

INVESTIGATION OF p-SCN-Bn-DOTA-TRASTUZUMAB LABELED WITH RADIOACTIVE AND NON-RADIOACTIVE LUTETIUM AND YTTRIUM: A CRUCIAL STEP FOR FUTURE APPLICATIONS

Marija Arev¹, Marija Mirković², Magdalena Radović², Katarina Davaliev³, Petre Makreski⁴, Paulina Apostolova¹, Sanja Vranješ-Đurić², Aleksandar Dimovski³, Predrag Džodić⁵, Emilija Janevik-Ivanovska¹

¹Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia

²Vinča Institute of Nuclear Sciences, University of Belgrade, 11001 Belgrade, Serbia

³Research Centre for Genetic Engineering and Biotechnology "Georgi D Efremov", Macedonian Academy of Sciences and Arts, 1000 Skopje, North Macedonia

⁴Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, 1000 Skopje, North Macedonia

⁵Faculty of Medicine, Department of Pharmacy, University of Niš, 18000 Niš, Serbia

The purpose of this work is to obtain stable radioimmunoconjugates of trastuzumab-DOTA (⁹⁰Y and ¹⁷⁷Lu labeled).

MATERIAL AND METHODS

Conjugation of antibodies

4 °C during 18 hours
Purification by ultrafiltration (6 times)
with 0.05 M ammonium acetate (pH 7)
Immunoconjugate concentration 1 mg/mL

Freeze-drying of immunoconjugates

I - 40 °C, 1 °C/min, 5 hours
II - 25 °C, 0.15 °C/min, 28 hours, 0.133 mbar
III - 25 °C, 0.2 °C/min, 14 hours
1% mannitol

Chemical Identification

Infrared and Raman spectroscopy
MALDI-TOF MS
(average number of BFCAs)
Integrity - SDS-PAGE

Radioactive labeling with Lu-177/Y-90

BFCa in the ratio 1:20
activity ⁹⁰Y 1,425 mCi, pH 4.5-5
activity ¹⁷⁷Lu 8.15 mCi, pH 6
Tr-DOTA (40 °C, 1 hour)

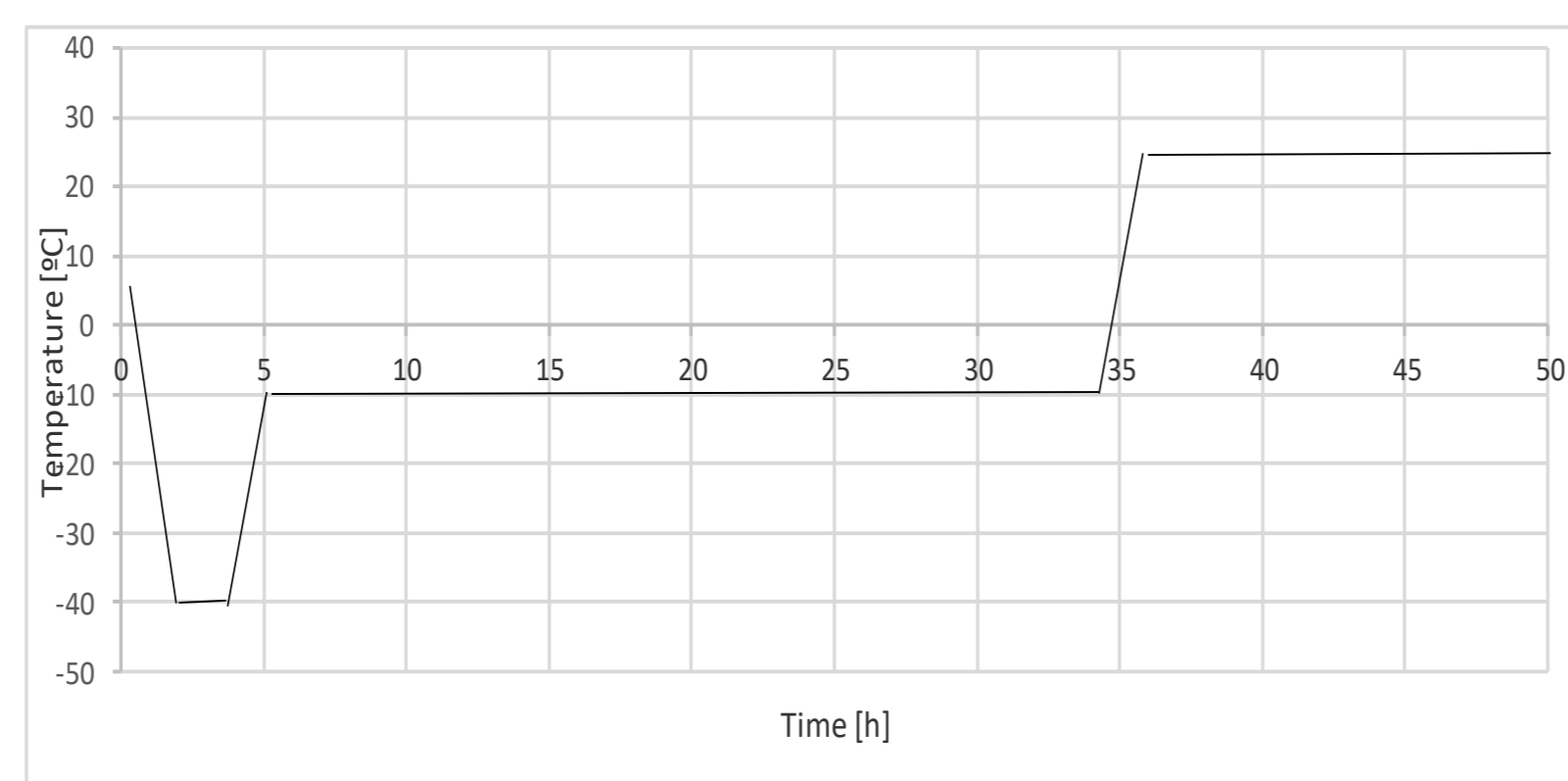
Radiochemical purity

was tested with ITLC-SG using three
mobile phases:
0.9% NaCl,
0.4 M methanol/sodium-acetate (1:1)
0.1 M acetic buffer.

Stability

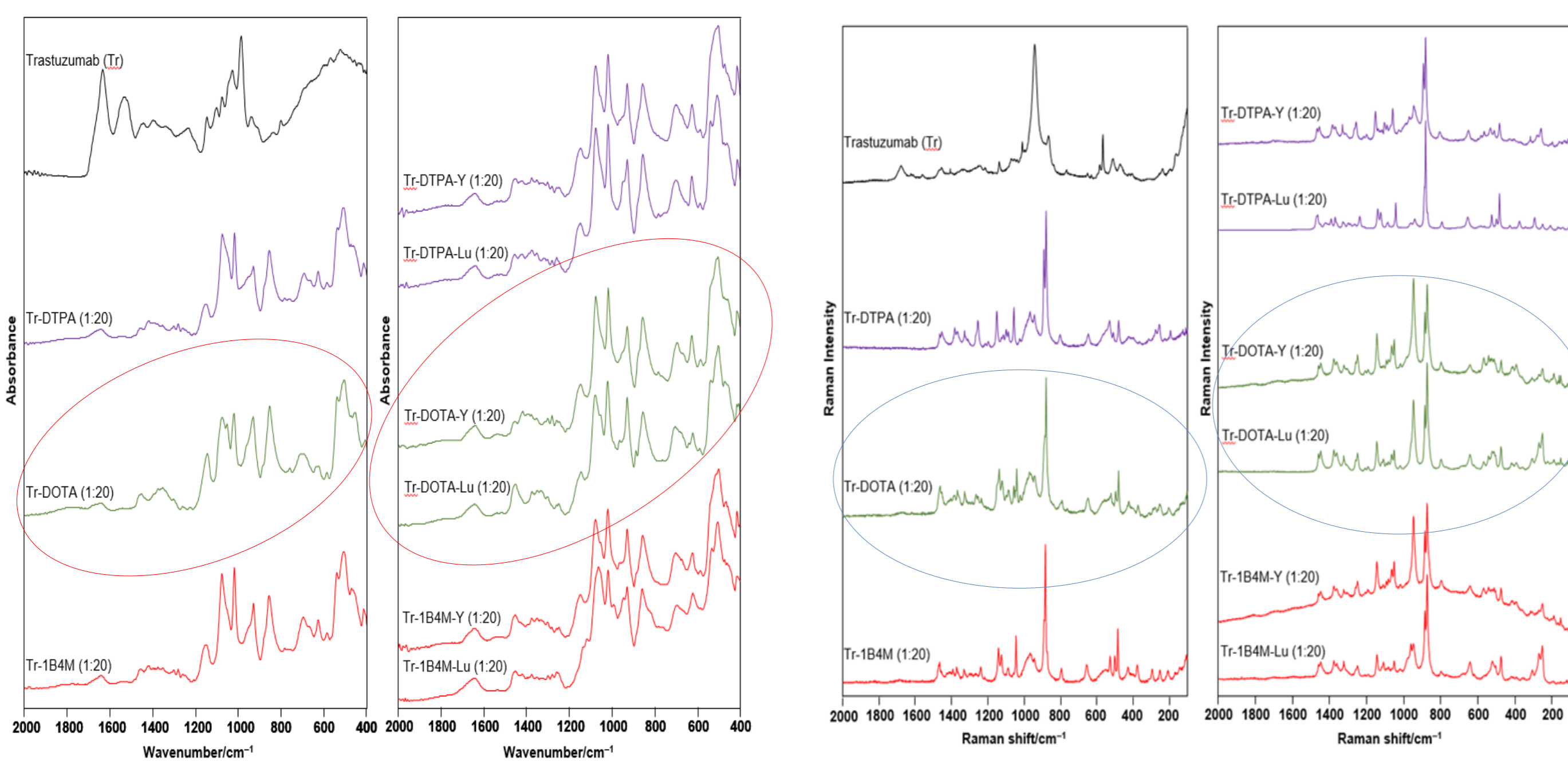
was tested in 0.9% NaCl (¹⁷⁷Lu) and
0.4 M methanol/sodium-acetate (1:1) (⁹⁰Y),
At room temperature for 1, 24, 48 and 72h.

RESULTS



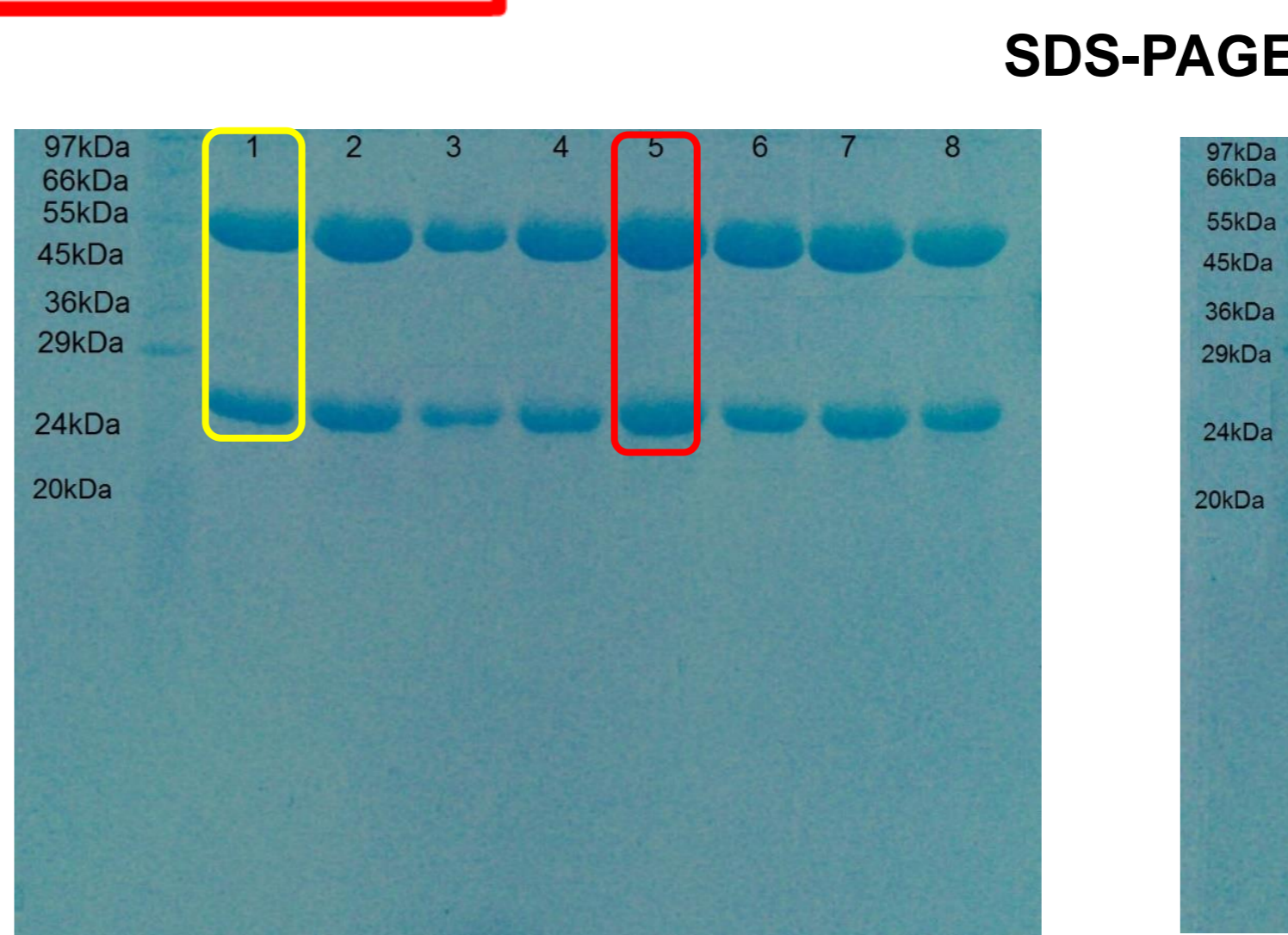
Lyophilization protocol

IR and RAMAN

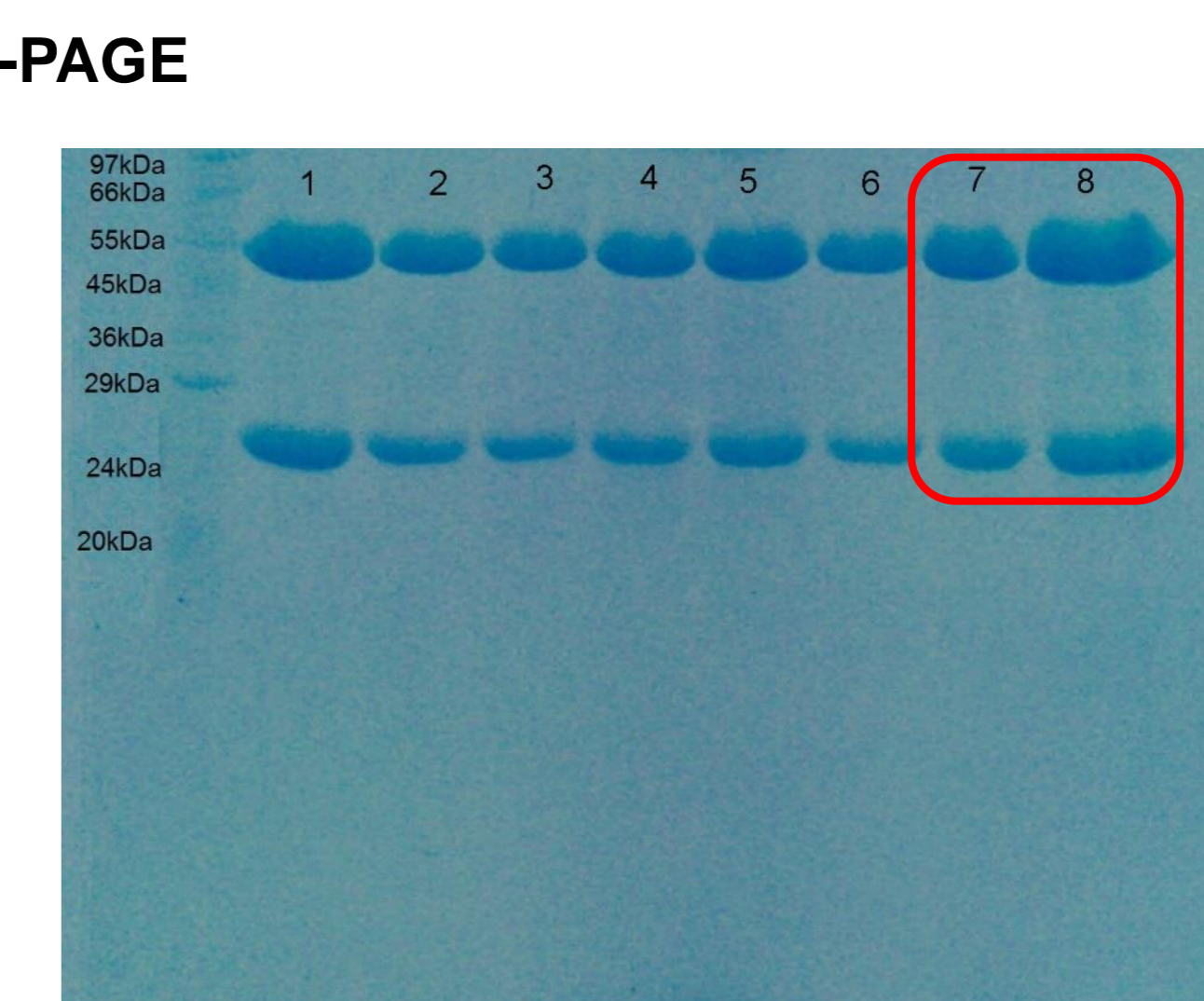


Characteristic IR bands				
Amide band I	Amide band II	Amide band III	Amide band IV and V	Amid band VI
1640-1645 cm ⁻¹	1480-1575 cm ⁻¹	1233-1300 cm ⁻¹	620-810 cm ⁻¹	500-595 cm ⁻¹

Characteristic Raman bands					
S-S	Tyr	Trp	Phe	Indol ring	Amid band I
400-700 cm ⁻¹	647cm ⁻¹ , 760-790 cm ⁻¹	757 cm ⁻¹ , 878 cm ⁻¹ , 1337 cm ⁻¹	1004-1060 cm ⁻¹ , 1610 cm ⁻¹	1560 cm ⁻¹	1668-1688 cm ⁻¹
					Amide band III
					1235-1260 cm ⁻¹

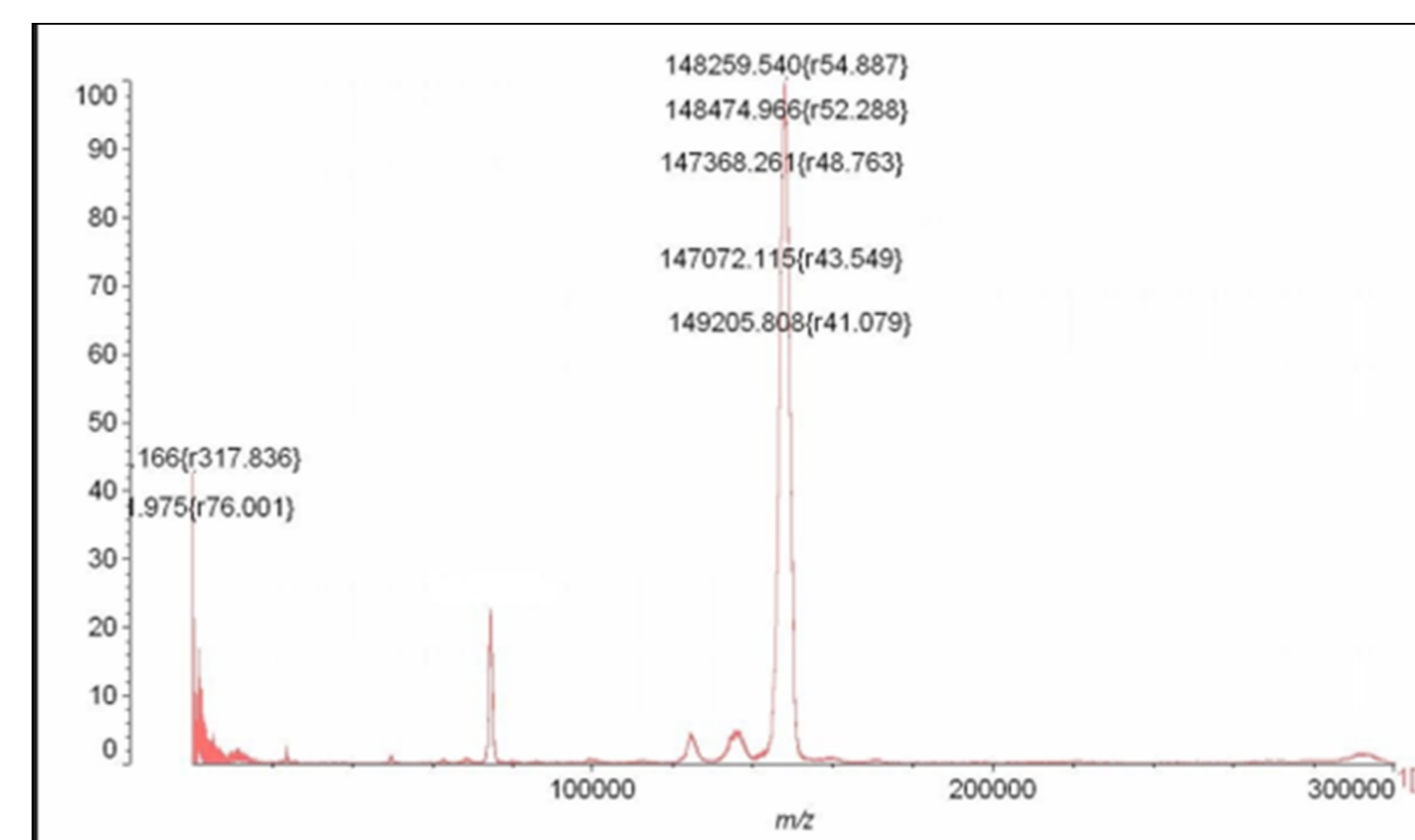


Reducing SDS-PAGE of Trastuzumab 1 mg/mL (1);
DOTA-Trastuzumab (1:20) (5)



Reducing SDS-PAGE of Y-DOTA-Trastuzumab (1:20) (7);
Lu-DOTA-Trastuzumab (1:20) (8)

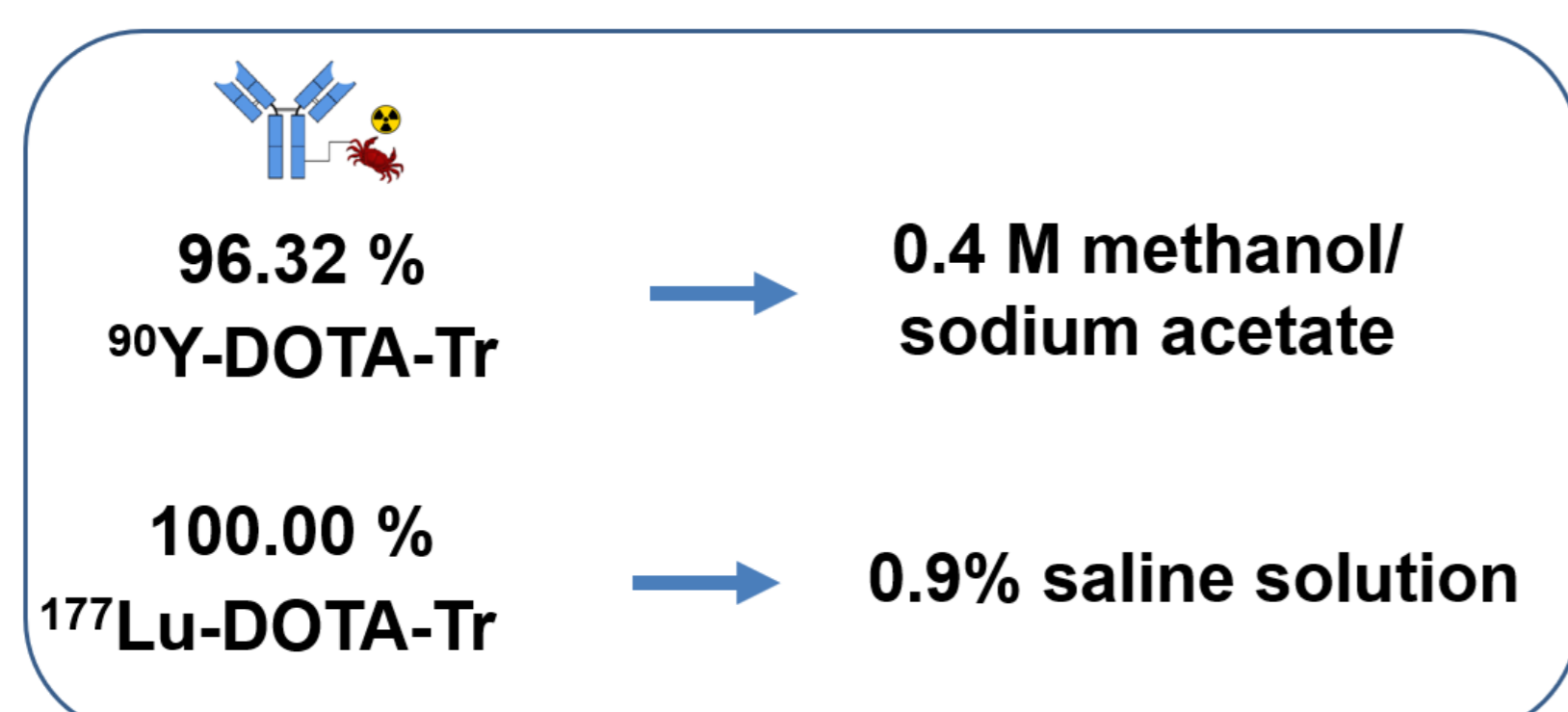
MALDI-TOF MS



Samples	H+ mass	Mass difference H+(conjugated-theoretical)	Mw of BFCs	No. of Chelators
1 Trastuzimab (Tr) theoretical	14,5531.50 Da			
2 Tr-DOTA 1:20	14,8259.54 Da	2,728.04	551.61 g/mol	4.9

STABILITY

RADIOCHEMICAL PURITY



Incubation time [h] Radiochemical Purity [%] Release of ⁹⁰Y and ¹⁷⁷Lu [%]

⁹⁰ Y-DOTA-Trastuzumab	1	96.32	3.68
	24	92.40	7.6
	48	88.15	11.85
	72	82.83	17.17
¹⁷⁷ Lu-DOTA-Trastuzumab	1	100	0
	24	99.14	0.86
	48	98.97	1.03
	72	98.52	1.48

CONCLUSION

Our study shows the successful formulation of stable radioimmunoconjugates, making this agent a potential use *in vivo* investigations.