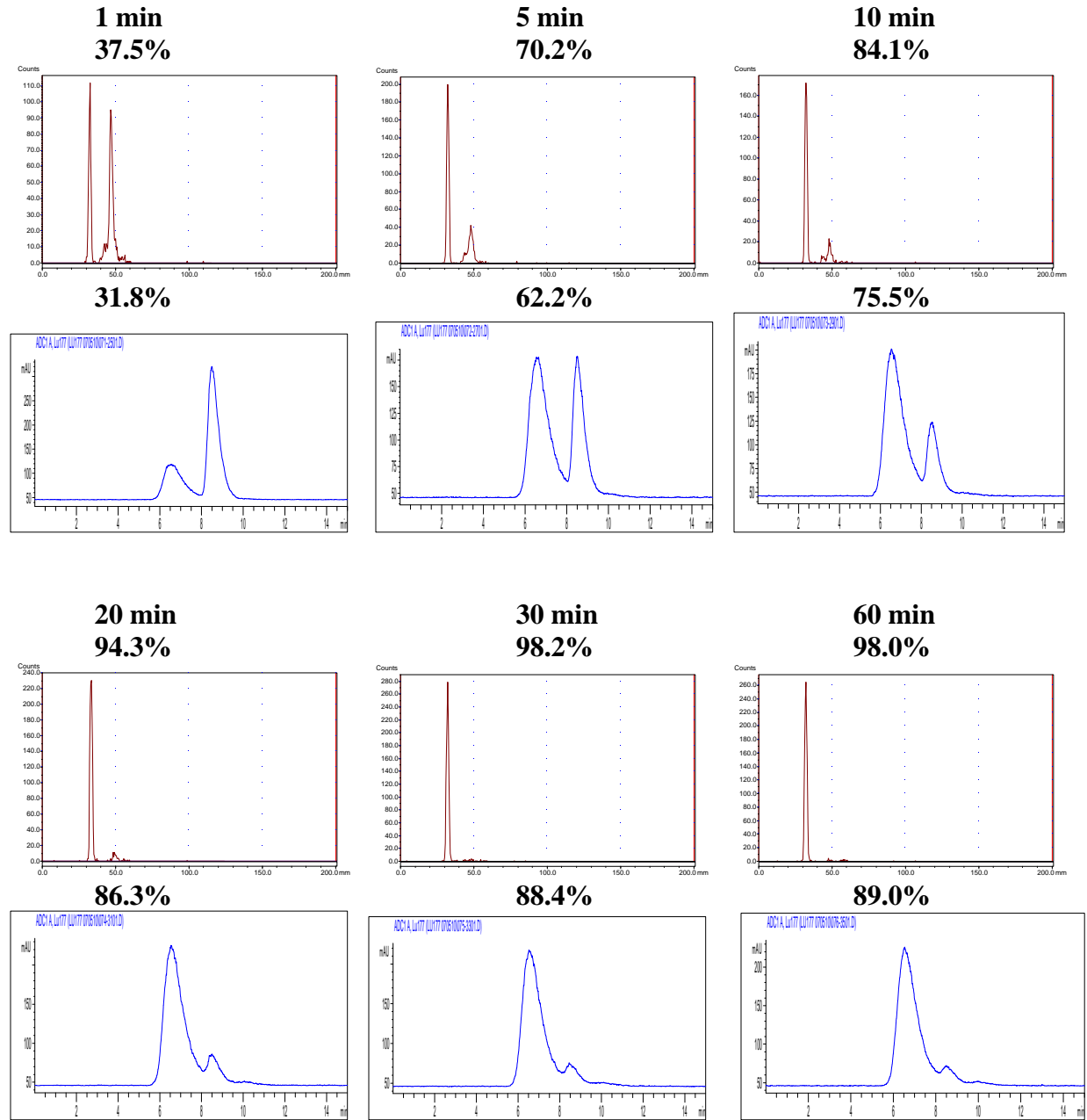


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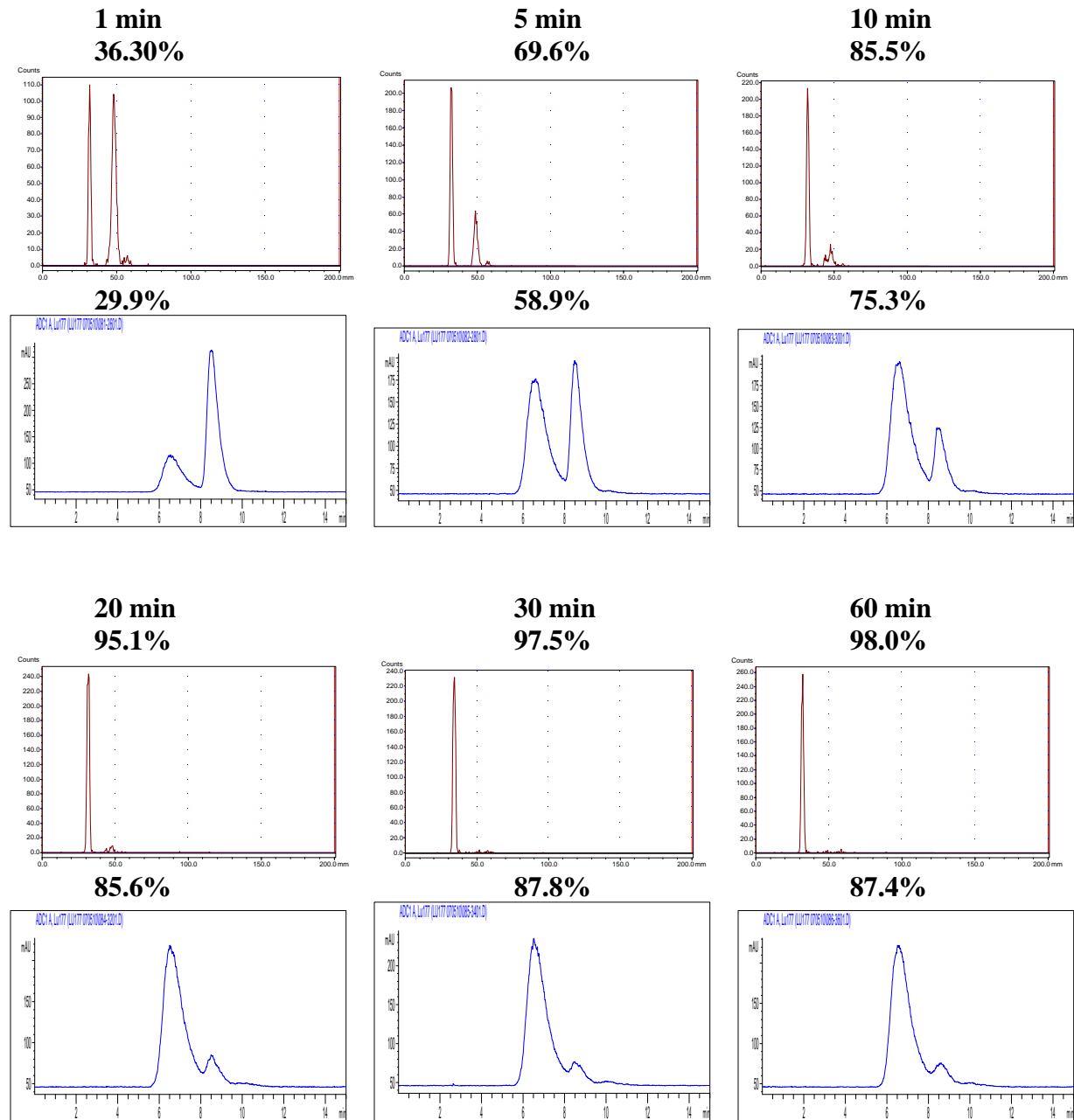


ii) 3p-C-DEPA-Trastuzumab

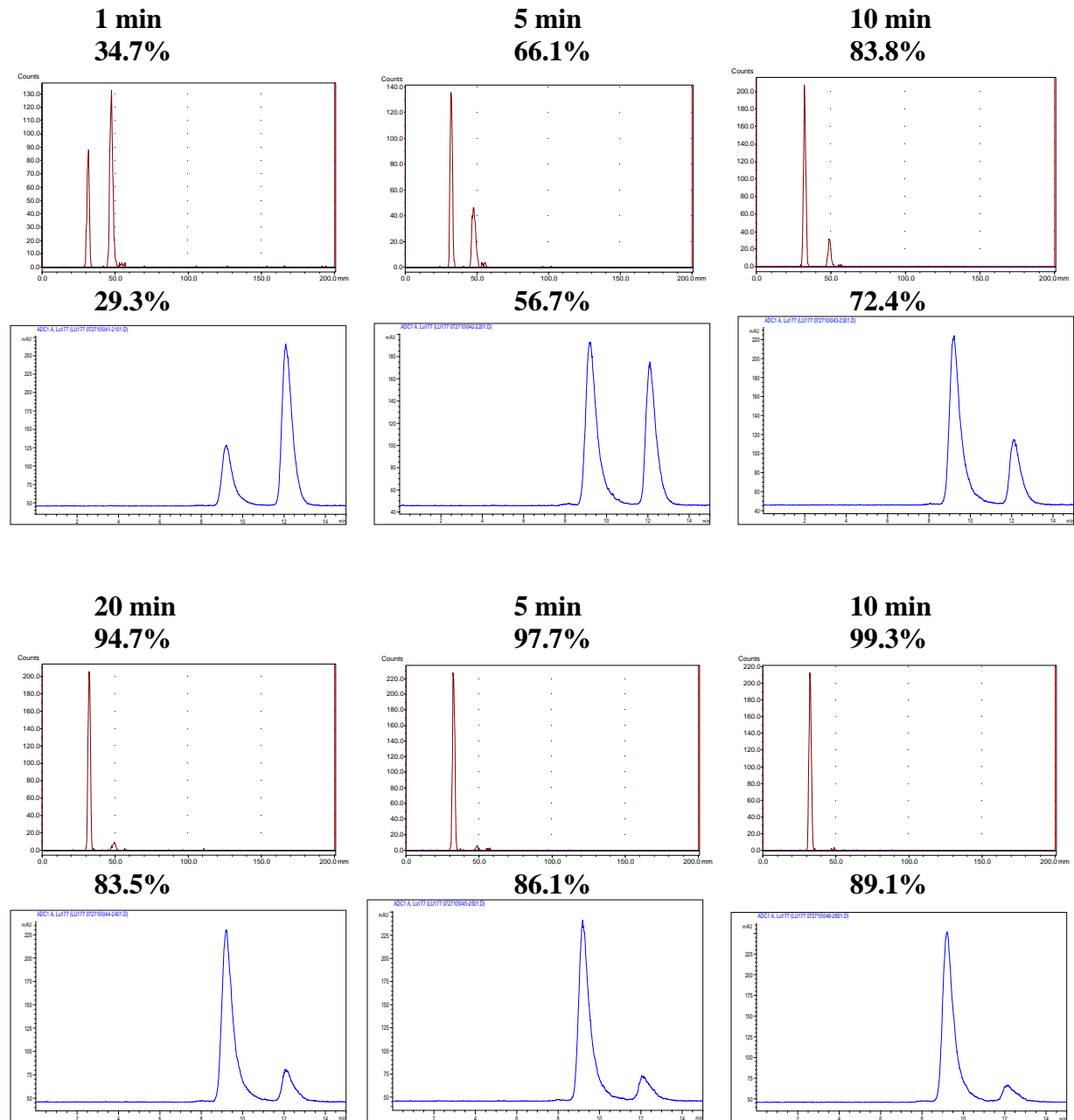
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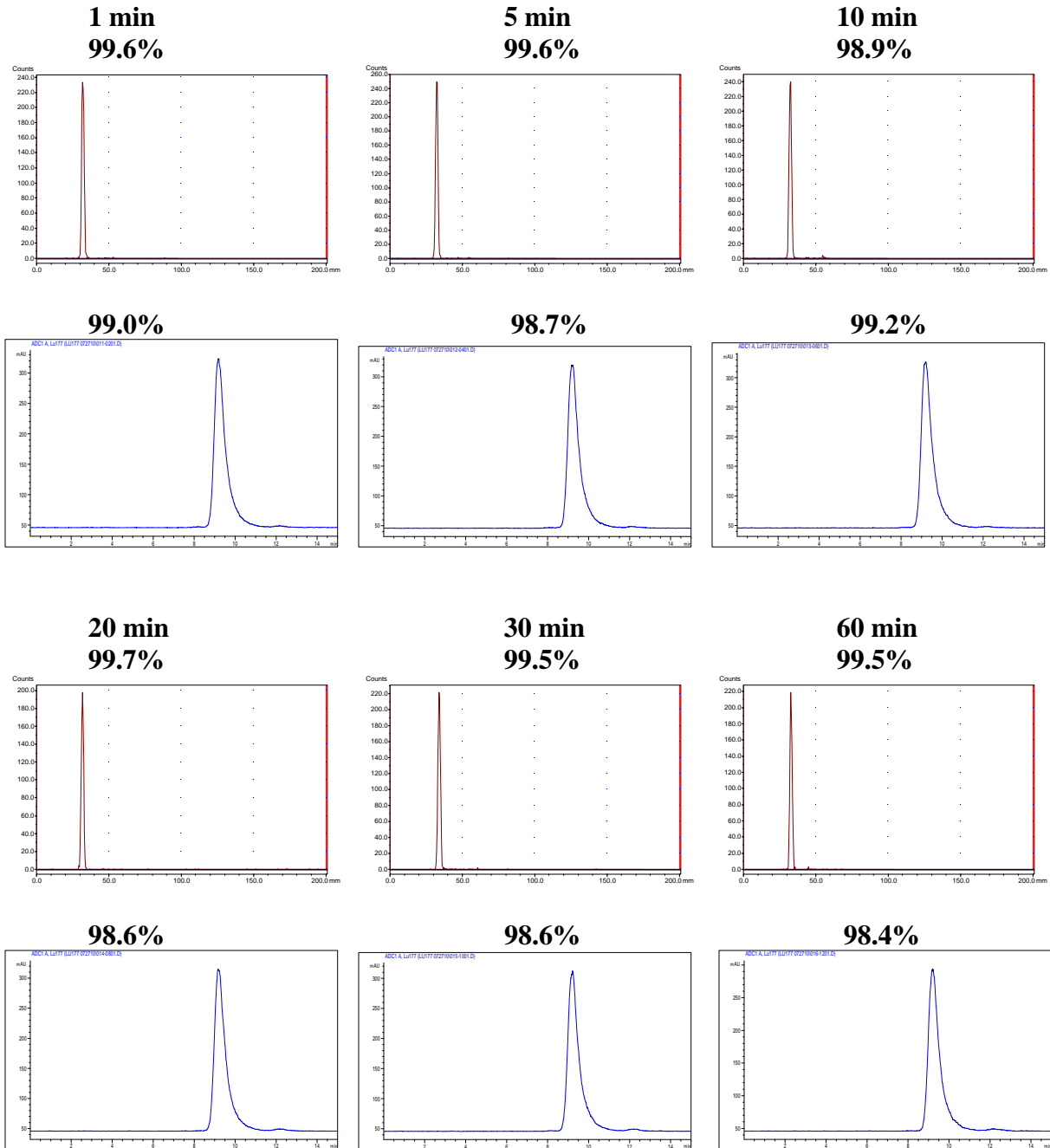


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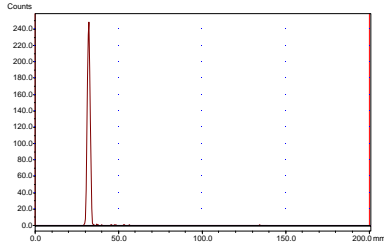
iii) C-DTPA-Trastuzumab

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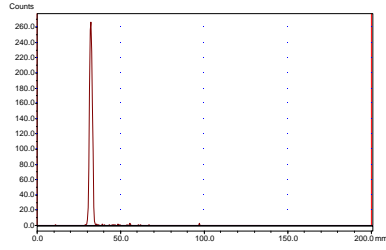


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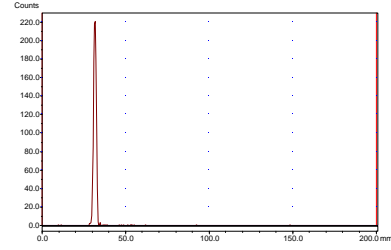
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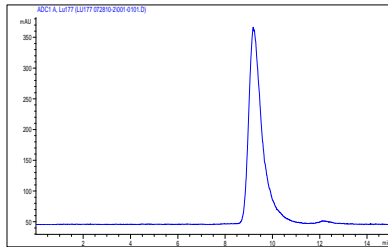
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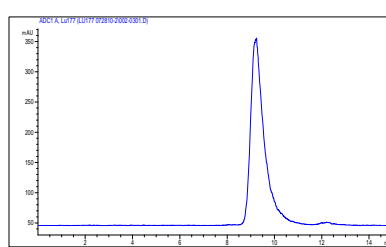
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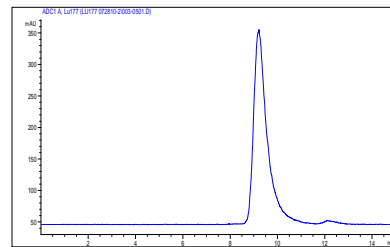
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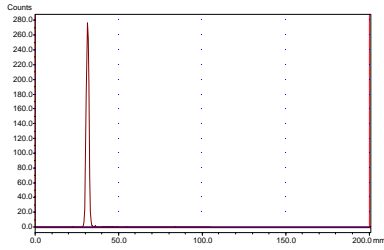
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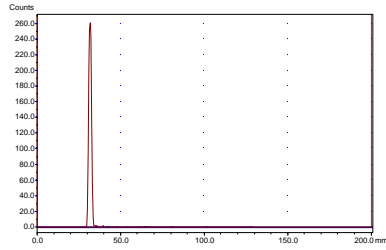
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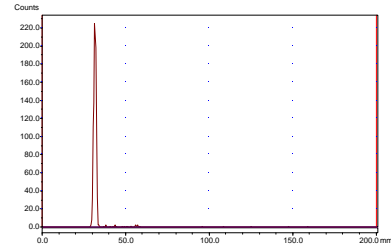
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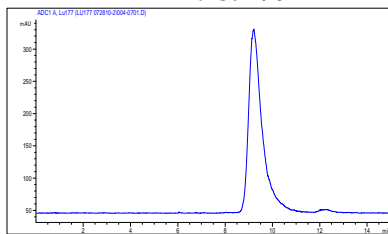
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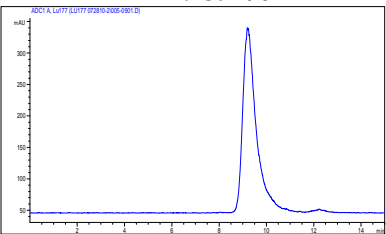
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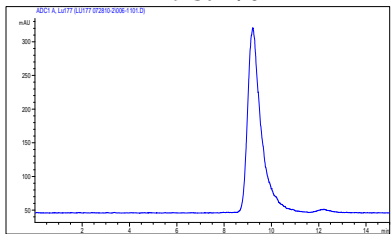
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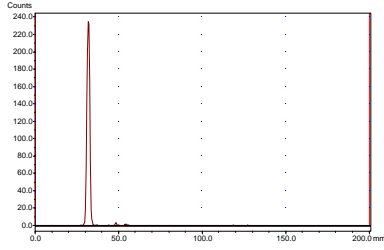


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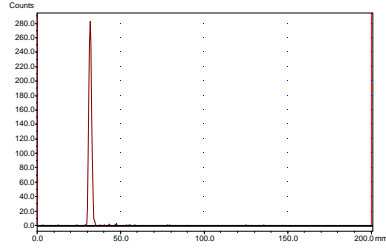


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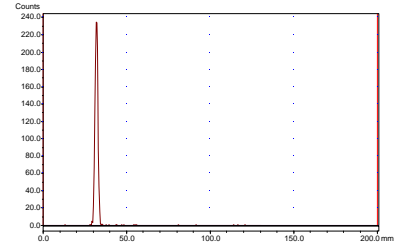
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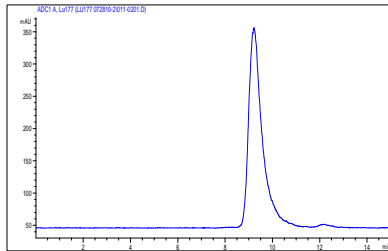
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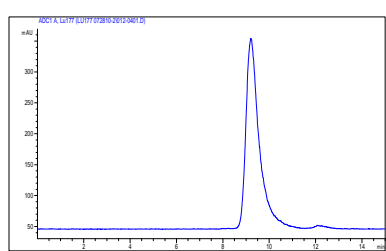
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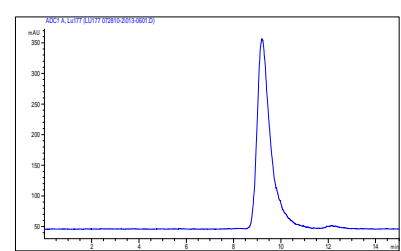
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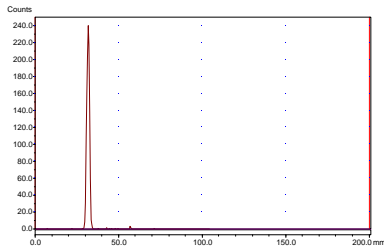
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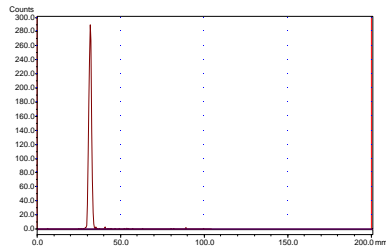
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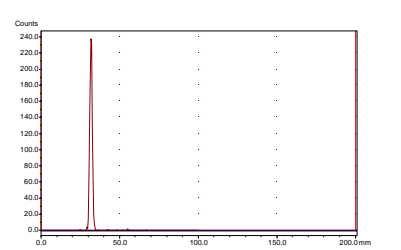
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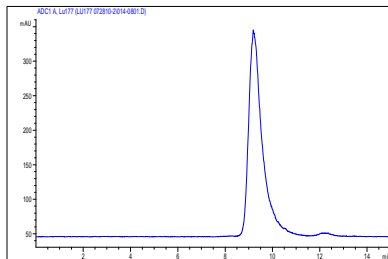
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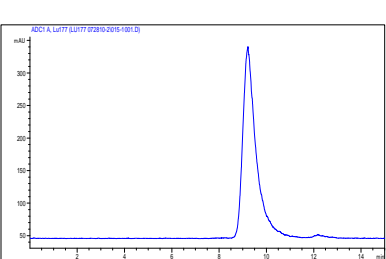
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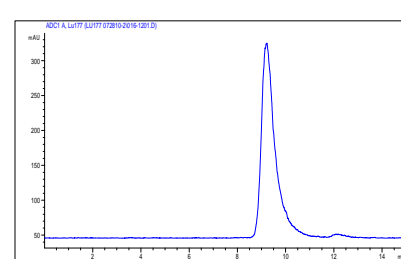
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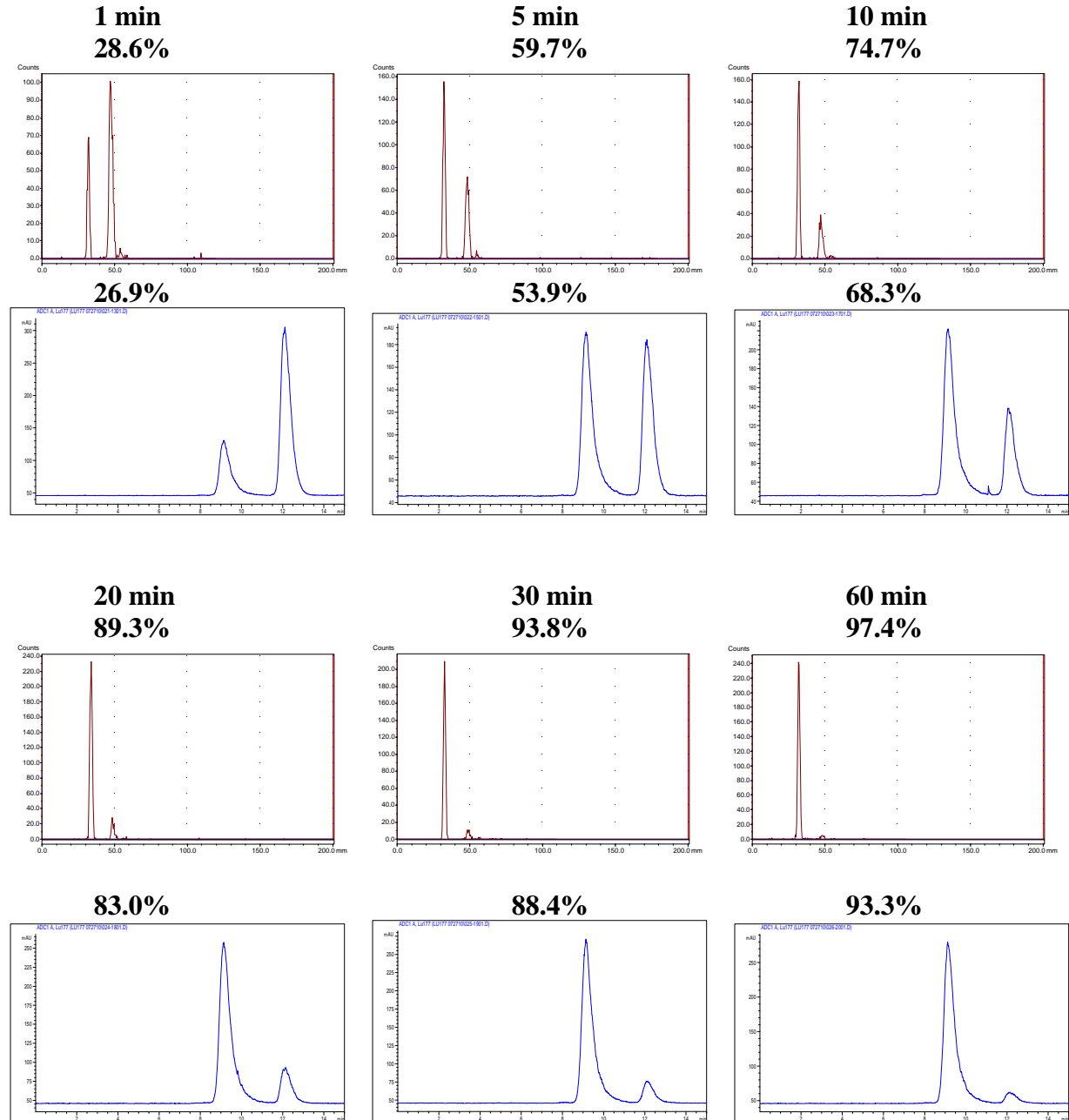


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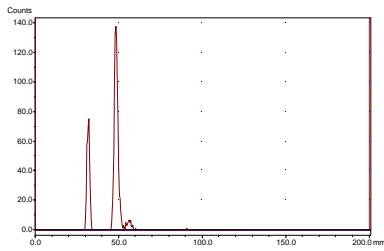
iv) C-DOTA-trastuzumab

Sample 1

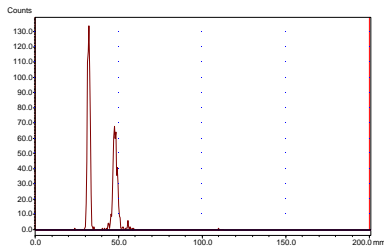


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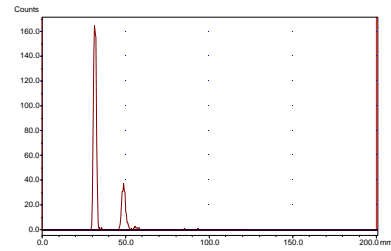
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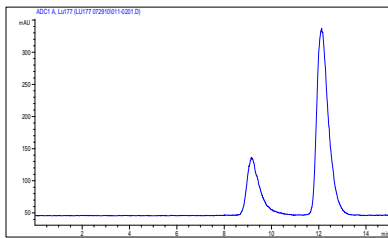
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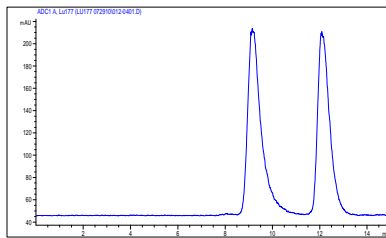
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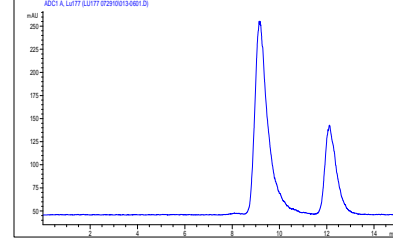
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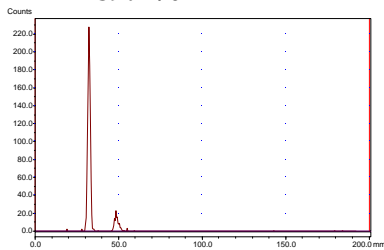
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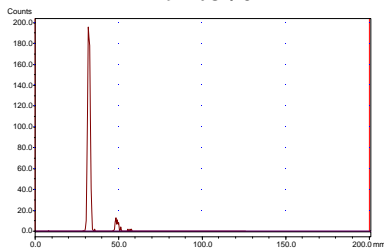
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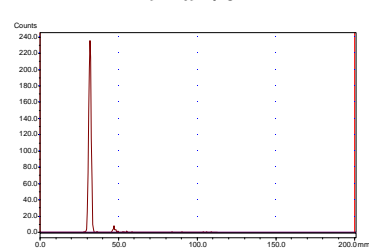
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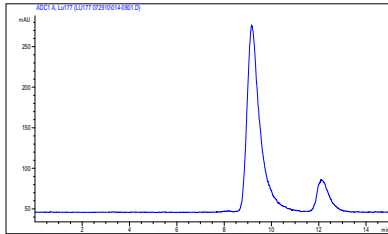
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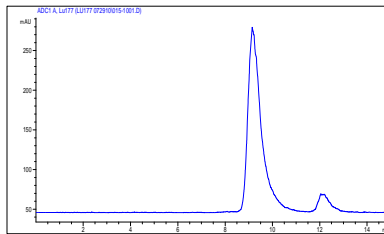
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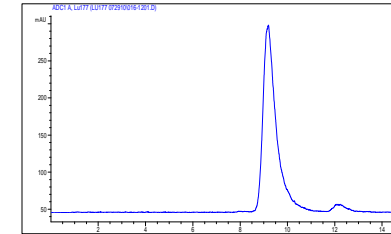
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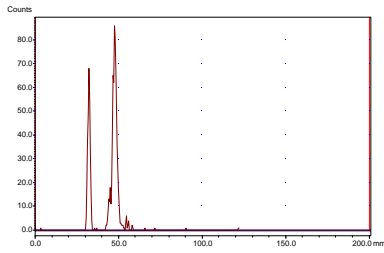


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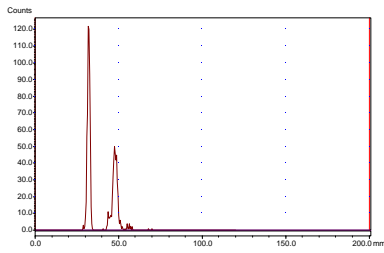


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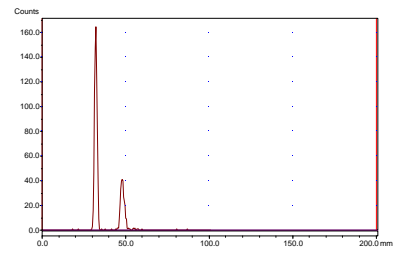
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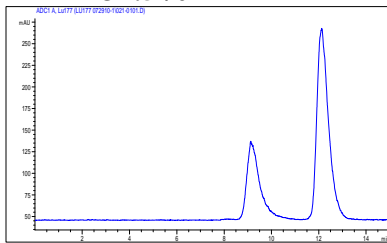
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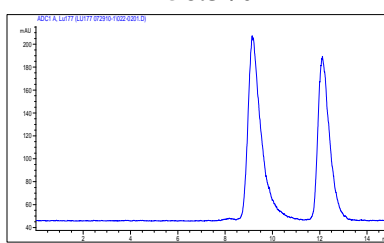
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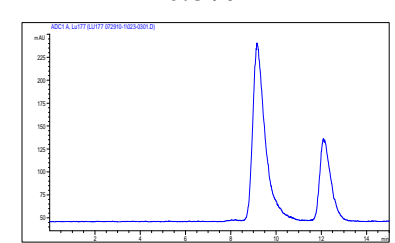
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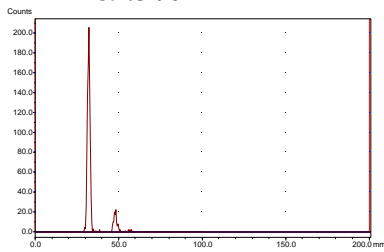
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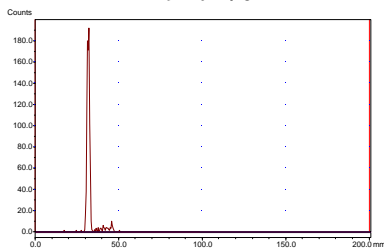
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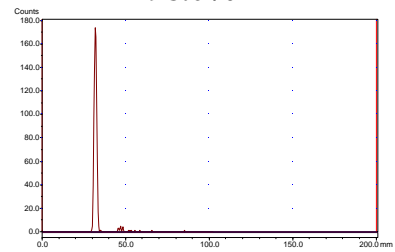
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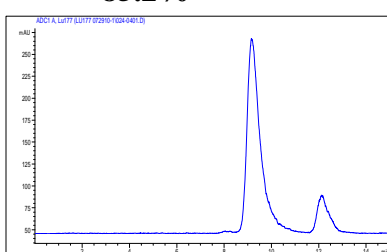
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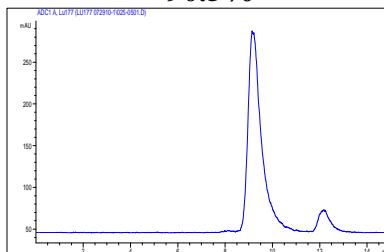
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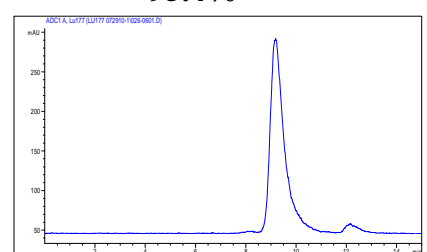
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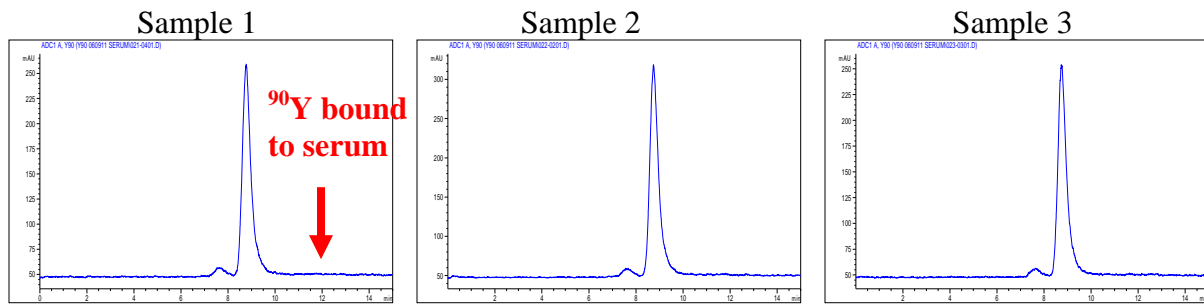


B. HPLC chromatograms for serum stability measurements

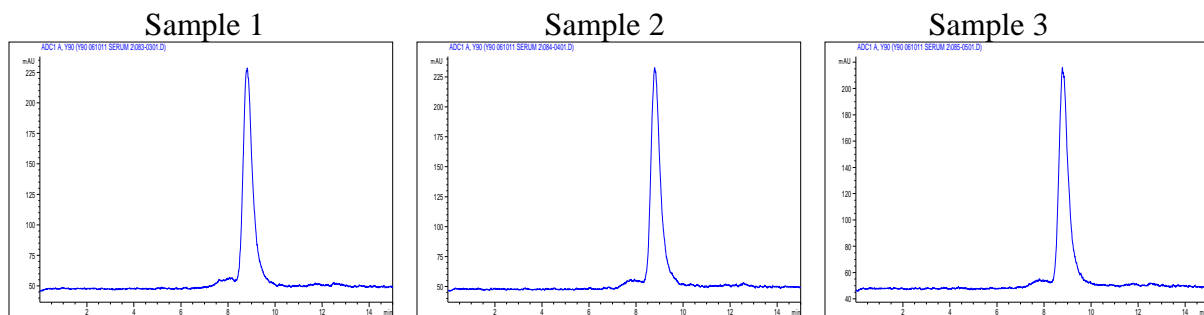
1. ^{90}Y -radiolabelled ligand-trastuzumab conjugates

i) ^{90}Y -3p-C-NETA-Trastuzumab

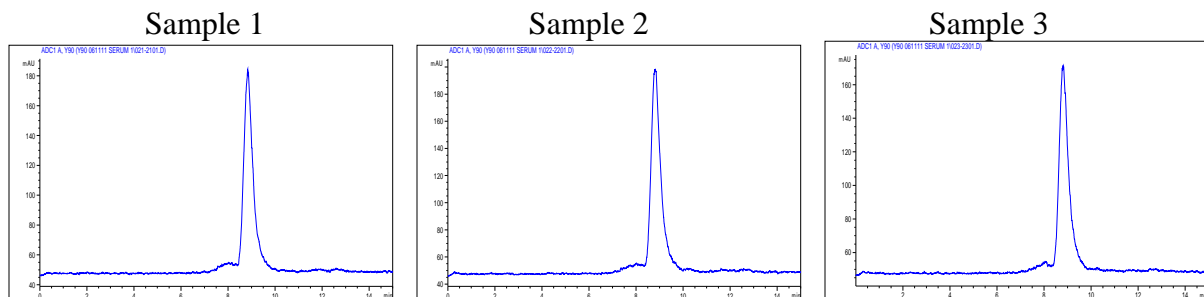
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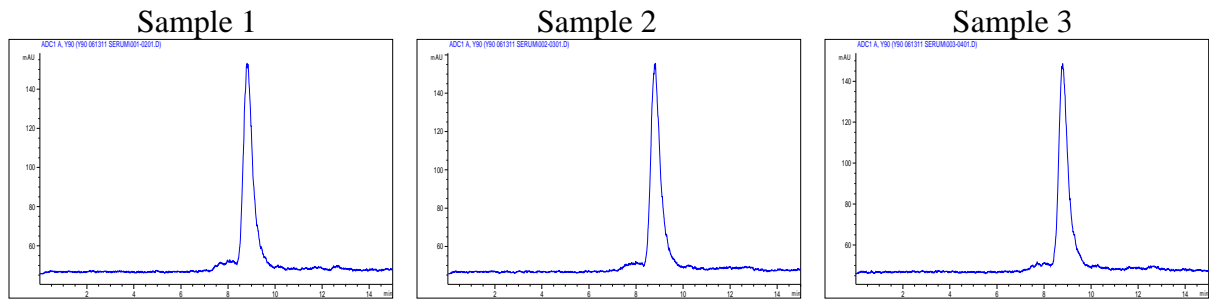
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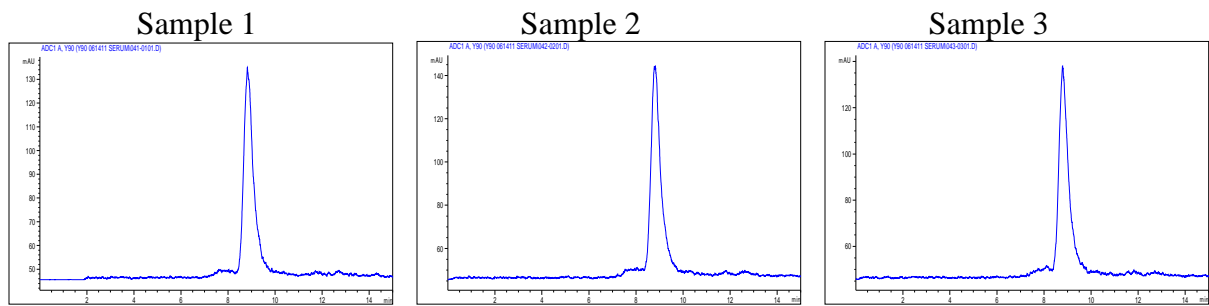
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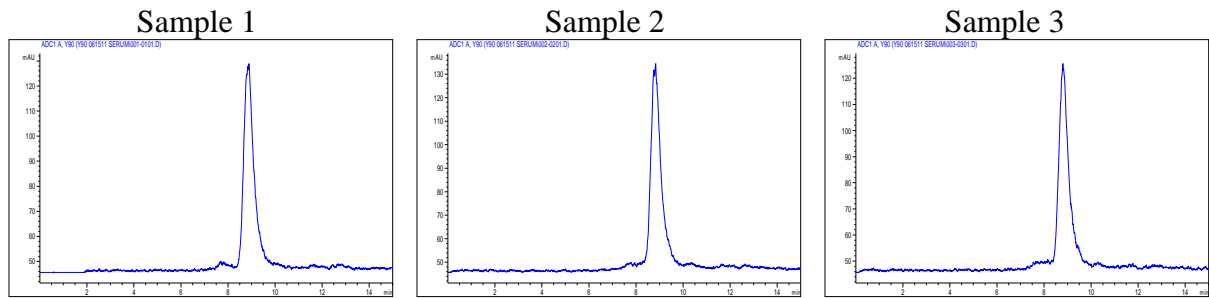
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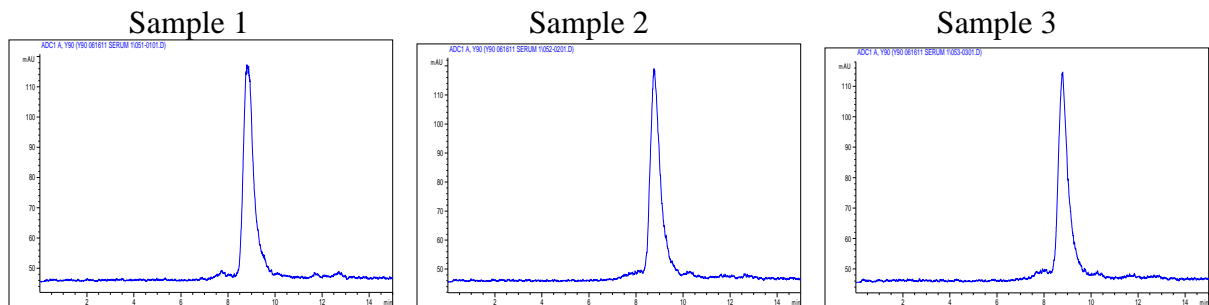
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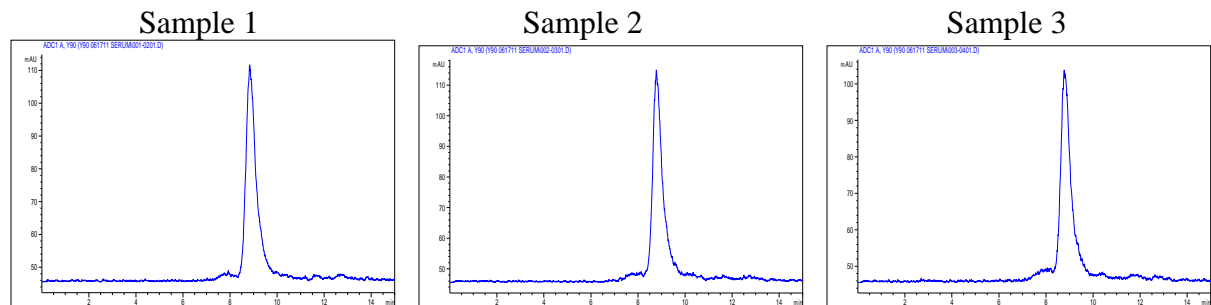
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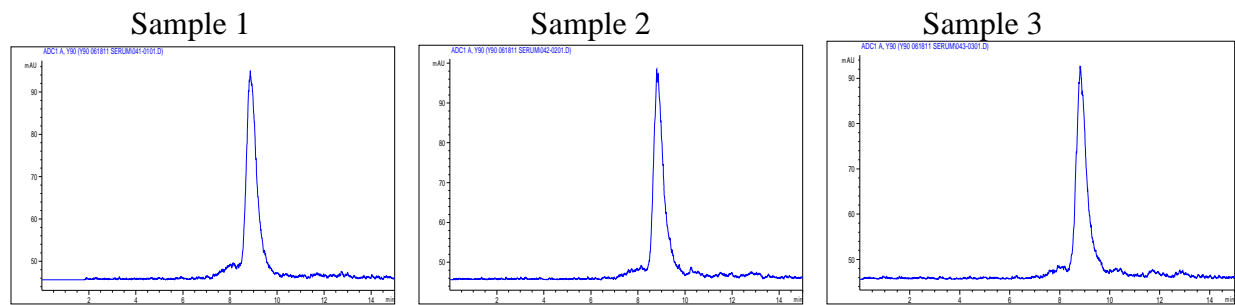
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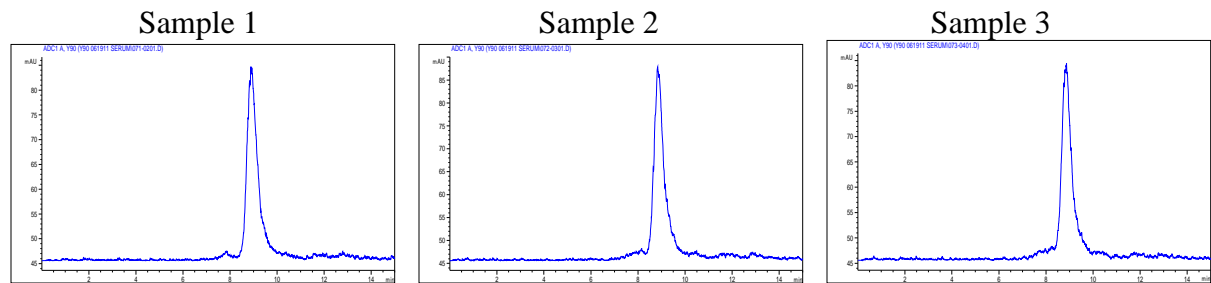
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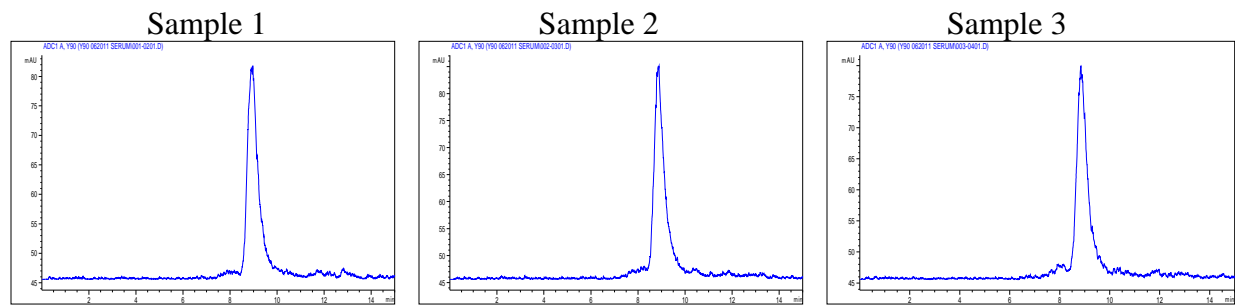
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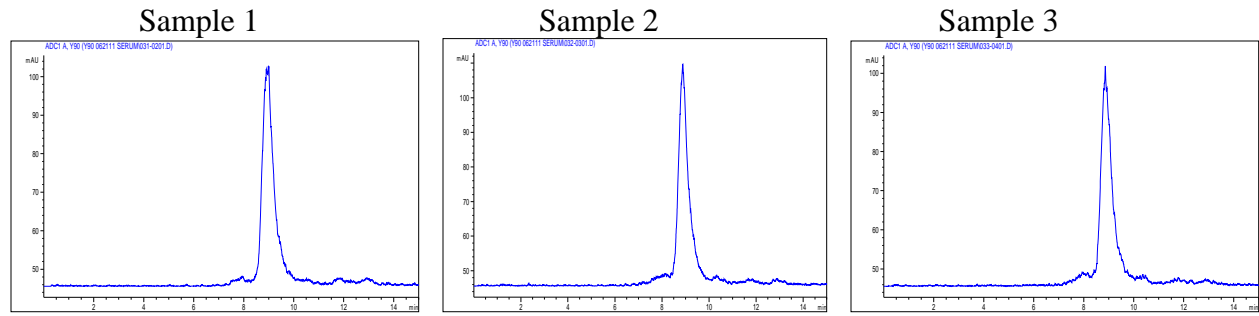
234 hr



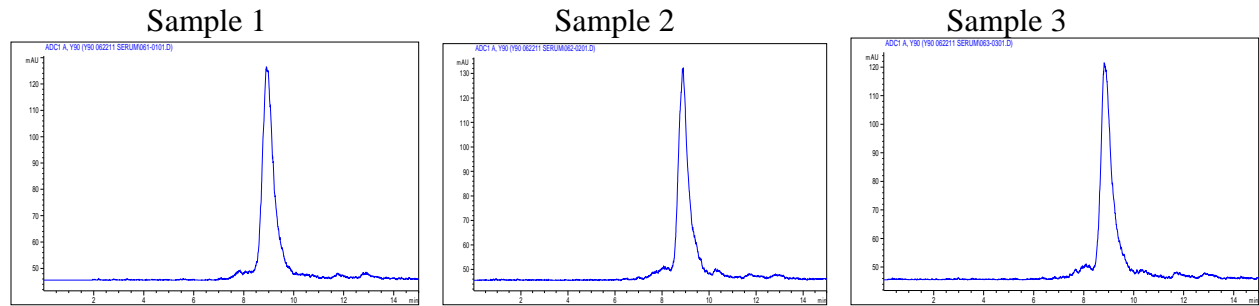
253 hr



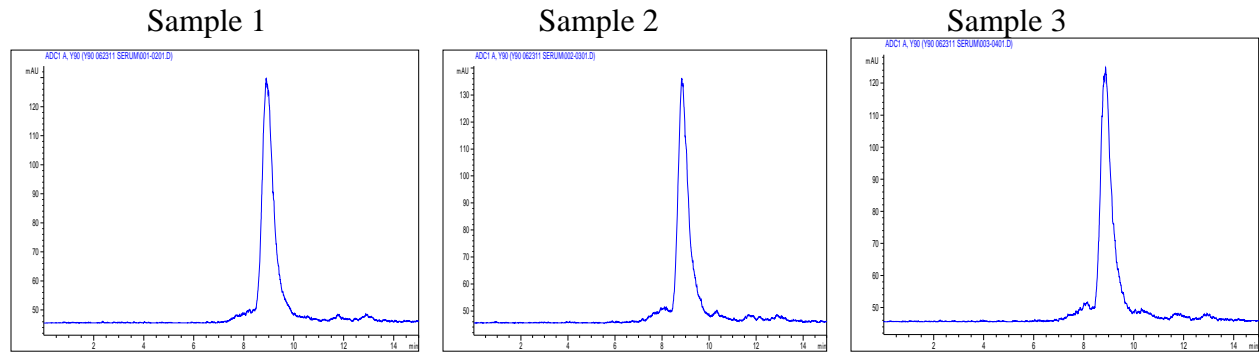
277 hr



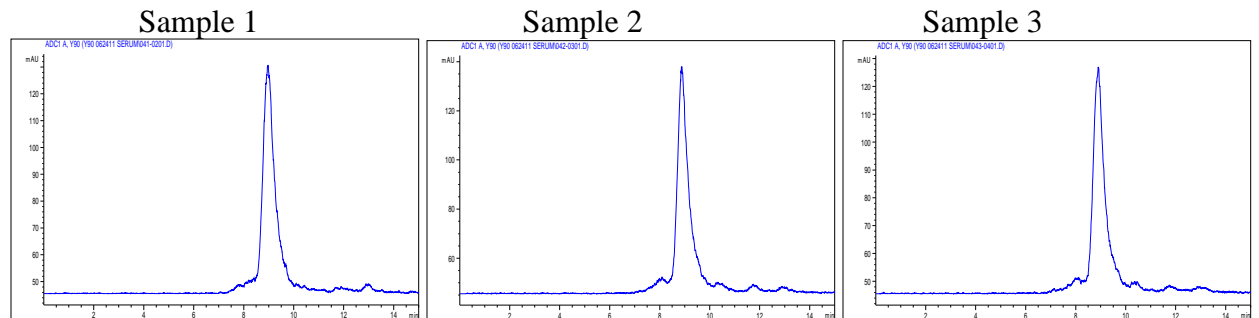
302 hr



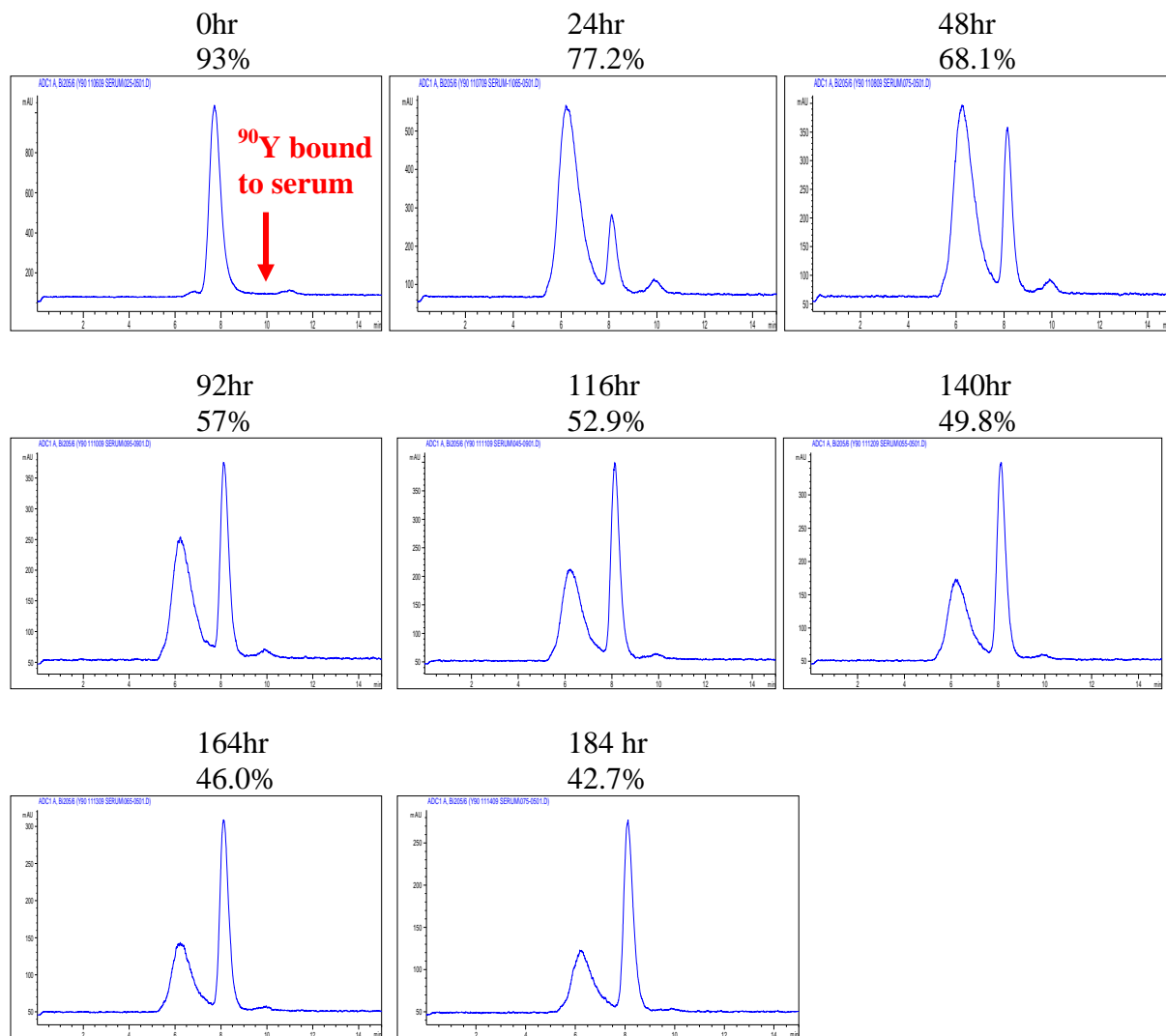
327 hr



349 hr

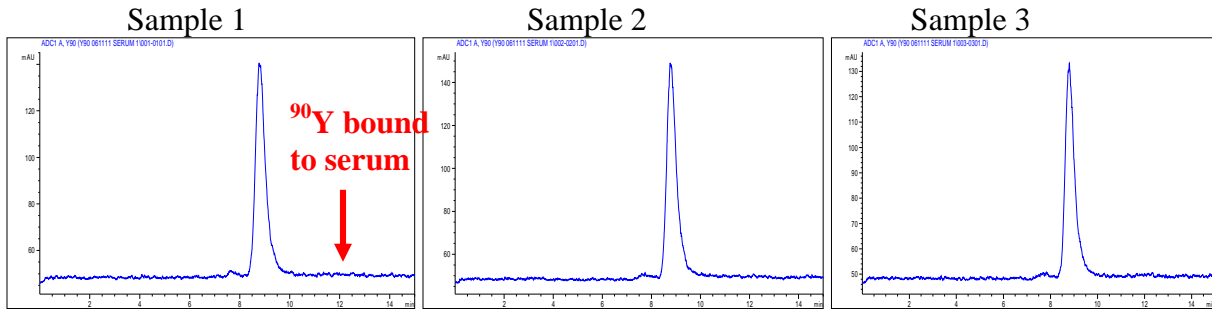


ii) ^{90}Y -3p-C-DEPA-Trastuzumab

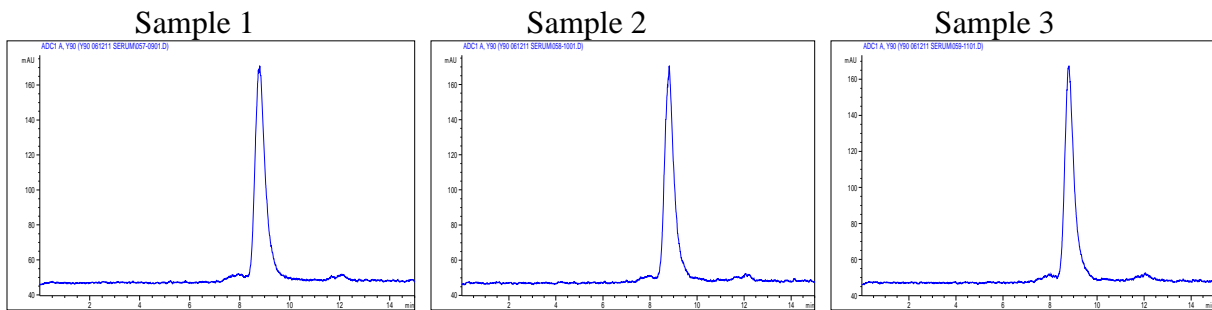


iii) ^{90}Y -C-DOTA-Trastuzumab

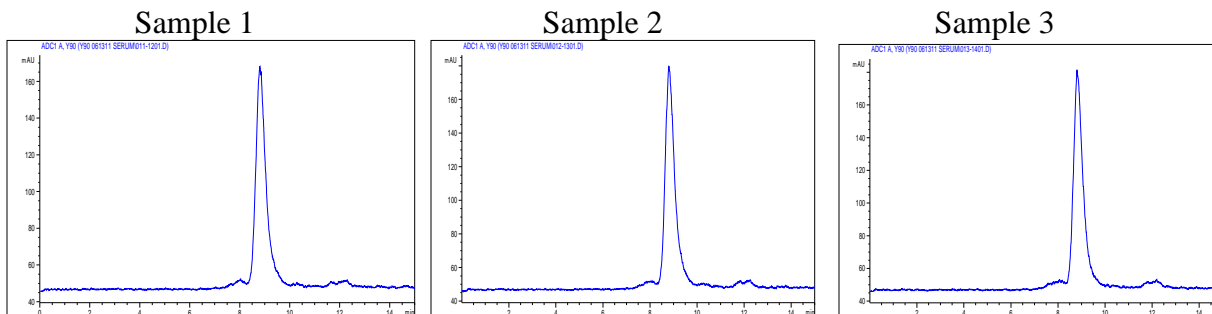
0 hr



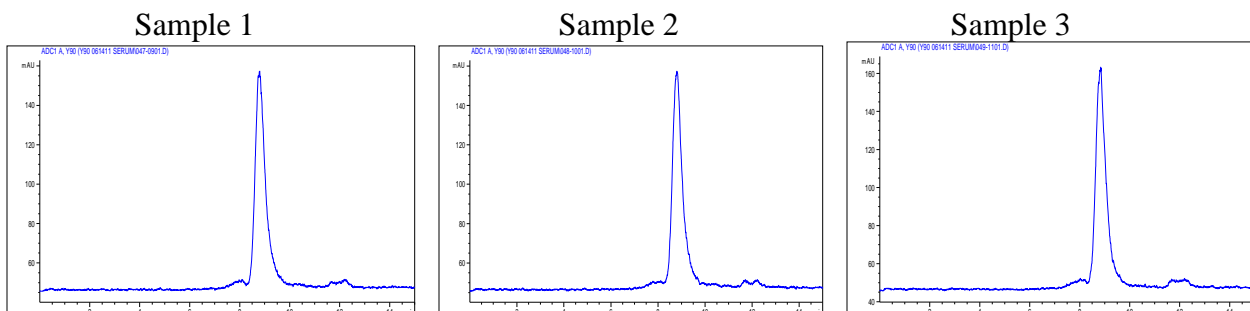
27 hr



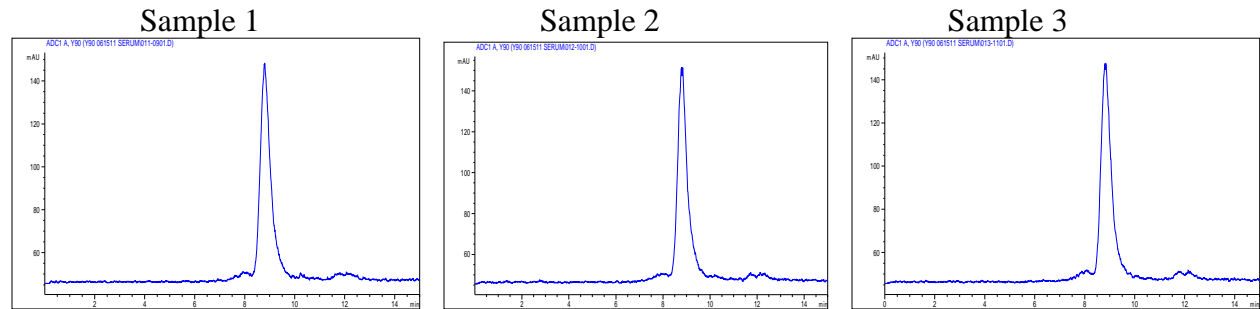
47 hr



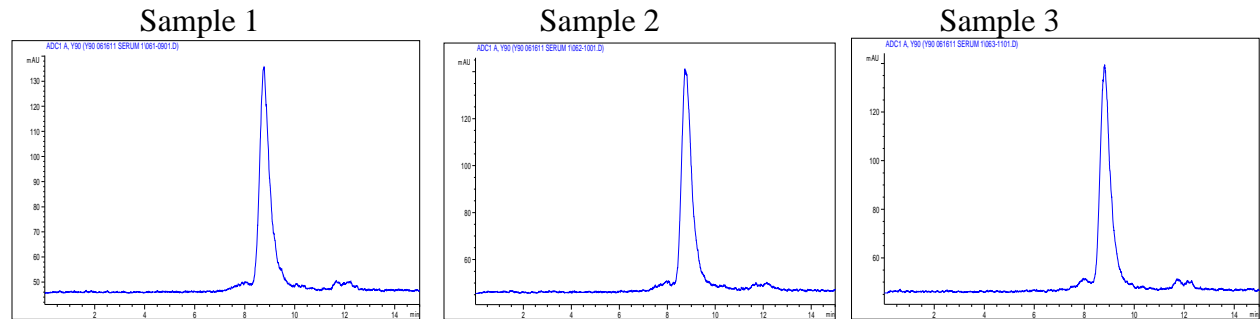
72 hr



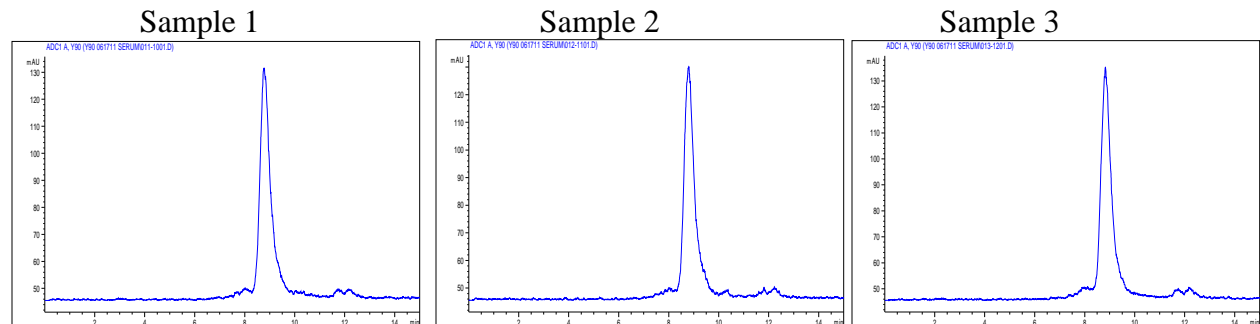
91 hr



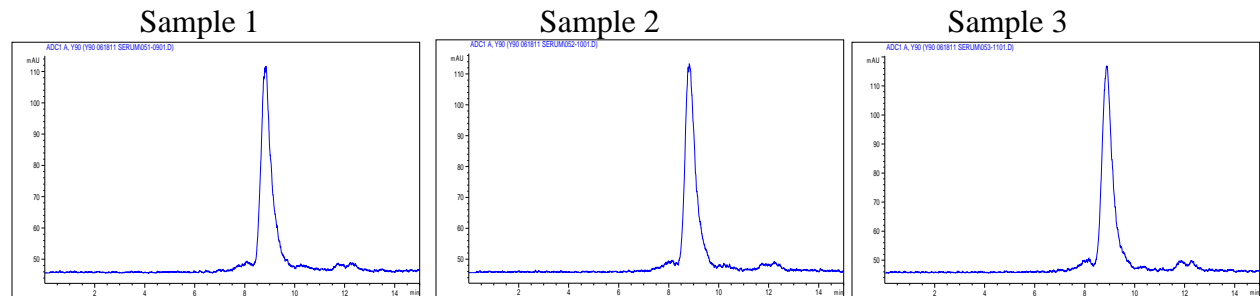
118 hr



140 hr

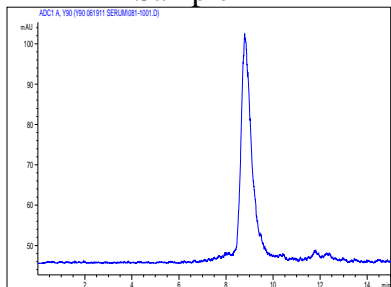


165 hr

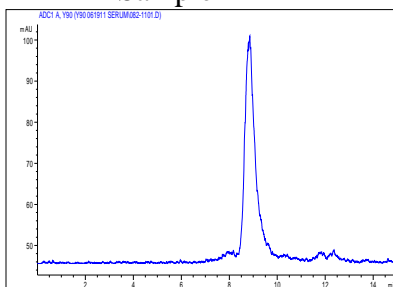


191 hr

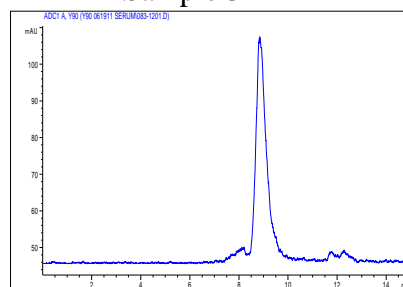
Sample 1



Sample 2

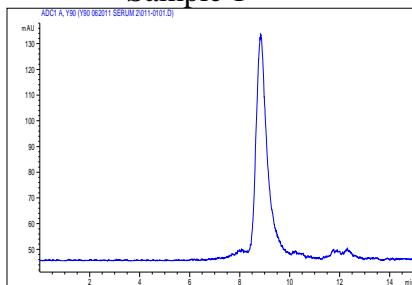


Sample 3

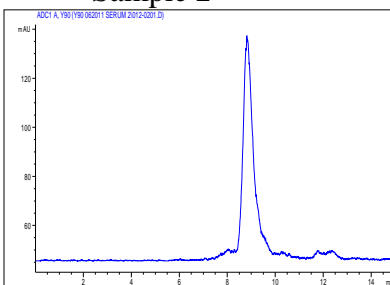


211 hr

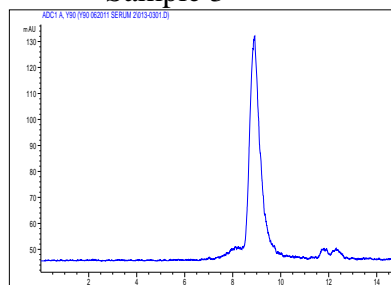
Sample 1



Sample 2

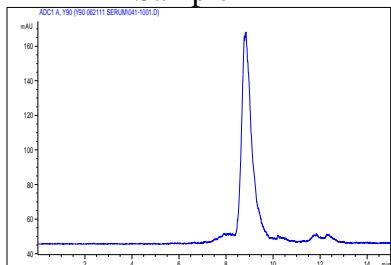


Sample 3

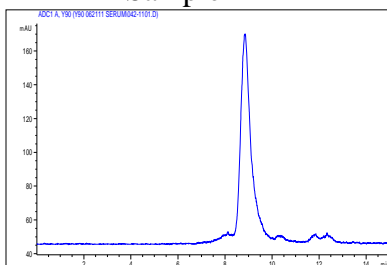


234 hr

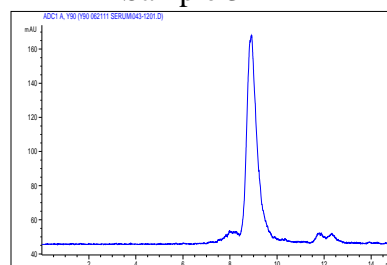
Sample 1



Sample 2

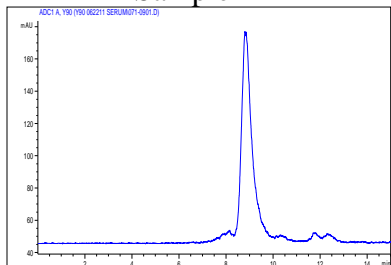


Sample 3

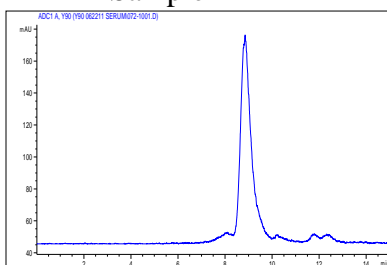


259 hr

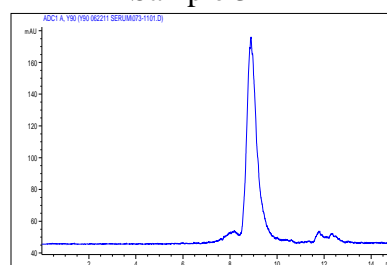
Sample 1



Sample 2

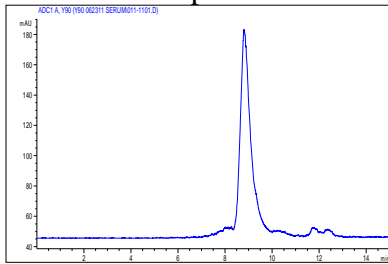


Sample 3

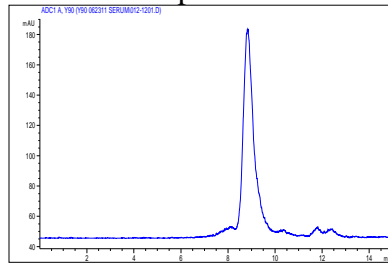


285 hr

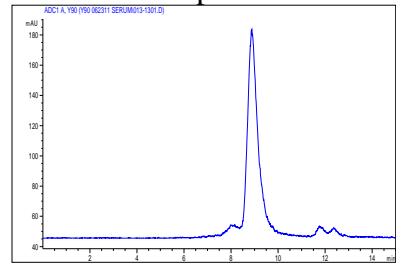
Sample 1



Sample 2

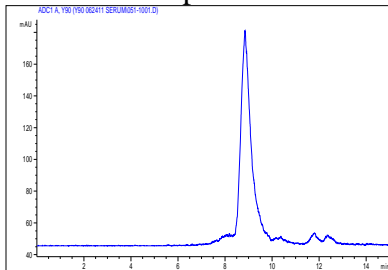


Sample 3

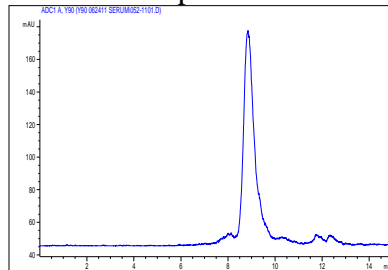


306 hr

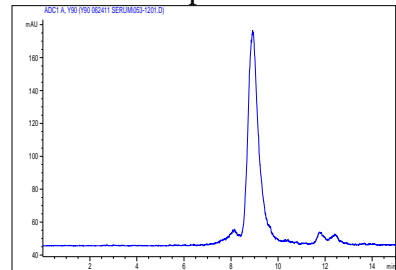
Sample 1



Sample 2

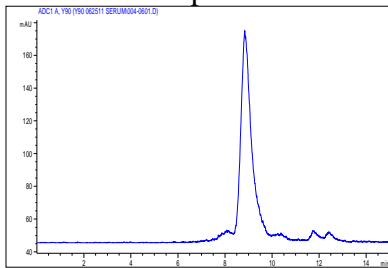


Sample 3

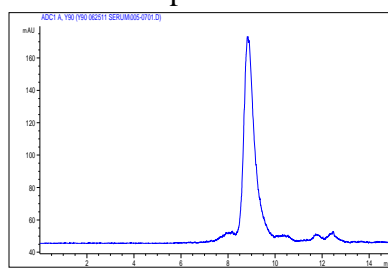


329 hr

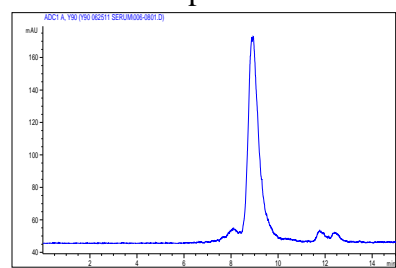
Sample 1



Sample 2

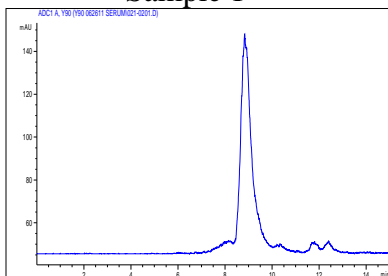


Sample 3

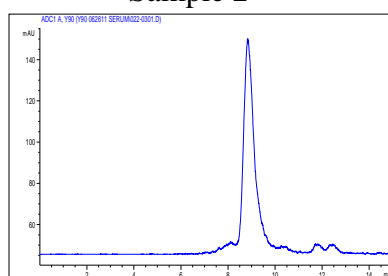


362 hr

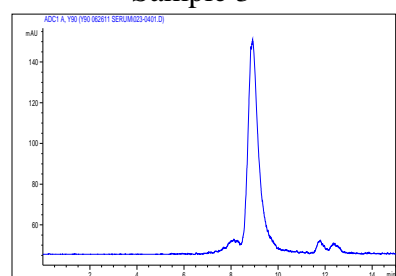
Sample 1



Sample 2



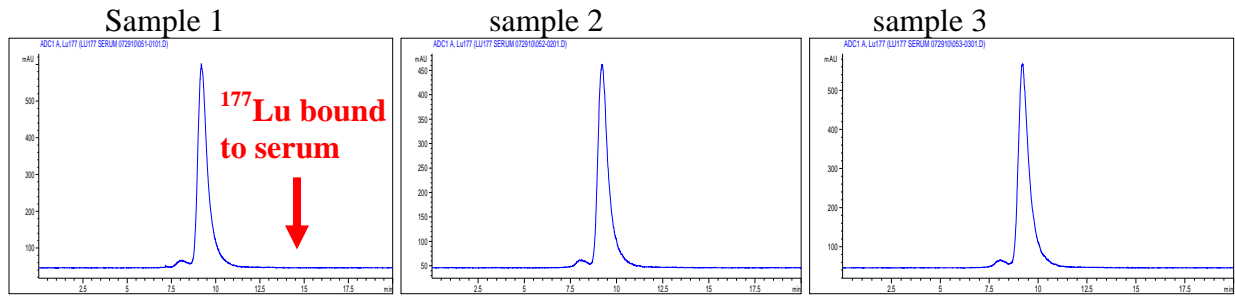
Sample 3



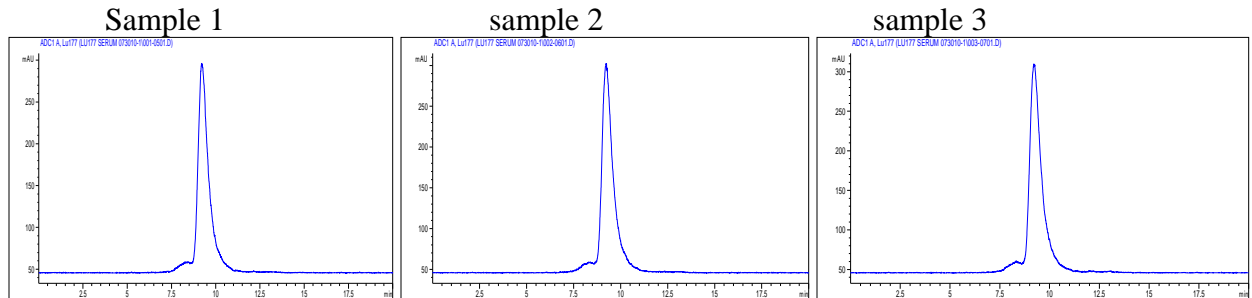
2. ^{177}Lu -radiolabeled ligand-trastuzumab conjugates

i) ^{177}Lu -3p-C-NETA-trastuzumab

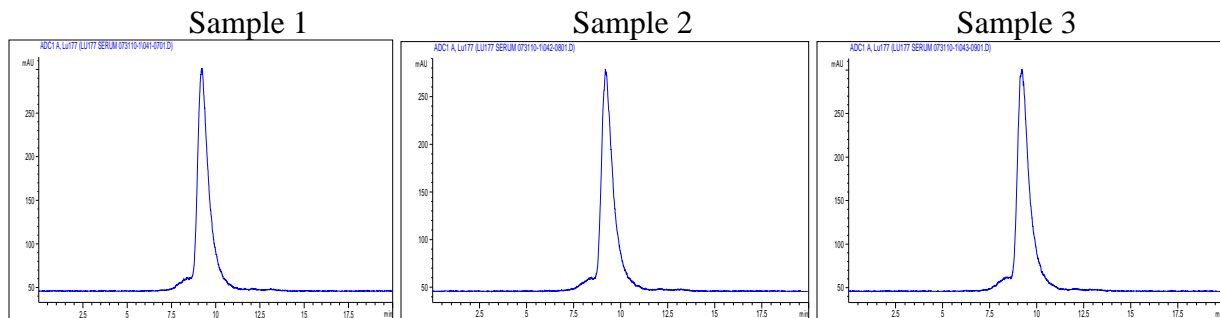
0 hr



20 hr

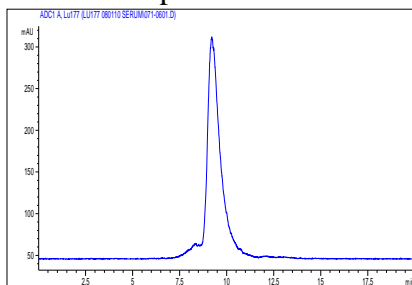


46 hr

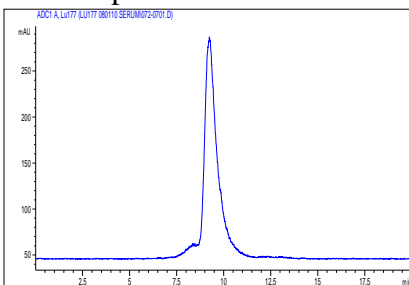


66 hr

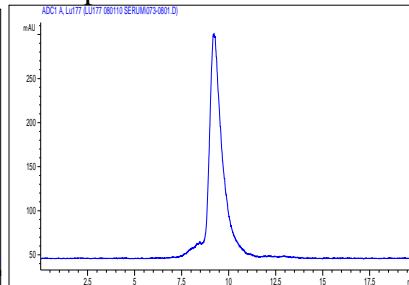
Sample 1



sample 2

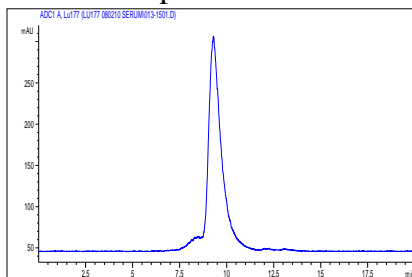


sample 3

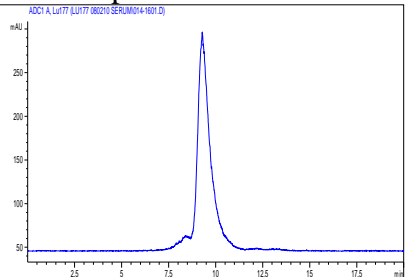


92 hr

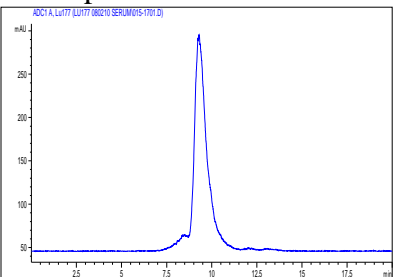
Sample 1



sample 2

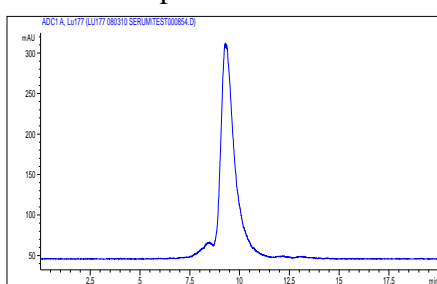


sample 3

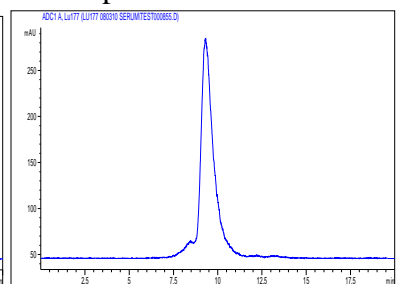


105 hr

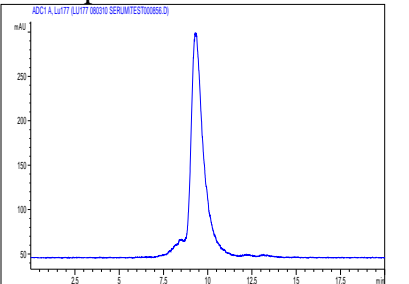
Sample 1



sample 2

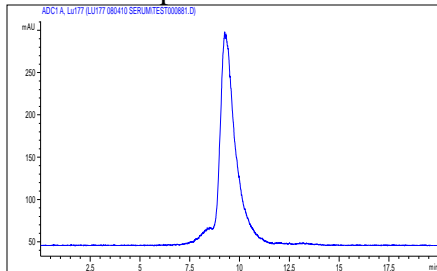


sample 3

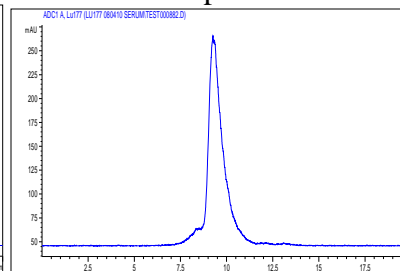


127 hr

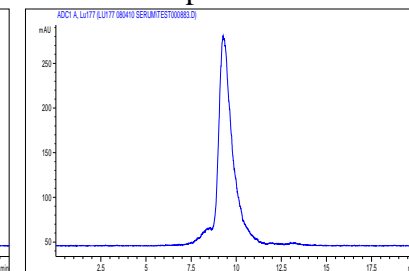
Sample 1



Sample 2

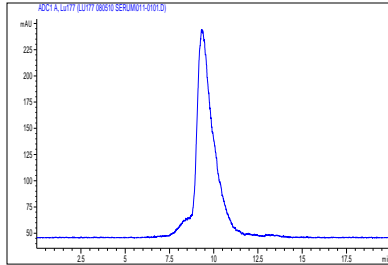


Sample 3

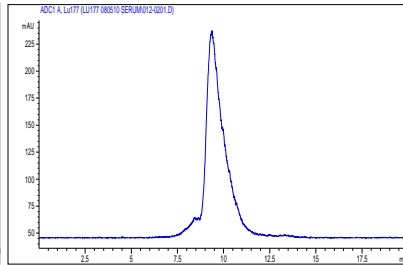


151 hr

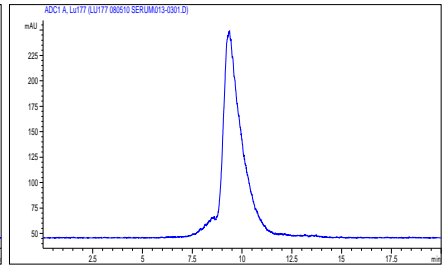
Sample 1



sample 2

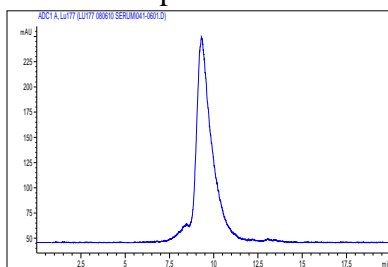


sample 3

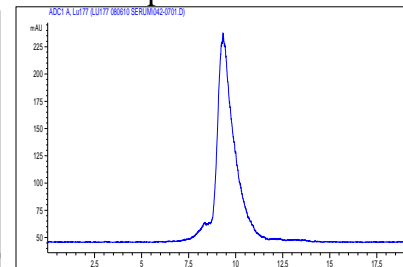


174 hr

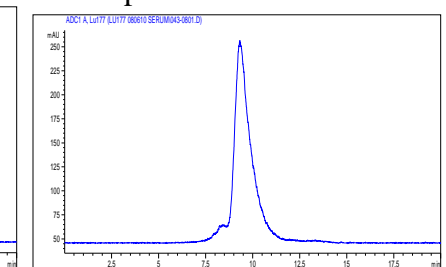
Sample 1



sample 2

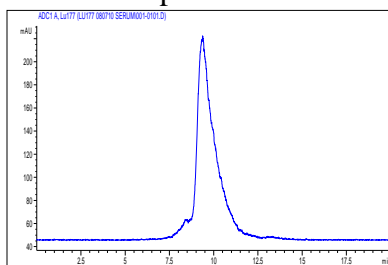


sample 3

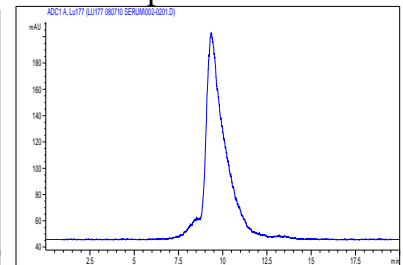


198 hr

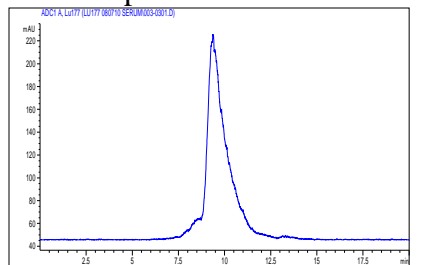
Sample 1



sample 2

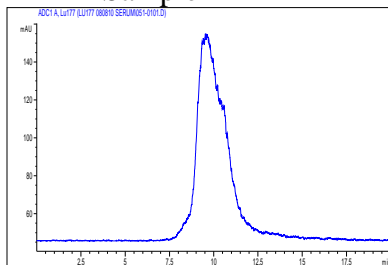


sample 3

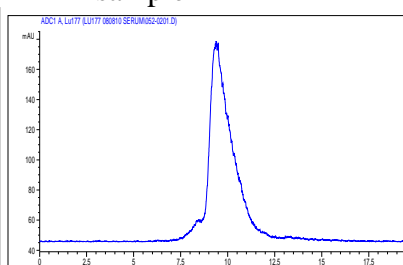


225 hr

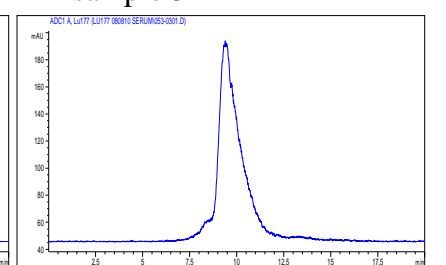
Sample 1



sample 2

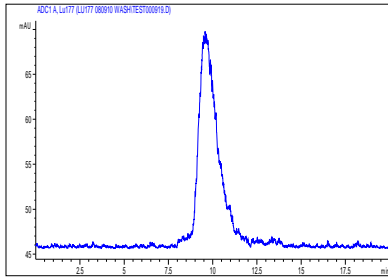


sample 3

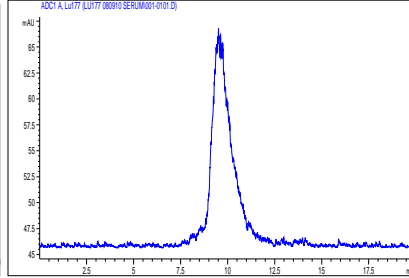


252 hr

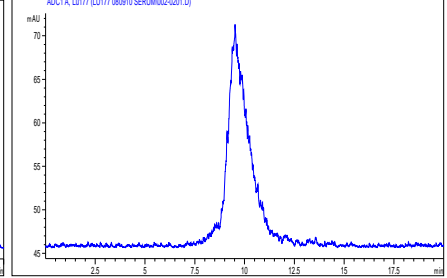
Sample 1



sample 2

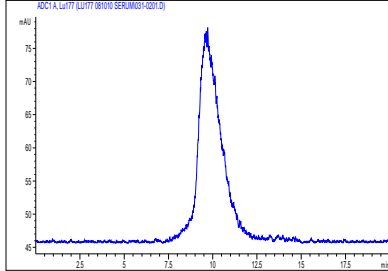


sample 3

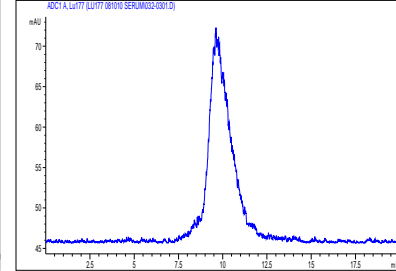


273 hr

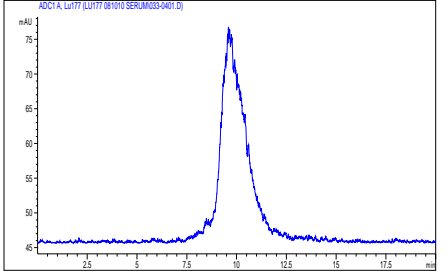
Sample 1



sample 2

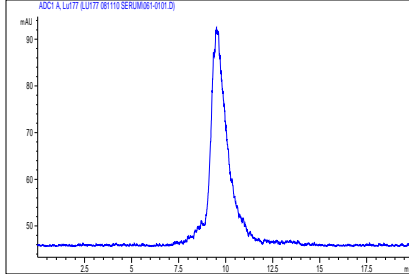


Sample 3

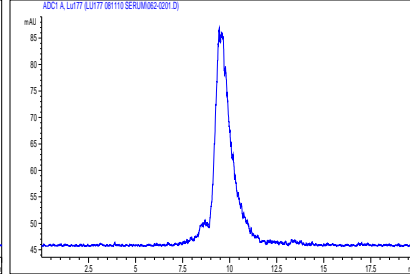


299 hr

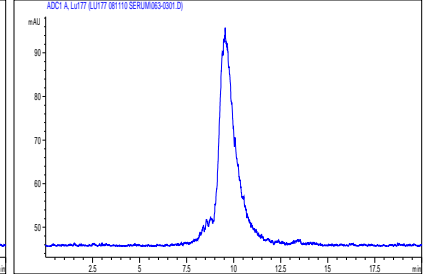
Sample 1



sample 2

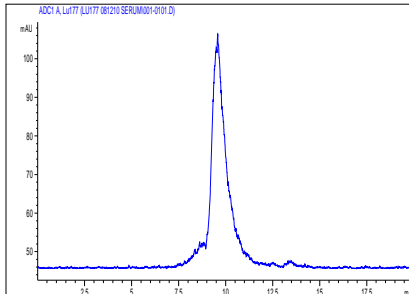


sample 3

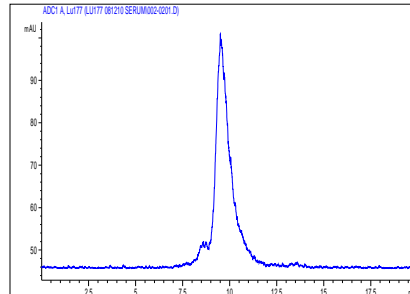


322 hr

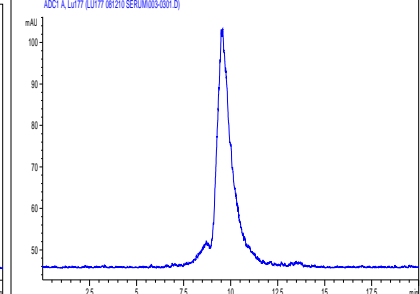
Sample 1



sample 2

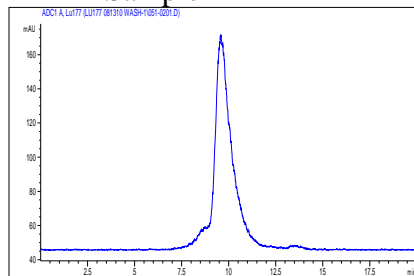


sample 3

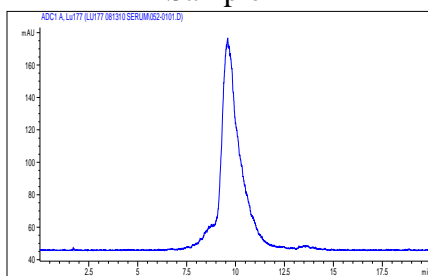


344 hr

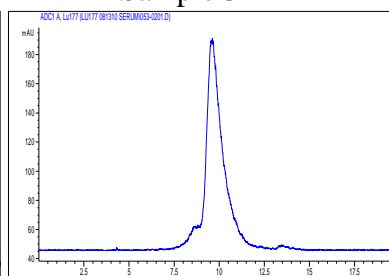
Sample 1



Sample 2



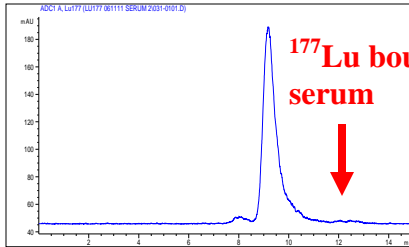
Sample 3



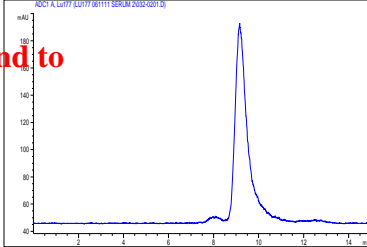
ii) ^{177}Lu -3p-C-DEPA-trastuzumab

0 hr

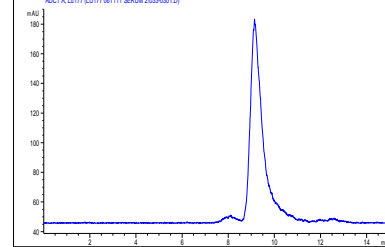
Sample 1
99.1%



Sample 2
98.7%

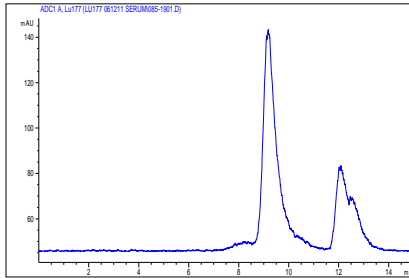


Sample 3
98.7%

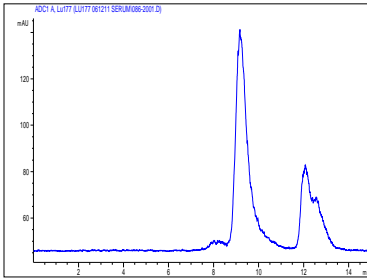


22 hr

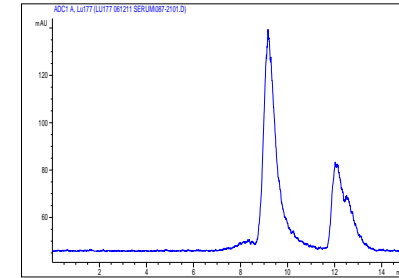
Sample 1
69.3%



Sample 2
68.9%

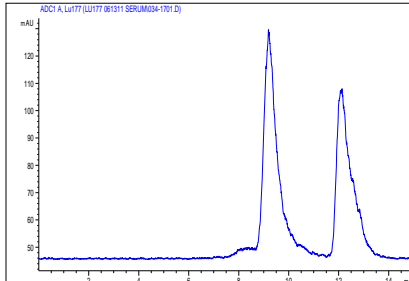


Sample 3
68.3%

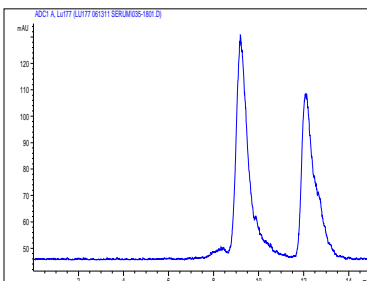


43 hr

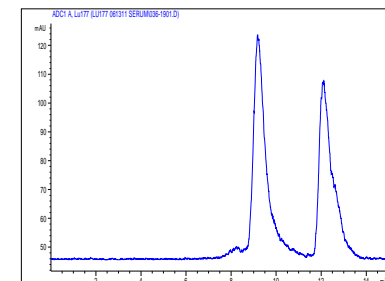
Sample 1
56.9%



Sample 2
56.9%

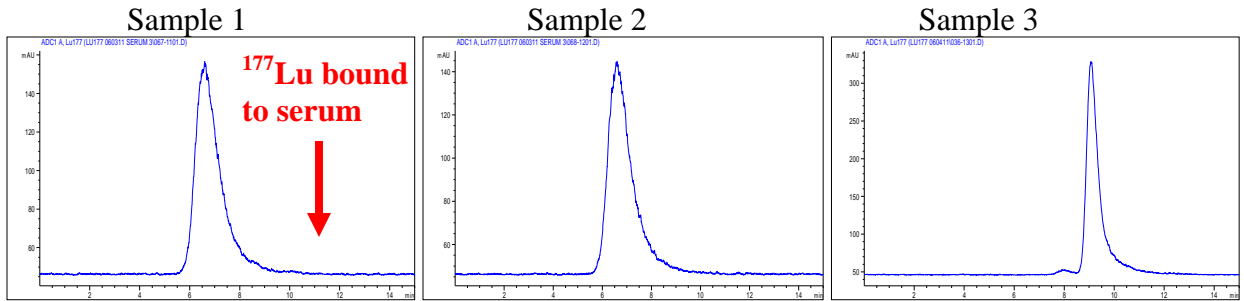


Sample 3
55.8%

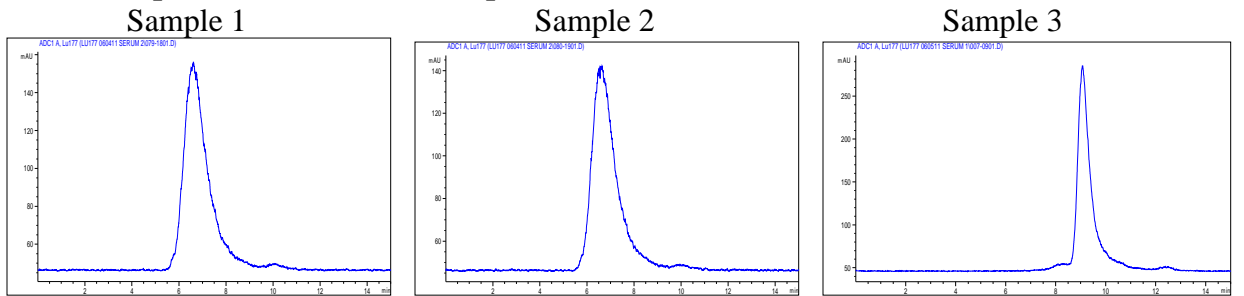


iii) ^{177}Lu -C-DOTA-Trastuzumab

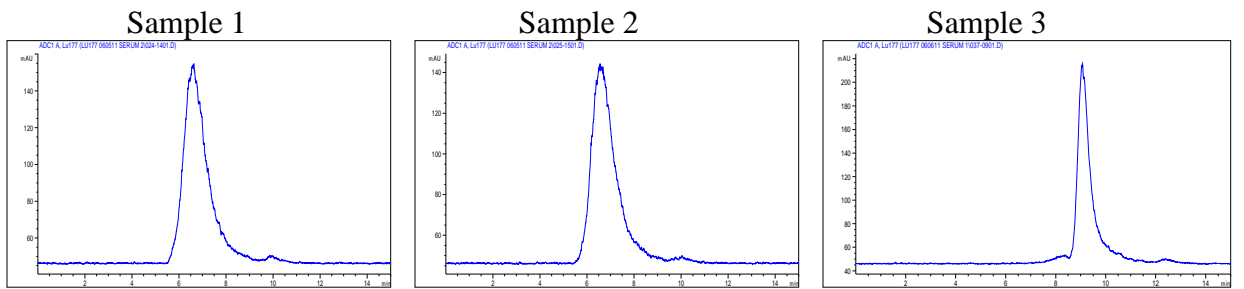
0 hr



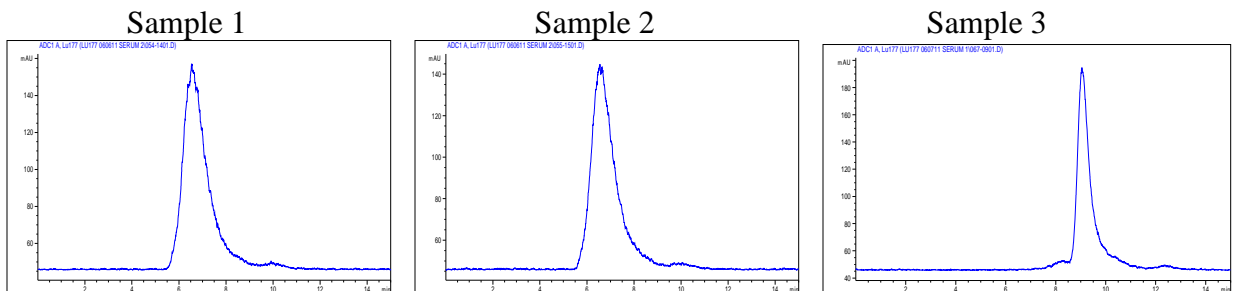
22 hr (samples 1 and 2), 20 hr (sample 3)



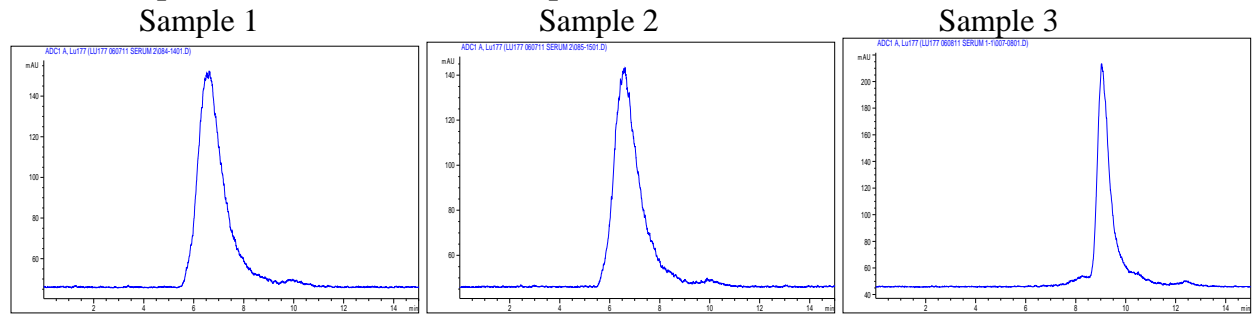
44 hr



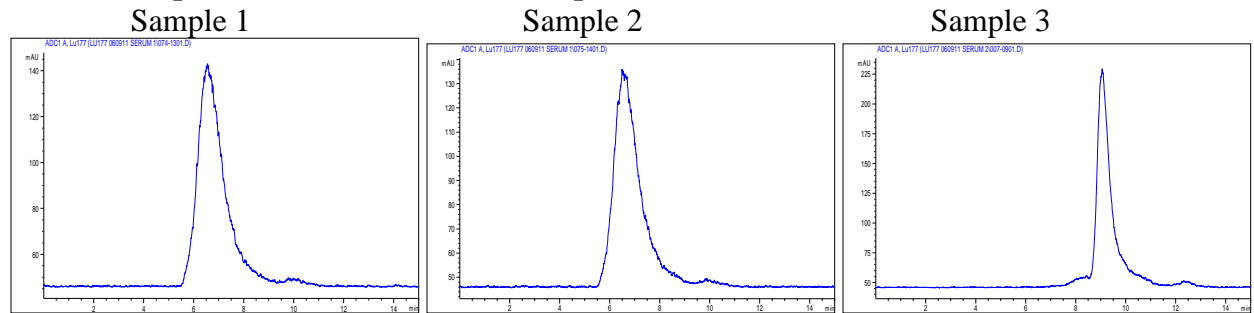
68 hr



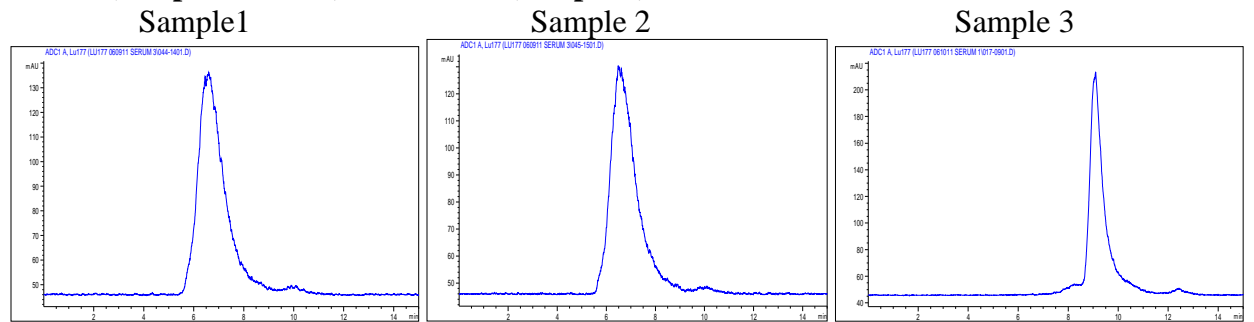
92 hr (samples 1 and 2) and 93 hr (sample 3)



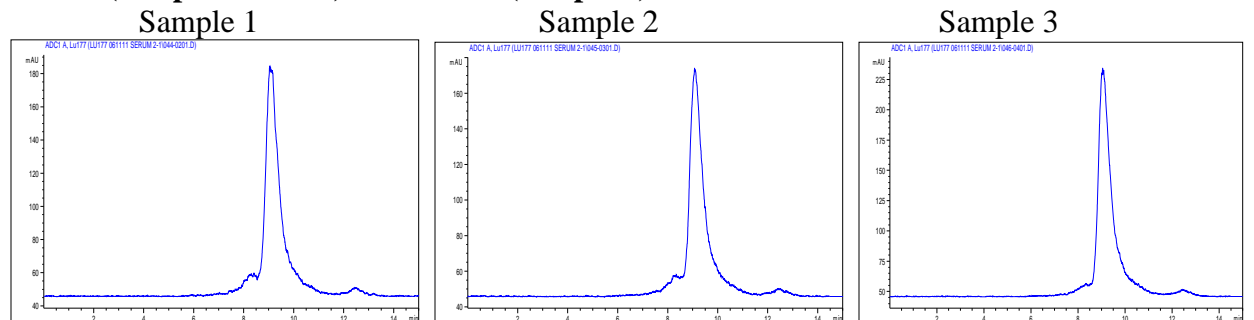
131 hr (samples 1 and 2) and 117 hr (sample 3)



143 hr (samples 1 and 2) and 147 hr (sample 3)

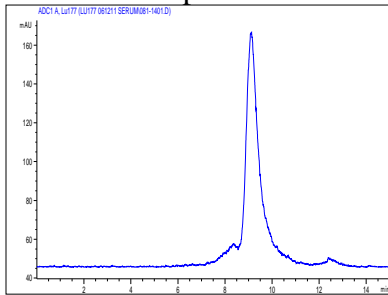


171 hr (samples 1 and 2) and 175 hr (sample 3)

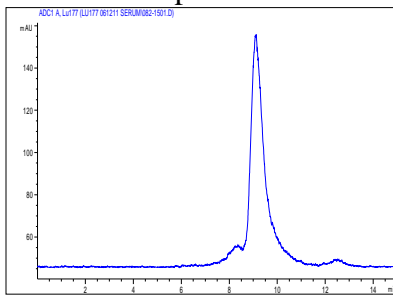


193 hr (samples 1 and 2) and 197 hr (sample 3)

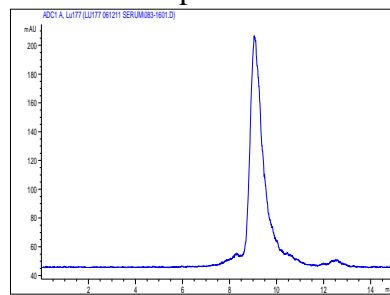
Sample 1



Sample 2

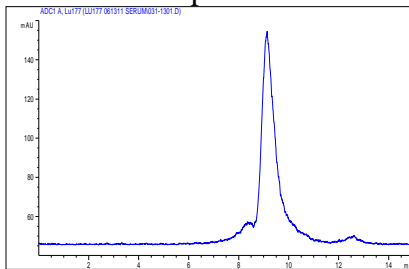


Sample 3

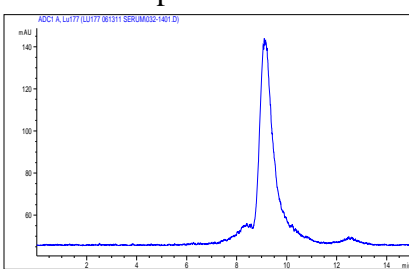


214 hr (samples 1 and 2) and 218 hr (sample 3)

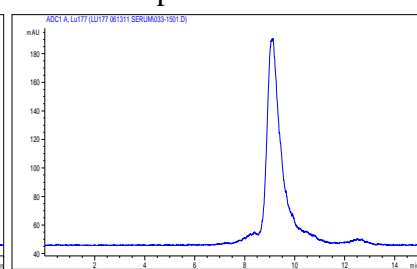
Sample 1



Sample 2

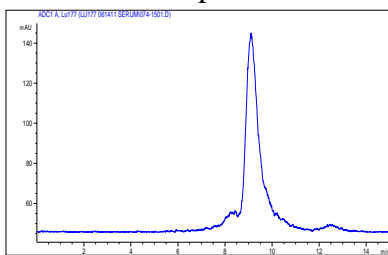


Sample 3

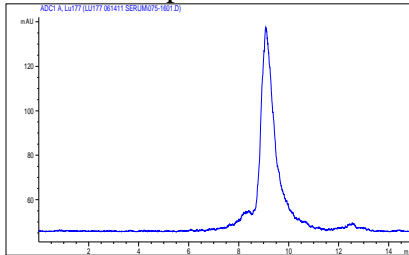


237 hr (samples 1 and 2) and 241 hr (sample 3)

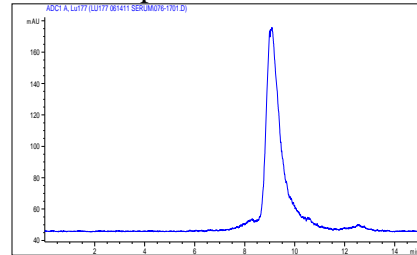
Sample 1



Sample 2

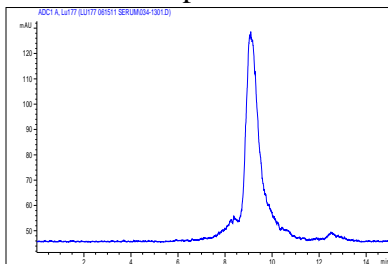


Sample 3

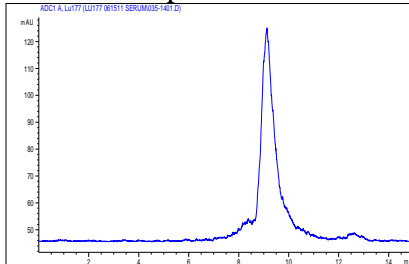


261 hr (samples 1 and 2) and 265 hr (sample 3)

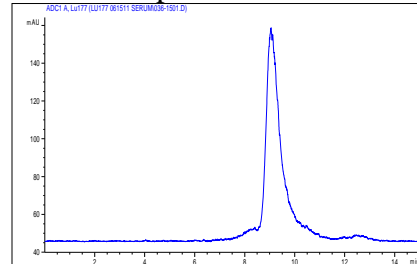
Sample 1



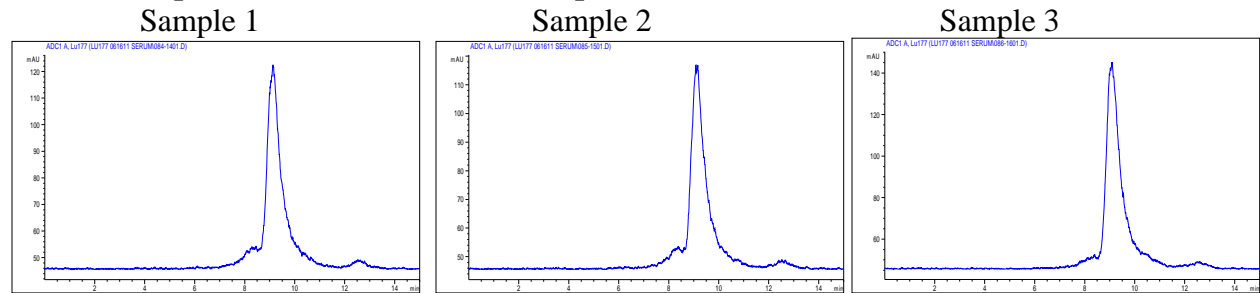
Sample 2



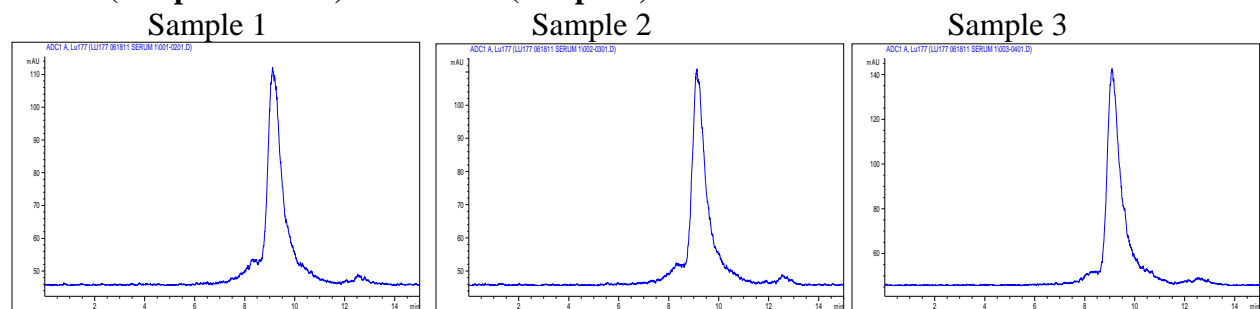
Sample 3



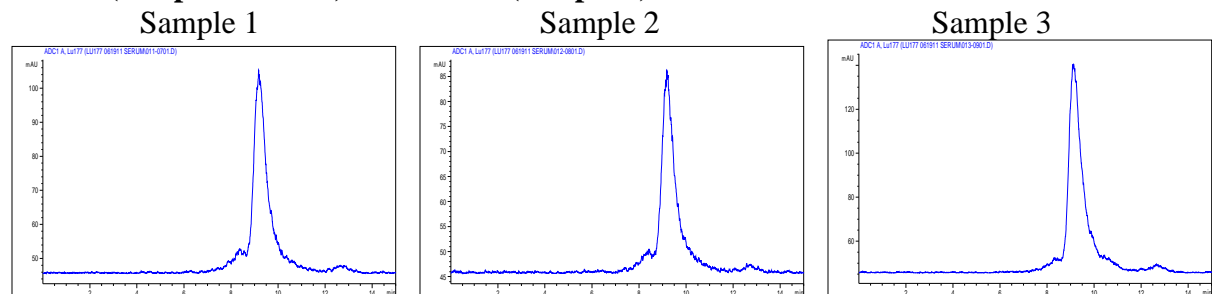
285 hr (samples 1 and 2) and 289 hr (sample 3)



322 hr (samples 1 and 2) and 326 hr (sample 3)



355 hr (samples 1 and 2) and 359 hr (sample 3)



C. *In vivo* biodistribution of ¹⁷⁷Lu-3p-C-NETA-trastuzumab conjugate in ZR-75-1-bearing mice (% ID/g, n = 3, intravenous injection)

Tissue	24 h			72 h			120 h		
blood	14.57	±	10.63	12.09	±	1.12	1.58	±	1.30
liver	9.35	±	6.85	5.09	±	0.69	2.20	±	0.06
kidney	3.50	±	2.93	2.30	±	0.69	1.09	±	0.12
bone	5.24	±	6.66	0.85	±	0.17	0.31	±	0.28
tumor	6.26	±	3.57	10.55	±	7.93	10.10	±	0.45