SUPPLEMENTARY MATERIAL

Rapid metabolism of the immuno-PET radioligand [^{68}Ga]Ga-NOTA-GZP upon *in vivo* administration in mice

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SUPPLEMENTARY MATERIAL

List of supplementary figures

Figure S1: Radio-TLC chromatogram of [^{68}Ga]Ga-NOTA-GZP in 1M ammonium acetate: MeOH (1:1) (A) and 0.1M citrate (B) as a mobile phase in SG iTLC paper as stationary phase.

Figure S2. LC-RAD-MS of the final formulated product ([^{68}Ga]Ga-NOTA-GZP). Extracted chromatogram for the precursor mass (NOTA-GzP) at *m/z* 977.4566 (A), radiochromatogram of the formulated radiotracer (B) and extracted chromatogram for the radiotracer mass ([^{68}Ga]Ga-NOTA-GZP) at *m/z* 1030.3692.

Figure S3. Mass spectra of produced radiotracer showing the precursor signal NOTA-GzP at *m/z* 977.4566 corresponding to $[M+H_2O+H]^+$ and the $[M+2H]^{+2}$ ion at *m/z* 489.2324. Spectra corresponding to the chromatogram in Figure S5-A

Figure S4. Mass spectra of the produced radiotracer [^{68}Ga]Ga-NOTA-GzP showing signals at *m/z* 1030.3711 corresponding to $[M+H]^+$, 1048.3811 corresponding to $[M+H_2O+H]^+$ and the $[M+2H]^{+2}$ ion at *m/z* 515.6902. Spectra corresponding to the chromatogram in Figure S5-C.

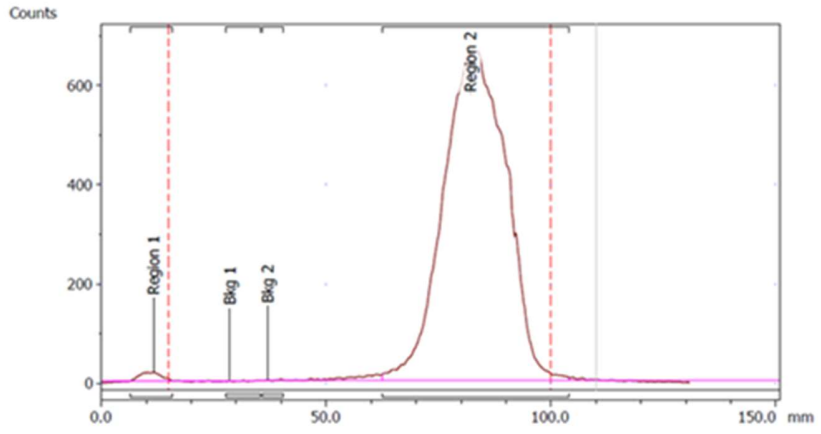
Figure S5: Radio-TLC chromatogram of [$^{68}Ga^{+3}$]$GaCl_3$ in 1M ammonium acetate: MeOH (1:1) (A) and 0.1M citrate (B) as a mobile phase in SG iTLC paper as stationary phase.

Figure S6: Radio-TLC chromatogram of [$^{68}Ga^{+3}$]Ga-NOTA in 1M ammonium acetate: MeOH (1:1) mobile phase in SG iTLC paper as stationary phase.

Figure S7. Structural comparison of two [^{68}Ga]Ga-NOTA-GZP granzyme B–targeted peptide conjugates. *Upper panel*: [^{68}Ga]Ga-NOTA-GZP in which the NOTA chelator is directly conjugated to the β-Ala–Gly–Gly–Ile–Glu–Phe–Asp–CHO granzyme B–targeting peptide through an amide linkage. *Lower panel*: benzyl-isothiocyanate-derived [^{68}Ga]Ga-NOTA-GZP analogue in which the chelator is connected to the peptide through a p-benzyl–thiourea linker. Dotted bonds indicate coordination of Ga-68 within the NOTA chelation cavity.

SUPPLEMENTARY MATERIAL

A

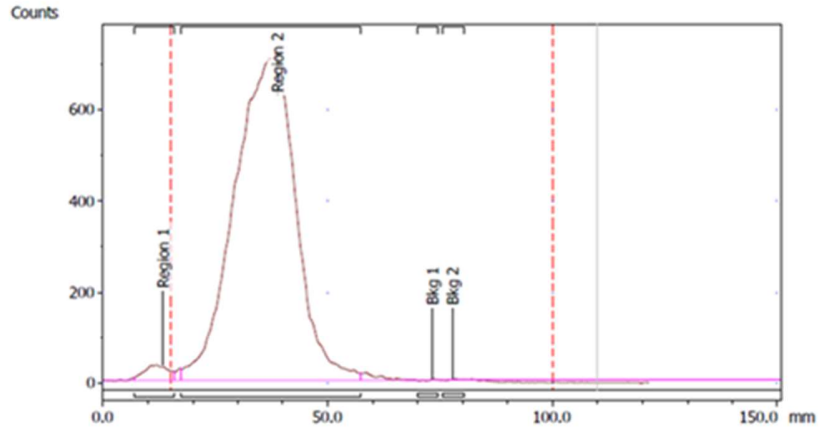


Regions: ^{68}Ga Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	6.4	15.7	-0.040	996	0.92	0.91
Bkg 1	27.7	35.4	0.160			
Bkg 2	35.8	40.4	0.259			
Region 2	62.4	104.1	0.790	107871	99.08	98.40
2 Peaks				108867	100.00	99.31

Total Area: 109629 Counts
Average Background: 6 Counts

B



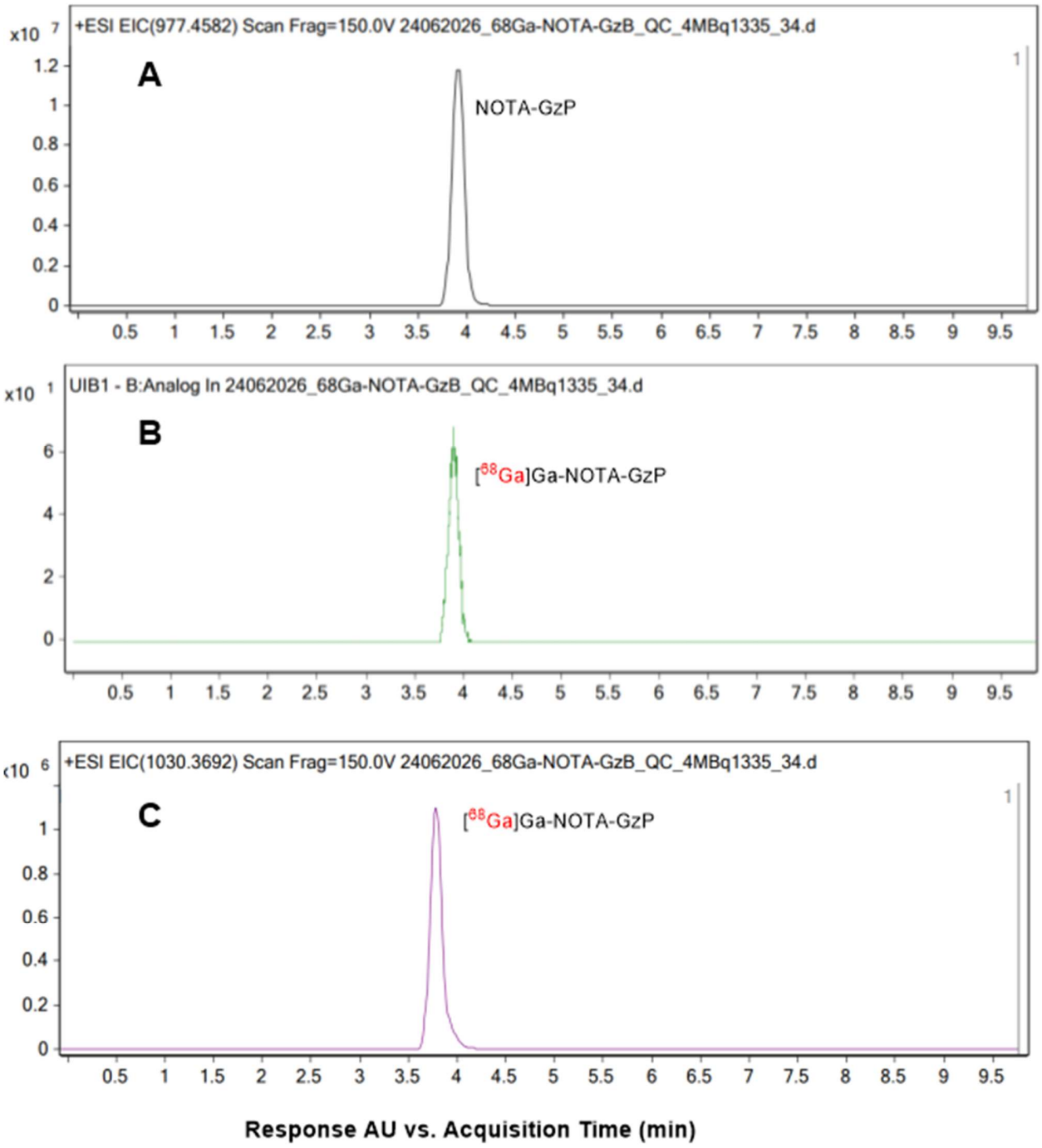
Regions: ^{68}Ga Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	7.0	15.9	-0.019	2005	1.76	1.77
Region 2	17.3	57.4	0.281	111581	98.24	98.59
Bkg 1	70.0	74.5	0.687			
Bkg 2	75.4	80.4	0.739			
2 Peaks				113586	100.00	100.36

Total Area: 113182 Counts
Average Background: 7 Counts

Figure S1: Radio-iTLC chromatogram of [^{68}Ga]Ga-NOTA-GZP in 1M ammonium acetate: MeOH (1:1) (A) and 0.1M citrate (B) as mobile phase in SG iTLC paper as stationary phase.

SUPPLEMENTARY MATERIAL



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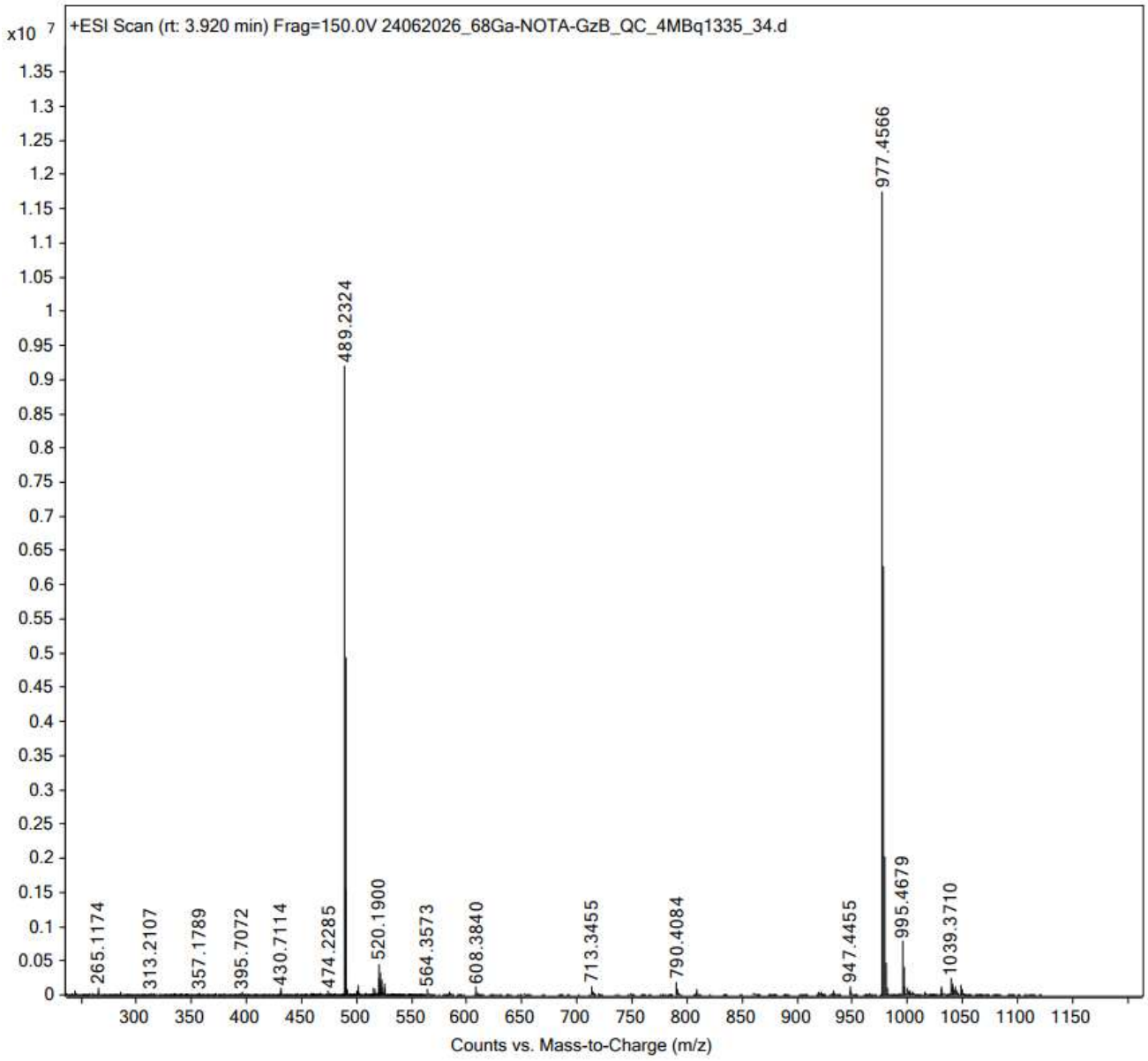
SUPPLEMENTARY MATERIAL



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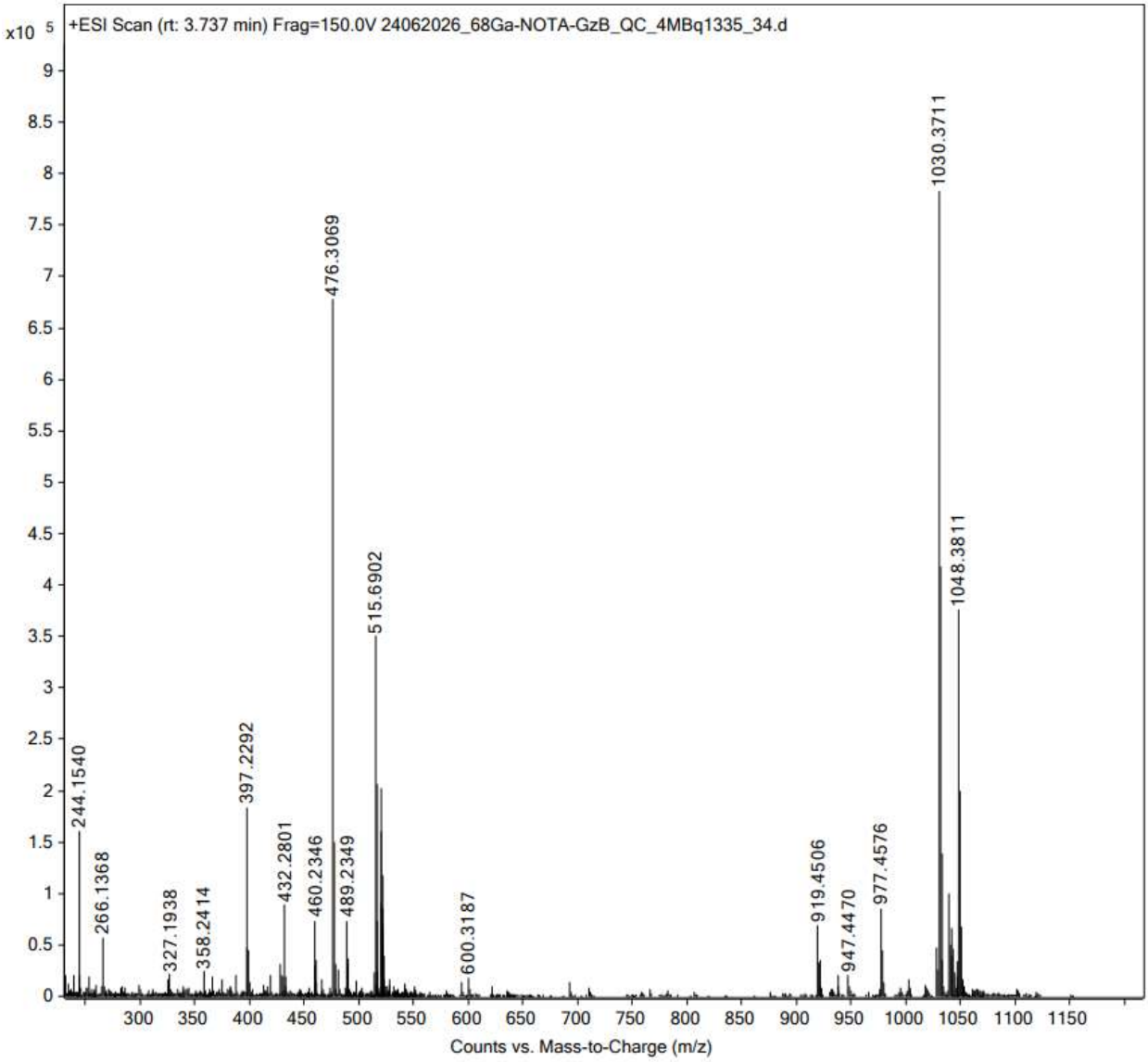

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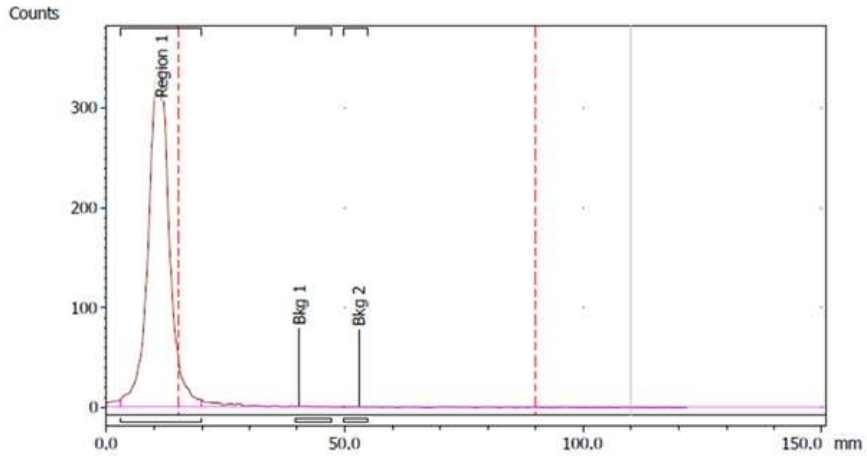
A

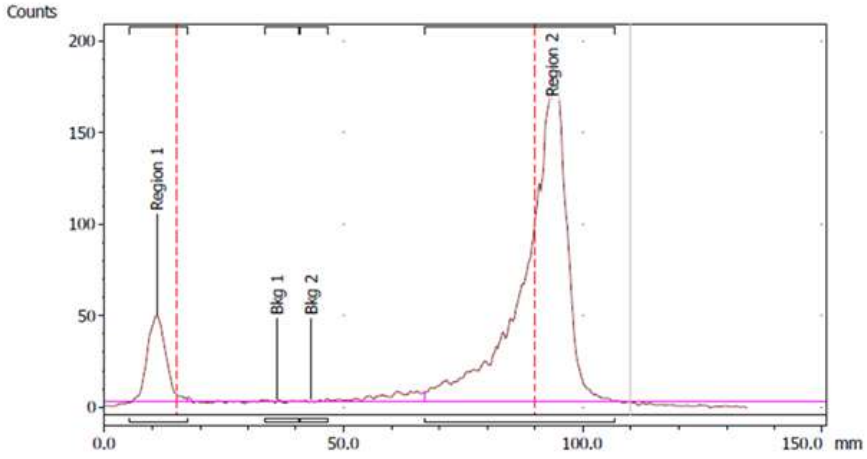


Regions: ^{68}Ga Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	2.9	19.8	-0.047	17189	100.00	98.15
Bkg 1	39.7	47.2	0.339			
Bkg 2	49.7	54.8	0.507			
1 Peak				17189	100.00	98.15

Total Area: 17514 Counts
Average Background: 1 Counts

B

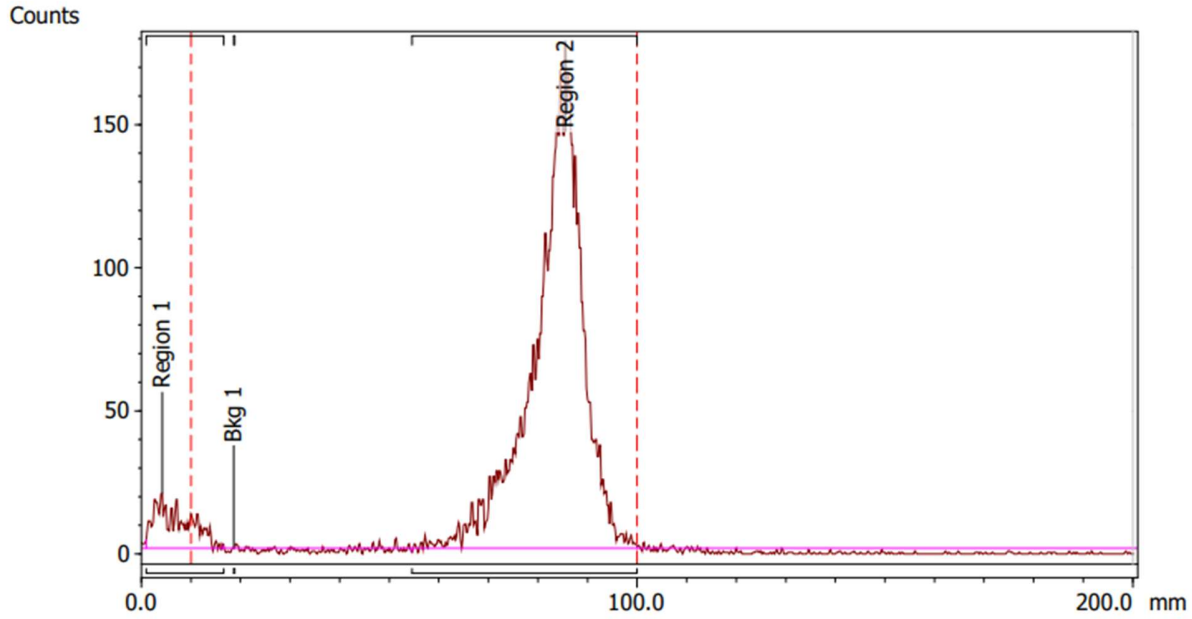


Regions: ^{68}Ga Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	5.1	17.3	-0.055	2045	10.51	10.32
Bkg 1	33.5	40.6	0.281			
Bkg 2	40.9	46.6	0.376			
Region 2	67.0	106.8	1.048	17424	89.49	87.94
2 Peaks				19469	100.00	98.26

Total Area: 19814 Counts
Average Background: 3 Counts

Figure S5: Radio-iTLC chromatogram of [$^{68}Ga^{+3}$]$GaCl_3$ in 1M ammonium acetate: MeOH (1:1) (A) and 0.1M citrate (B) as mobile phase in SG iTLC paper as stationary phase.



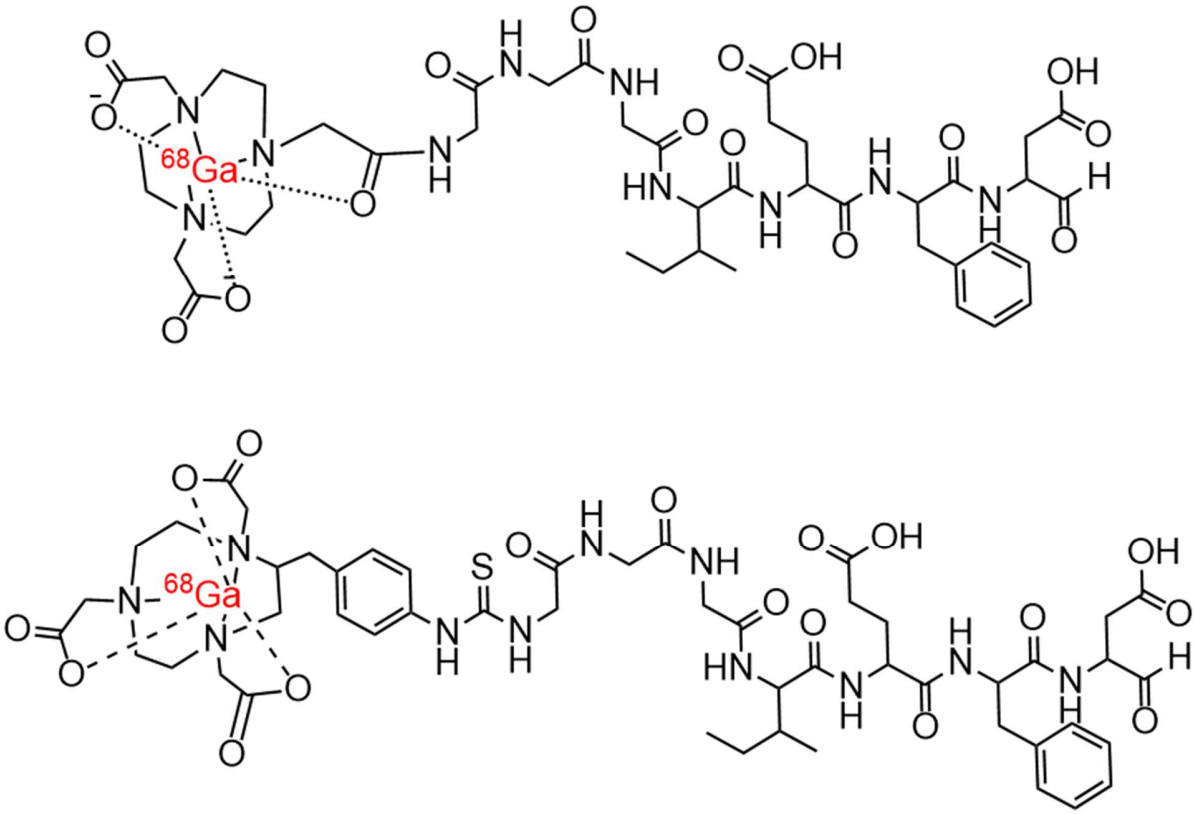
Regions: ^{68}Ga Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	1.0	16.6	-0.065	609	6.67	7.37
Bkg 1	18.6	18.8	0.096			
Region 2	54.6	100.0	0.840	8520	93.33	103.07
2 Peaks				9129	100.00	110.44

Total Area: 8266 Counts
Average Background: 2 Counts

Figure S6: Radio-iTLC chromatogram of [$^{68}Ga^{+3}$]Ga-NOTA in 1M ammonium acetate: MeOH (1:1) mobile phase in SG iTLC paper as stationary phase.

SUPPLEMENTARY MATERIAL



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