Supplementary Materials

^{89}Zr-radiolabelling of p-NCS-Bz-DFO-anti-HER2 affibody immunoconjugate: Characterization and assessment of *in vitro* potential in HER2-positive breast cancer imaging

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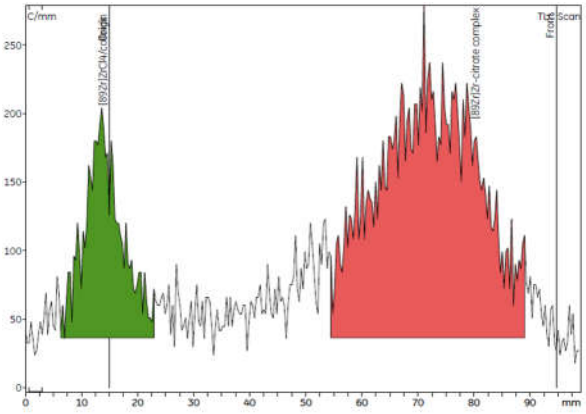
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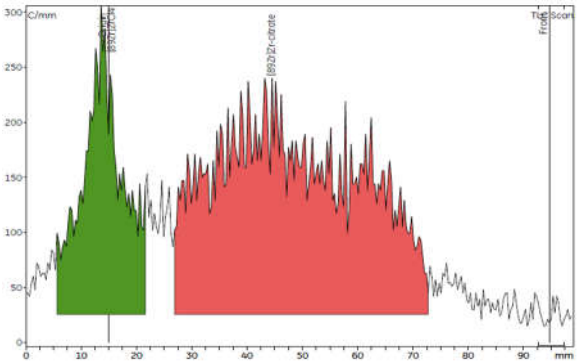
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]ZrCl4/colloids	-0.021	12.09	DD(M)	1196.857	22.69	-0.11	4.7
[89Zr]Zr-citrate complex	0.713	41.17	DD(M)	4077.143	77.31	3.80	4.6
Sum in ROI	-	-	-	5274.000	100.00	-	-
Total area	-	-	-	9903.285	-	-	-
Area (total) RF	-	-	-	8469.000	-	-	-

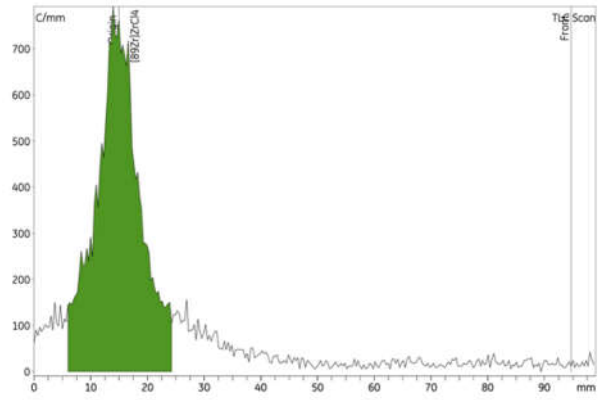
(B)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]ZrCl4	0.017	22.81	DD(M)	2067.000	25.96	-0.09	7.2
[89Zr]Zr-citrate	0.363	65.04	DD(M)	5895.000	74.04	1.93	4.9
Sum in ROI	-	-	-	7962.000	100.00	-	-
Total area	-	-	-	9063.499	-	-	-
Area (total) RF	-	-	-	7476.000	-	-	-

(C)

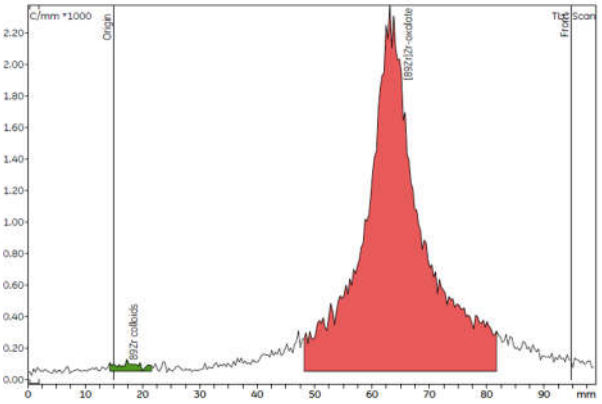


Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %
[89Zr]ZrCl4	0.008	70.53	DD(M)	6835.000	100.00
Sum in ROI	-	-	-	6835.000	100.00
Total area	-	-	-	9691.000	-
Area (total) RF	-	-	-	5586.000	-
-	-	-	-	-	-

Figure S1. Radio-TLC chromatograms for the [^{89}Zr]ZrCl$_4$ solution (target dissolved in HCl) using the three mobile phases: (A) 0.1M sodium citrate (method 1), (B) 20 mM citric acid (method 2), (C) methanol: ammonium acetate 1M= 7:3 (method 3)

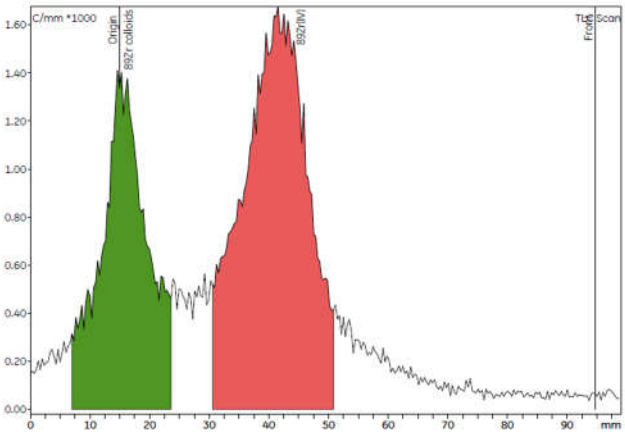
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr colloids	0.038	0.79	DD(M)	276.60	1.07	0.20	0.0
[89Zr]Zr-oxalate	0.604	73.28	DD(M)	25666.80	98.93	3.22	21.8
Sum in ROI	-	-	-	25943.40	100.00	-	-
Total area	-	-	-	35024.40	-	-	-
Area (total) RF	-	-	-	33688.00	-	-	-

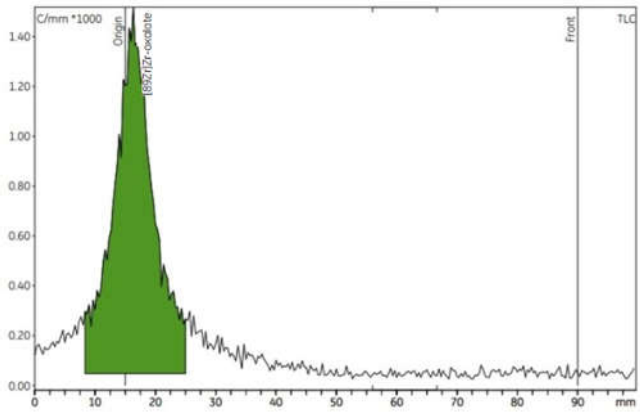
(B)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr colloids	0.004	27.97	DD(M)	12385.00	36.94	0.02	13.9
89Zr(IV)	0.338	47.74	DD(M)	21140.00	63.06	1.80	11.3
Sum in ROI	-	-	-	33525.00	100.00	-	-
Total area	-	-	-	44280.00	-	-	-
Area (total) RF	-	-	-	37513.00	-	-	-

(C)

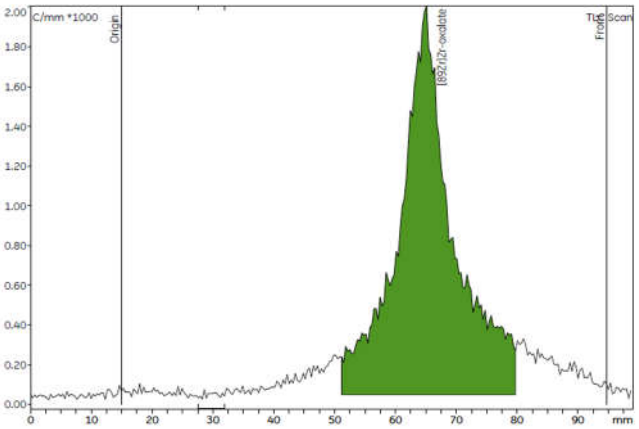


Integration TLC

Substance	R/F	%Total %	Type	Area Counts	%Area %
[89Zr]Zr-oxalate	0.018	69.40	DD(M)	10894.25	100.00
Sum in ROI	-	-	-	10894.25	100.00
Total area	-	-	-	15698.88	-
Area (total) RF	-	-	-	9246.13	-
Remainder (Tot)	-	-	-	4804.63	30.60

Figure S2. Radio-TLC chromatograms for the [^{89}Zr]Zr-oxalate (1.5 pH) solution using the three mobile phases: (A) 0.1M sodium citrate (method 1), (B) 20 mM citric acid (method 2), (C) methanol: ammonium acetate 1M= 7:3 (method 3)

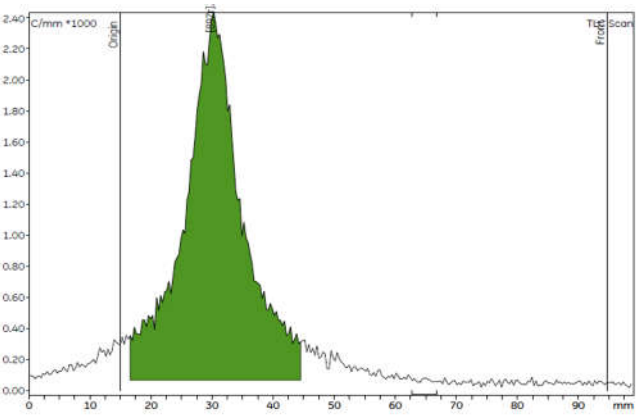
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.629	73.39	DD(M)	20127.15	100.00	3.36	24.2
Sum in ROI	-	-	-	20127.15	100.00	-	-
Total area	-	-	-	27425.46	-	-	-
Area (total) RF	-	-	-	23953.62	-	-	-
Remainder (Tot)	-	-	-	7298.31	26.61	-	-

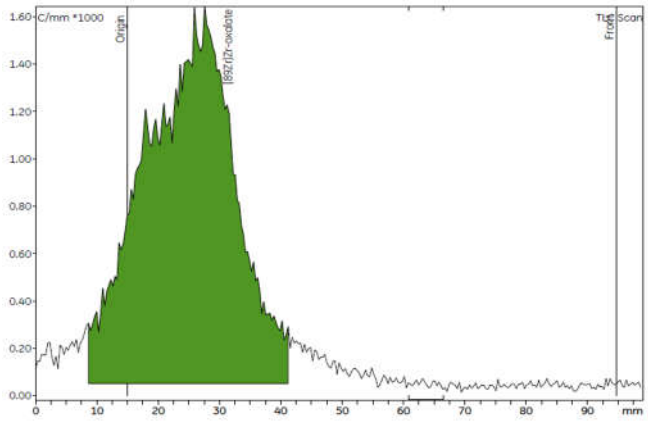
(B)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.196	82.59	DD(M)	25947.00	100.00	1.04	29.4
Sum in ROI	-	-	-	25947.00	100.00	-	-
Total area	-	-	-	31416.83	-	-	-
Area (total) RF	-	-	-	27680.00	-	-	-
Remainder (Tot)	-	-	-	5469.83	17.41	-	-

(C)

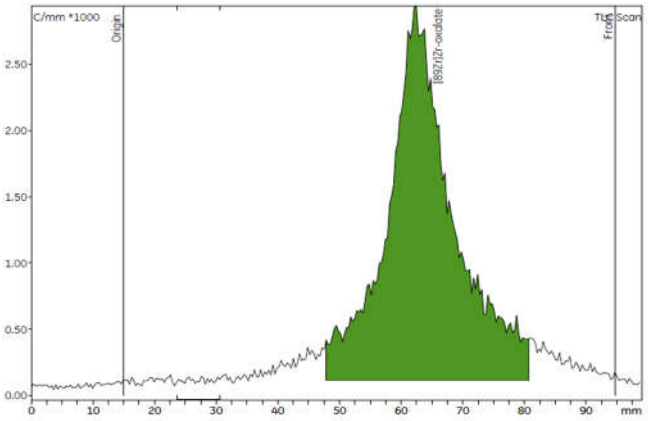


Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.163	86.44	DD(M)	26979.71	100.00	0.87	13.4
Sum in ROI	-	-	-	26979.71	100.00	-	-
Total area	-	-	-	31211.29	-	-	-
Area (total) RF	-	-	-	25663.65	-	-	-
Remainder (Tot)	-	-	-	4231.59	13.56	-	-

Figure S3. Radio-TLC chromatograms for the [^{89}Zr]Zr-oxalate (5.0 pH) solution using the three mobile phases: (A) 0.1M sodium citrate (method 1), (B) 20 mM citric acid (method 2), (C) methanol: ammonium acetate 1M= 7:3 (method 3)

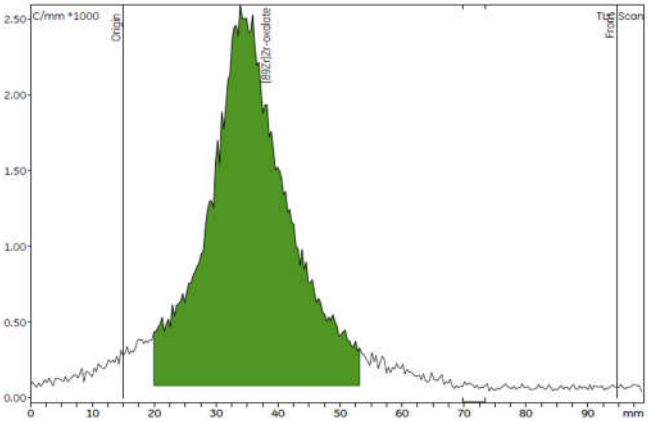
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.588	75.30	DD(M)	34454.86	100.00	3.13	27.4
Sum in ROI	-	-	-	34454.86	100.00	-	-
Total area	-	-	-	45758.43	-	-	-
Area (total) RF	-	-	-	38056.71	-	-	-
Remainder (Tot)	-	-	-	11303.57	24.70	-	-

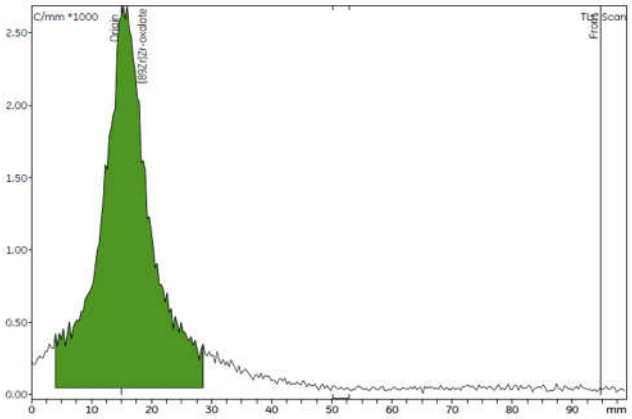
(B)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.246	85.83	DD(M)	35794.45	100.00	1.31	24.8
Sum in ROI	-	-	-	35794.45	100.00	-	-
Total area	-	-	-	41705.73	-	-	-
Area (total) RF	-	-	-	38352.09	-	-	-
Remainder (Tot)	-	-	-	5911.27	14.17	-	-

(C)

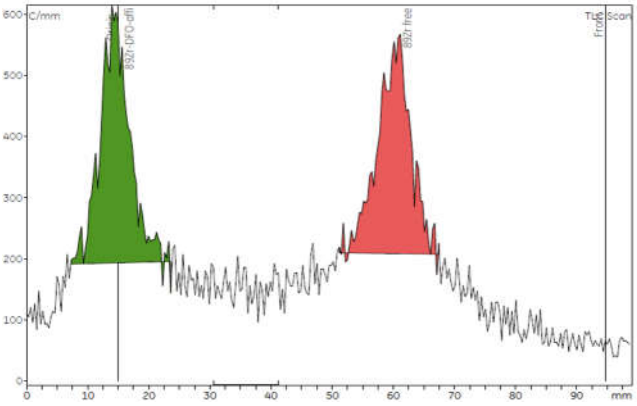


Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
[89Zr]Zr-oxalate	0.004	83.28	DD(M)	24706.25	100.00	0.02	32.3
Sum in ROI	-	-	-	24706.25	100.00	-	-
Total area	-	-	-	29667.25	-	-	-
Area (total) RF	-	-	-	16598.00	-	-	-
Remainder (Tot)	-	-	-	4961.00	16.72	-	-

Figure S4. Radio-TLC chromatograms for the [^{89}Zr]Zr-oxalate (7.0 pH) solution using the three mobile phases: (A) 0.1M sodium citrate (method 1), (B) 20 mM citric acid (method 2), (C) methanol: ammonium acetate 1M= 7:3 (method 3)

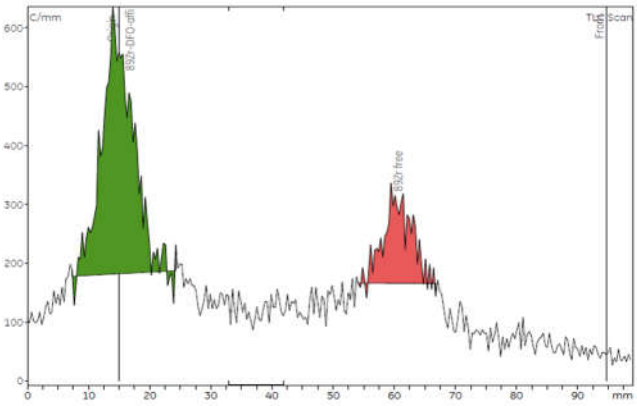
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	-0.008	16.32	BB(M)	2296.000	50.99	-0.04	7.4
89Zr free	0.575	15.69	BB(M)	2206.500	49.01	3.07	6.0
Sum in ROI	-	-	-	4502.500	100.00	-	-
Total area	-	-	-	14067.344	-	-	-
Area (total) RF	-	-	-	3373.501	-	-	-
Remainder (Tot)	-	-	-	9564.84	67.99	-	-

(B)

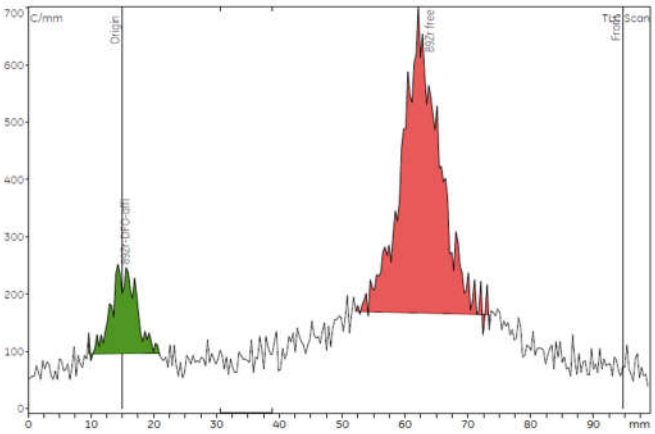


Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	-0.004	22.23	BB(M)	2501.286	75.55	-0.02	6.8
89Zr free	0.567	7.19	BB(M)	809.429	24.45	3.02	3.0
Sum in ROI	-	-	-	3310.714	100.00	-	-
Total area	-	-	-	11254.036	-	-	-
Area (total) RF	-	-	-	2268.222	-	-	-
Remainder (Tot)	-	-	-	7943.32	70.58	-	-

Figure S5. Radio TLC chromatograms obtained after (A) 1 hour and (B) 12 hours, respectively, following the analysis of the [^{89}Zr]Zr-p-SCN-Bz-DFO-anti-HER2 affibody solution labelled at 7.5-8.0 pH (Experiment 3)

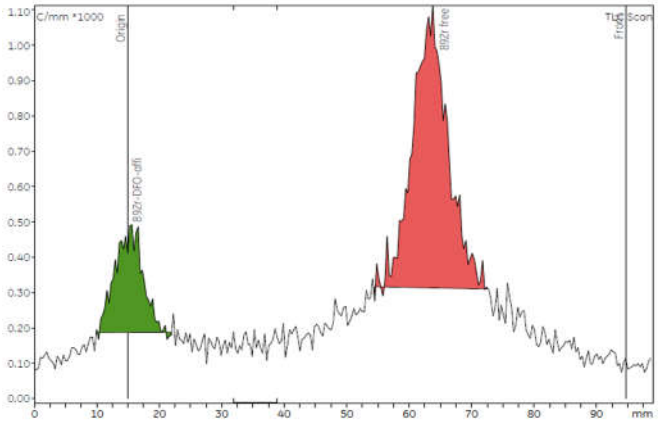
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	0.000	5.73	BB(M)	705.571	16.35	0.00	3.1
89Zr free	0.592	29.31	BB(M)	3609.857	83.65	3.16	9.9
Sum in ROI	-	-	-	4315.428	100.00	-	-
Total area	-	-	-	12316.760	-	-	-
Area (total) RF	-	-	-	6506.600	-	-	-
Remainder (Tot)	-	-	-	8001.33	64.96	-	-

(B)

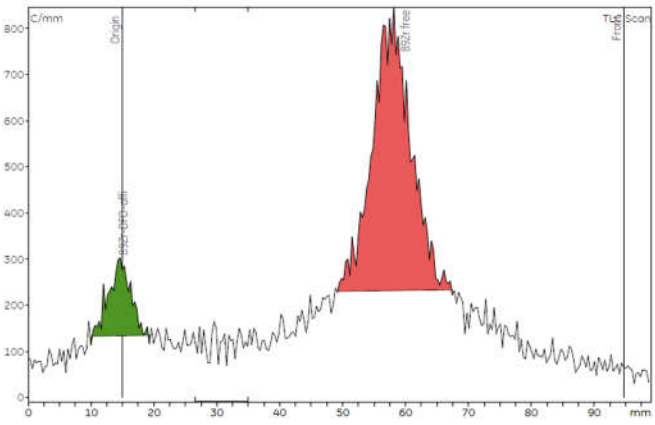


Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	0.013	7.45	BB(M)	1535.000	22.92	0.07	3.2
89Zr free	0.608	25.07	BB(M)	5162.000	77.08	3.24	10.8
Sum in ROI	-	-	-	6697.000	100.00	-	-
Total area	-	-	-	20592.904	-	-	-
Area (total) RF	-	-	-	10360.142	-	-	-
Remainder (Tot)	-	-	-	13895.90	67.48	-	-

Figure S6. Radio TLC chromatograms obtained after (A) 1 hour and (B) 12 hours, respectively, following the analysis of the [^{89}Zr]Zr-p-SCN-Bz-DFO-anti-HER2 affibody solution labelled at 8.0-8.5 pH (Experiment 3)

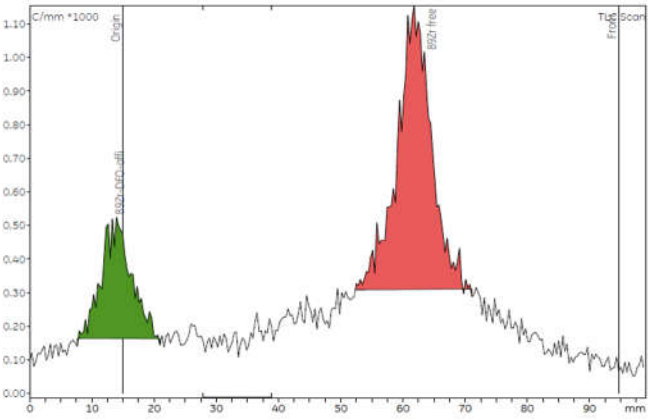
(A)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	-0.004	4.33	BB(M)	663.000	13.41	-0.02	3.3
89Zr free	0.542	27.94	BB(M)	4279.286	86.59	2.89	9.9
Sum in ROI	-	-	-	4942.285	100.00	-	-
Total area	-	-	-	15314.560	-	-	-
Area (total) RF	-	-	-	7325.201	-	-	-
Remainder (Tot)	-	-	-	10372.27	67.73	-	-

(B)



Integration TLC Scan

Substance	R/F	%Total %	Type	Area Counts	%Area %	Cap fac	S/N ratio
89Zr-DFO-affi	-0.013	9.31	BB(M)	1982.857	26.97	-0.07	5.5
89Zr free	0.592	25.22	BB(M)	5369.000	73.03	3.16	12.5
Sum in ROI	-	-	-	7351.857	100.00	-	-
Total area	-	-	-	21289.575	-	-	-
Area (total) RF	-	-	-	9933.181	-	-	-
Remainder (Tot)	-	-	-	13937.72	65.47	-	-

Figure S7. Radio TLC chromatograms obtained after (A) 1 hour and (B) 12 hours, respectively, following the analysis of the [^{89}Zr]Zr-p-SCN-Bz-DFO-anti-HER2 affibody solution labelled at 9.0 pH (Experiment 3)