Development of Potential Lutetium-177 Radiopharmaceuticals: Design, Radiolabelling and Nonclinical Evaluation
CRP F-22078

Design and standardization of the technology for the production of ready-to-use kits for conjugated antibody Daratumumab and protein FAPI-based radiopharmaceuticals, intended for labelling with Lu-177- proof of concept for comparative oncology in the One Health approach

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14.10.2025
2nd RCM, IAEA, Vienna

Vector molecules

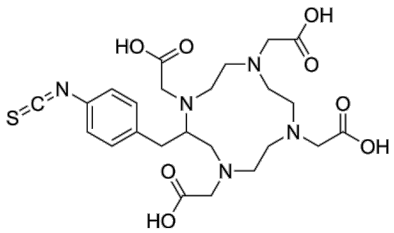
Daratumumab

- A fully human monoclonal IgG1κ antibody
- Anti-CD38 antigen
- patients with relapsed/ refractory myeloma and patients with newly diagnosed myeloma.

FAPI - 04

- A peptide-based molecule for targeting of FAP (serine proteinase) highly expressed on the surface of the major cell population in tumor stroma, particularly on the subpopulation of activated fibroblasts termed cancer-associated fibroblasts.

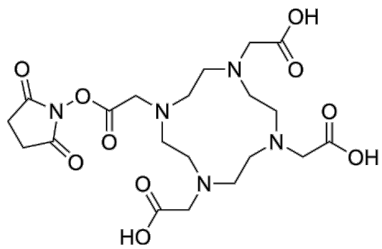
Chelators



p-SCN-Bn-DOTA

CAS No. : **127985-74-4**

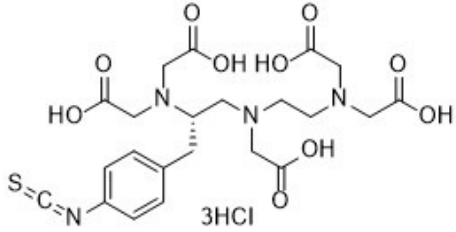
2-(4-Izothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid



DOTA-NHS ester

CAS No. : **170908-81-3**

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-N-hydroxysuccinimide ester



p-SCN-Bn-1B4M-DTPA

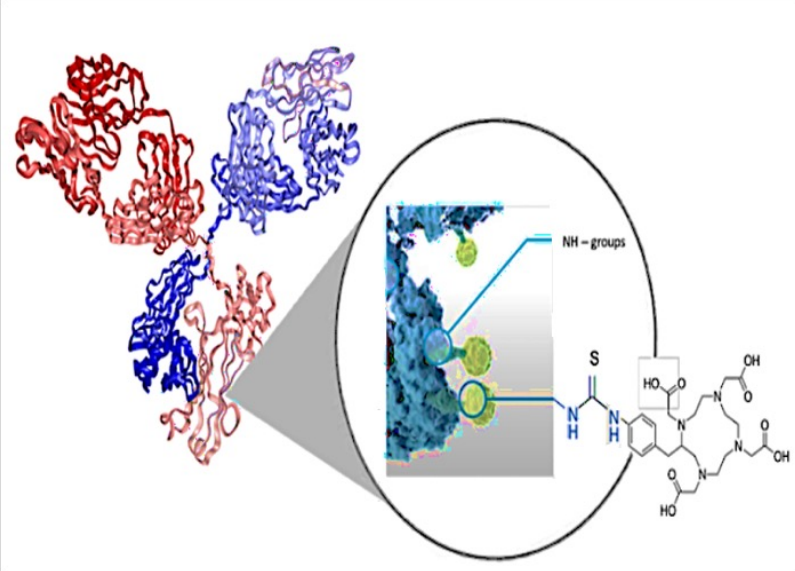
CAS No. : **102650-30-6**

2-(4-Isothiocyanatobenzyl)-6-methyl-diethylene-triamine pentaacetic acid

Reaction of immunoconjugation

Reaction condition

- Purification from the excipients and concentration of the purified antibody
- 0,1M Phosphate buffer pH 8.0
- Overnight shaking at 4°C (16h)
- Immunoconjugate purification with 0,15M ammonium acetate



MALDI analysis (Chelator-to-antibody ratio)

	Immunoconjugate	No of chelators attached to each antibody	Labeling yield
1.	Daratumumab-DOTA-NHS 1:10	0.04	< 30%
2.	Daratumumab-DOTA-NHS 1:20	0.09	
3.	Daratumumab-DOTA-NHS 1:30	0.20	
4.	Daratumumab-DOTA-SCN 1:10	0.23	
5.	Daratumumab-DOTA-SCN 1:20	0.87	
6.	Daratumumab-DOTA-SCN 1:30	0.39	
7.	Daratumumab-1B4M DTPA 1:10	0.12	
8.	Daratumumab-1B4M DTPA 1:20	0.30	
9.	Daratumumab-1B4M DTPA 1:30	0.57	

Reaction of immunoconjugation

Reaction condition

- Purification from the excipients and concentration of the purified antibody
- 0,1M *Phosphate* buffer pH 8.5 and 0,1M *Carbonate* buffer pH 8.5
- Gentle shaking at 37°C for 1,5h
- Immunoconjugate purification with 0,5M ammonium acetate pH 7.0

Radiolabeling conditions

- Liquid samples in 0,5M ammonium acetate
- Adjusted pH ~4
- Radiolabeling in incubator at 37°C for 1h
- iTLC for quality control with 0.1M Na-acetate

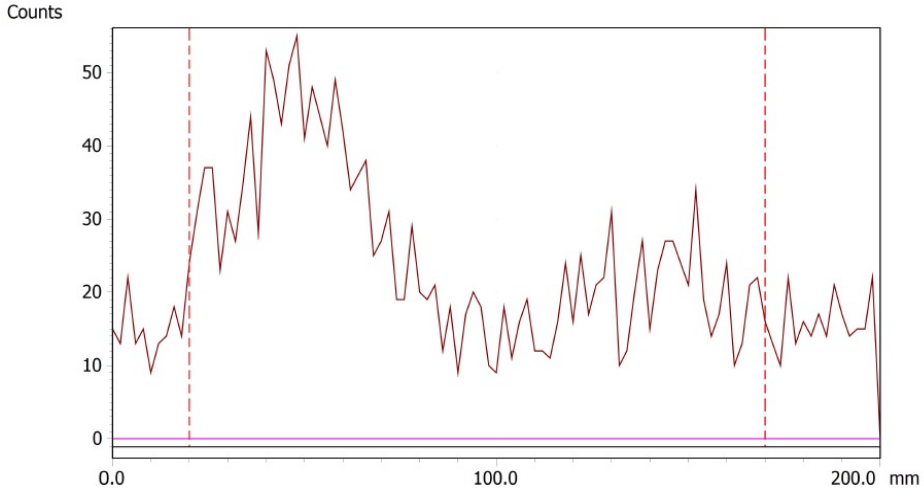
as mobile phase pH 4.5

MALDI analysis (Chelator-to-antibody ratio)

	Immunoconjugate	No of chelators attached to each antibody, Phosphate buffer	No of chelators attached to each antibody, Carbonate buffer	Radiochemical purity (%) After incubation at 37°C
1.	Daratumumab-DOTA-NHS 1:10	0,12	1,15	
2.	Daratumumab-DOTA-NHS 1:20	0,32	1,07	
3.	Daratumumab-DOTA-NHS 1:30	0,68	1,43	10,20%
4.	Daratumumab-DOTA-SCN 1:10	1,70	3,19	
5.	Daratumumab-DOTA-SCN 1:20	2,19	3,54	
6.	Daratumumab-DOTA-SCN 1:30	3,19	4,65	68,28%
7.	Daratumumab-1B4M DTPA 1:10	1,21	1,23	
8.	Daratumumab-1B4M DTPA 1:20	0,83	1,52	95,21%
9.	Daratumumab-1B4M DTPA 1:30	1,19	1,72	

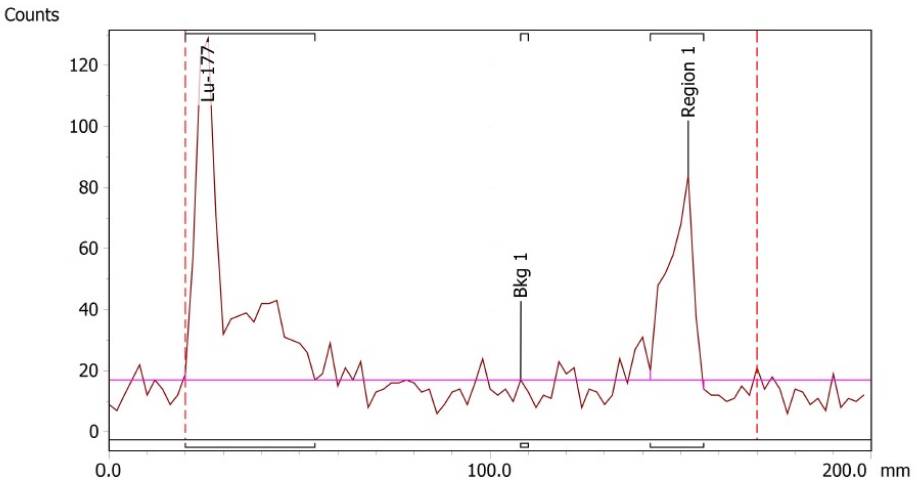
Project: Marija-Lu-Dox DARA DOTA NHS x 30 acetat 27. 5. 2025. Run 1.Measurement User:

Chromatogram: ^{177}Lu



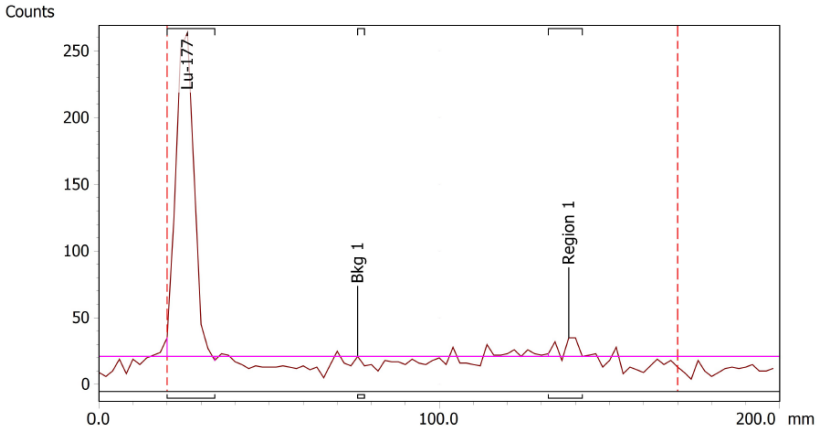
Project: Marija-Lu-Dox DARA DOTA SCN x 30 28. 5. 2025.2 dan Run 1.Measurement User

Chromatogram: ^{177}Lu



Project: Marija-Lu-Dox DARA DTPA x 20 acetat 28. 5. 2025.2 dan Run 1.Measurement

Chromatogram: ^{177}Lu

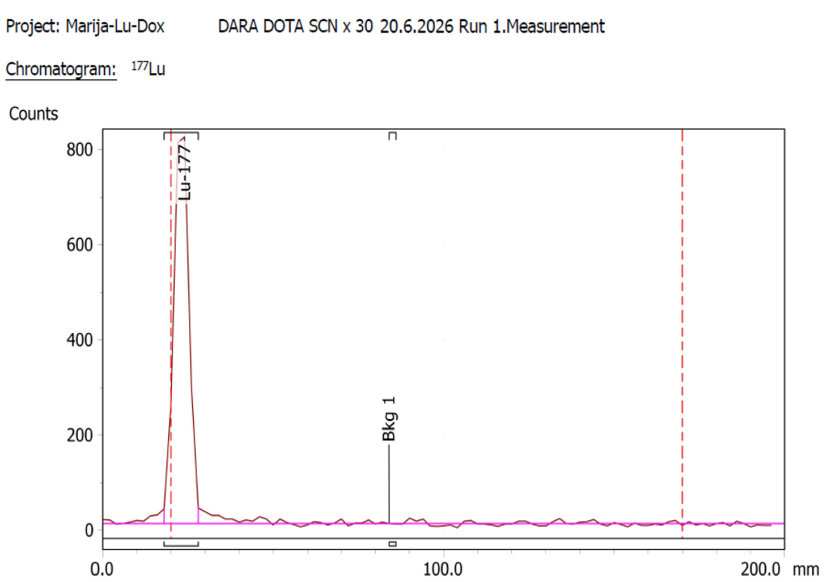


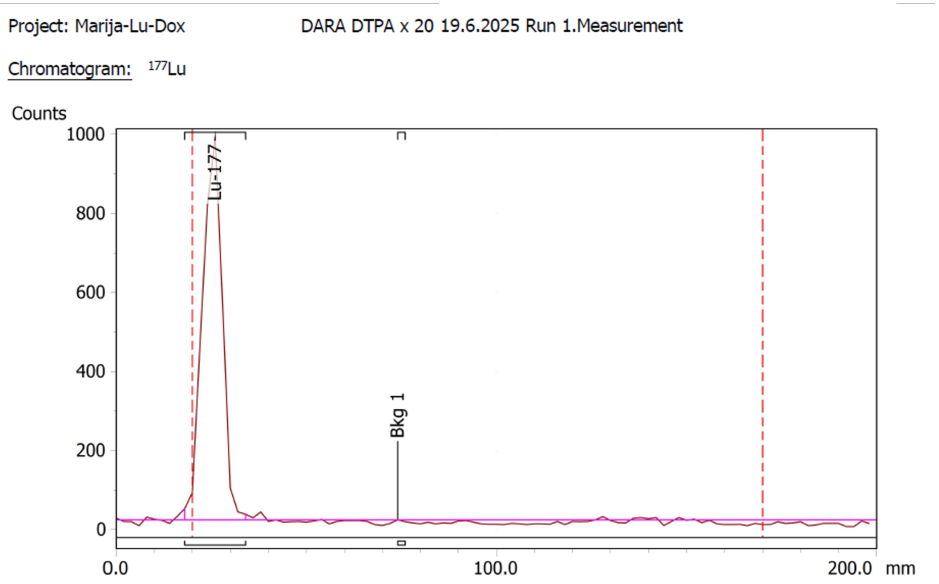
User: Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	20.0	54.0	0.040	536	68.28	94.53
Bkg 1	108.0	110.0	0.587			
Region 1	142.0	156.0	0.880	249	31.72	43.92
2 Peaks				785	100.00	138.45

Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	20.0	34.0	0.040	755	95.21	235.94
Bkg 1	76.0	78.0	0.373			
Region 1	132.0	142.0	0.787	38	4.79	11.88
2 Peaks				793	100.00	247.81



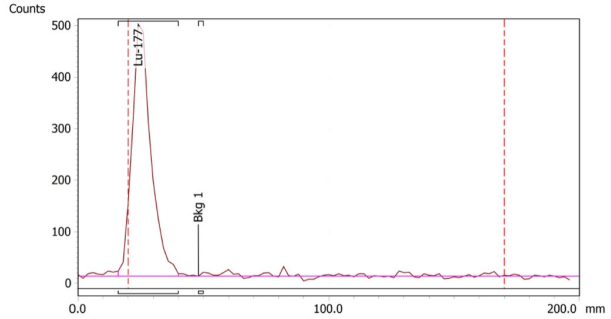


	Immunoconjugate	No of chelators attached to each antibody, Phosphate buffer	No of chelators attached to each antibody, Carbonate buffer	Radiochemical purity (%) After incubation at 37°C	Radiochemical purity (%) After shaking at 37°C
1.	Daratumumab-DOTA-NHS 1:10	0,12	1,15		
2.	Daratumumab-DOTA-NHS 1:20	0,32	1,07		
3.	Daratumumab-DOTA-NHS 1:30	0,68	1,43	10,20%	X
4.	Daratumumab-DOTA-SCN 1:10	1,70	3,19		
5.	Daratumumab-DOTA-SCN 1:20	2,19	3,54		
6.	Daratumumab-DOTA-SCN 1:30	3,19	4,65	68,28%	100%
7.	Daratumumab-1B4M DTPA 1:10	1,21	1,23		
8.	Daratumumab-1B4M DTPA 1:20	0,83	1,52	95,21%	100%
9.	Daratumumab-1B4M DTPA 1:30	1,19	1,72		

Stability of the radioimmunoconjugates in Human Serum Albumin

Project: Marija-Lu-Dox DARA DOTA SCN x 30 HSA acetat 19.6.2026 Run 1.Measurement User:

Chromatogram: ^{177}Lu

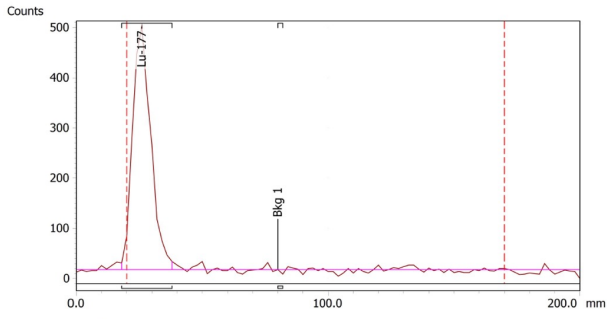


Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	16.0	40.0	0.027	2153	100.00	94.47
Bkg 1	48.0	50.0	0.187			
1 Peak				2153	100.00	94.47

Project: Marija-Lu-Dox DARA DOTA SCN x 30 HSA 20.6.2026 Run 1.Measurement Use

Chromatogram: ^{177}Lu

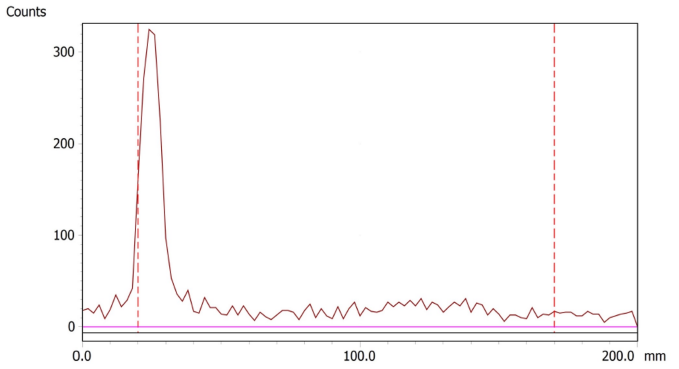


Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	18.0	38.0	0.040	2041	100.00	102.72
Bkg 1	80.0	82.0	0.400			
1 Peak				2041	100.00	102.72

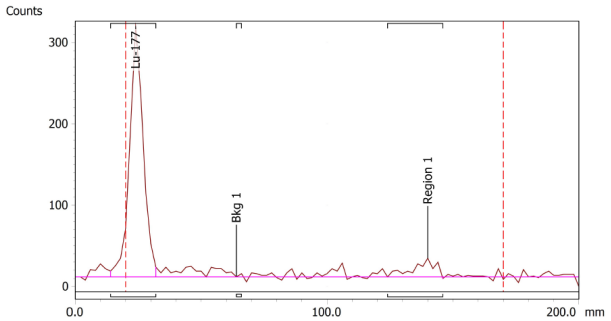
Project: Marija-Lu-Dox DARA DOTA SCN x 30 HSA stabilnost 23.6.2026 Run 1.Measurement User:

Chromatogram: ^{177}Lu



Project: Marija-Lu-Dox DARA DTPA x 20 acetat HSA 19.6.2025 Run 1.Measurement User

Chromatogram: ^{177}Lu

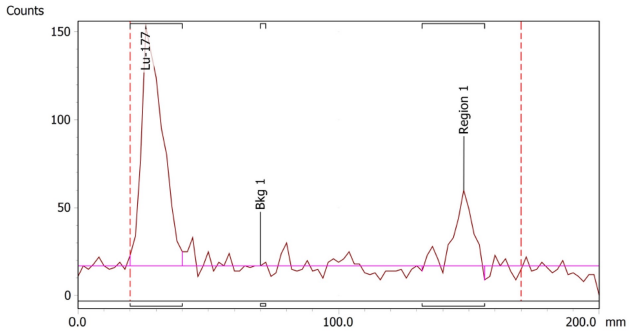


Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	14.0	32.0	0.027	969	89.72	71.15
Bkg 1	64.0	66.0	0.293			
Region 1	124.0	146.0	0.800	111	10.28	8.15
2 Peaks				1080	100.00	79.30

Project: Marija-Lu-Dox DARA DTPA x 20 2 den HSA 20.6.2026 Run 1.Measurement User:

Chromatogram: ^{177}Lu

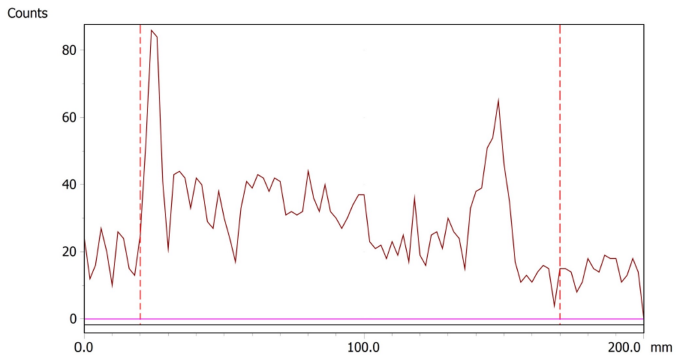


Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	20.0	40.0	0.040	636	78.52	85.37
Bkg 1	70.0	72.0	0.333			
Region 1	132.0	156.0	0.853	174	21.48	23.36
2 Peaks				810	100.00	108.72

Project: Marija-Lu-Dox DARA DTPA x 20 HSA 23.6.2025 Run 1.Measurement User:

Chromatogram: ^{177}Lu

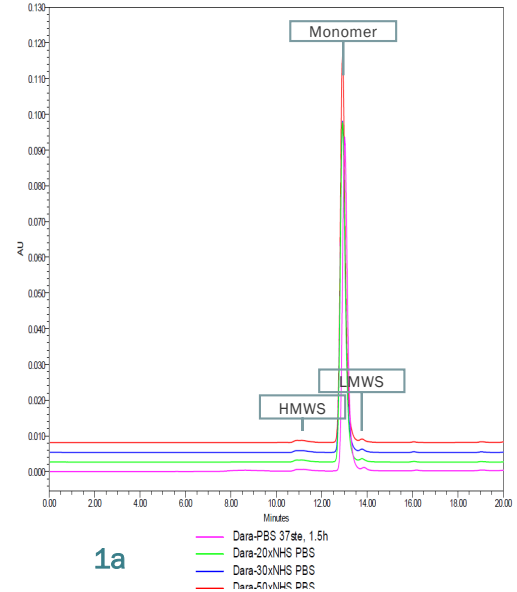


Stability after the immunoconjugation reaction

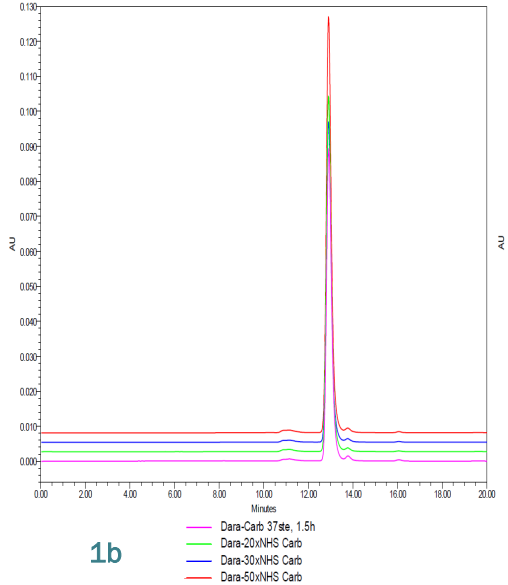
Size Exclusion Chromatograpy

- HPLC system with quaternary pump
- UV detection at 280 nm
- Column: Xbridge Premier Protein 250A, 7,8x300mm, 2,5µm
- Mobile phase: 90% 50mM Phosphate buffer pH 8.0, 150mM NaCl, 50mM KCL / 10% ACN
- Colum T: ambient; Sample T: 10°C, Injection Volume: 10µL
- Run time: 25min

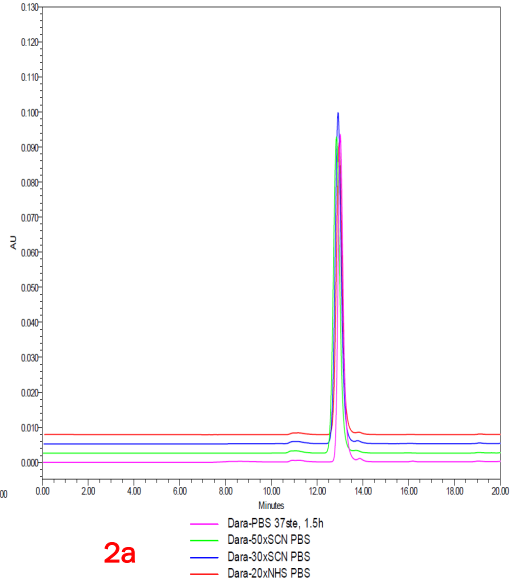
	Immunoconjugate	Phosphate buffer pH=8.5		Carbonate buffer pH=8.5	
		Main peak	Aggregates	Main peak	Aggregates
1.	Daratumumab-DOTA-NHS 1:10	99,55	/	99,51	/
2.	Daratumumab-DOTA-NHS 1:20	99,54	/	99,48	/
3.	Daratumumab-DOTA-NHS 1:30	97,60	1.39	97,94	1.53
4.	Daratumumab-DOTA-SCN 1:10	97,73	1.36	97,68	1.53
5.	Daratumumab-DOTA-SCN 1:20	97,87	1.37	97,06	1.47
6.	Daratumumab-DOTA-SCN 1:30	96,95	1.36	96,94	0.96
7.	Daratumumab-1B4M DTPA 1:10	97,93	1.36	97,07	1.49
8.	Daratumumab-1B4M DTPA 1:20	97,65	1.34	96,83	1.49
9.	Daratumumab-1B4M DTPA 1:30	97,30	1.34	96,62	1.48



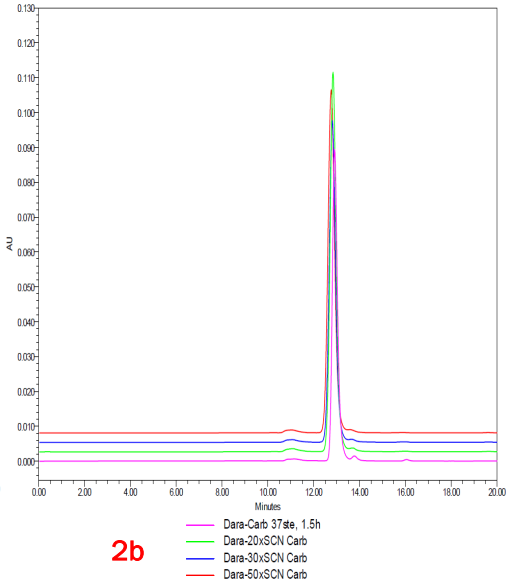
1a



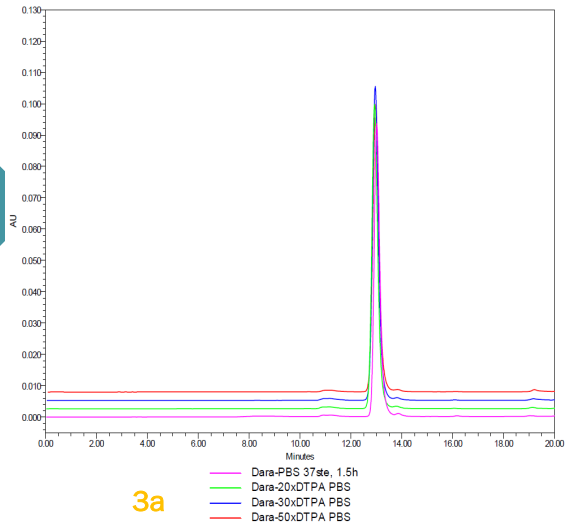
1b



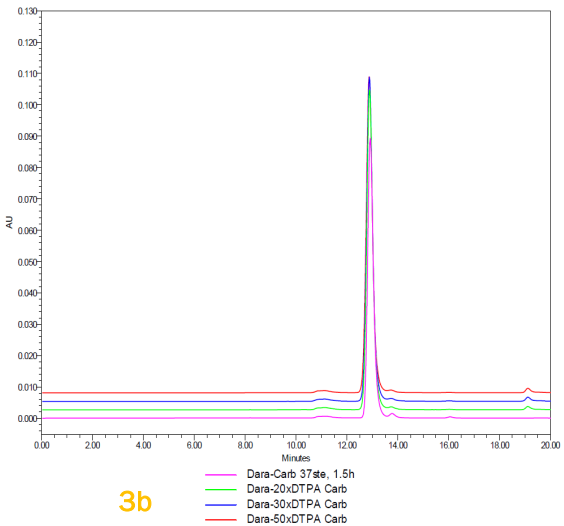
2a



2b



3a



3b

Representative overlay chromatograms

1a Dara NHS immunoconjugates in different molar excesses **prepared in PBS**

1b Dara NHS immunoconjugates in different molar excesses **prepared in Carbonate buffer**

2a Dara SCN immunoconjugates in different molar excesses **prepared in PBS**

2b Dara SCN immunoconjugates in different molar excesses prepared in **Carbonate buffer**

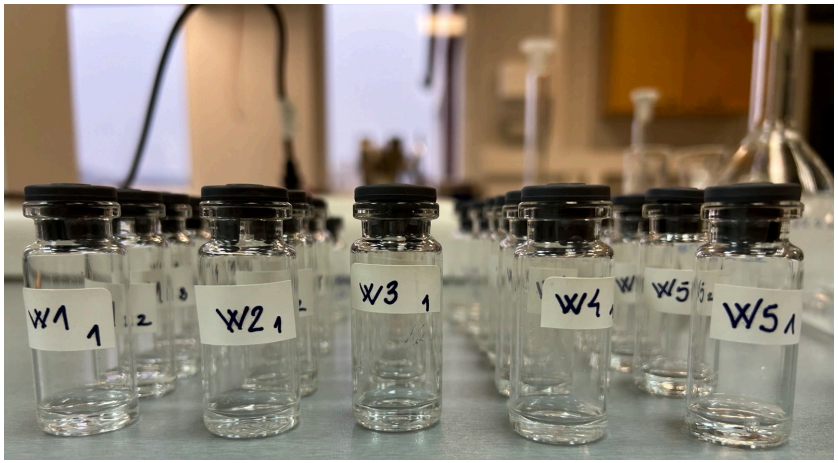
3a Dara 1B4M DTPA immunoconjugates in different molar excesses **prepared in PBS**

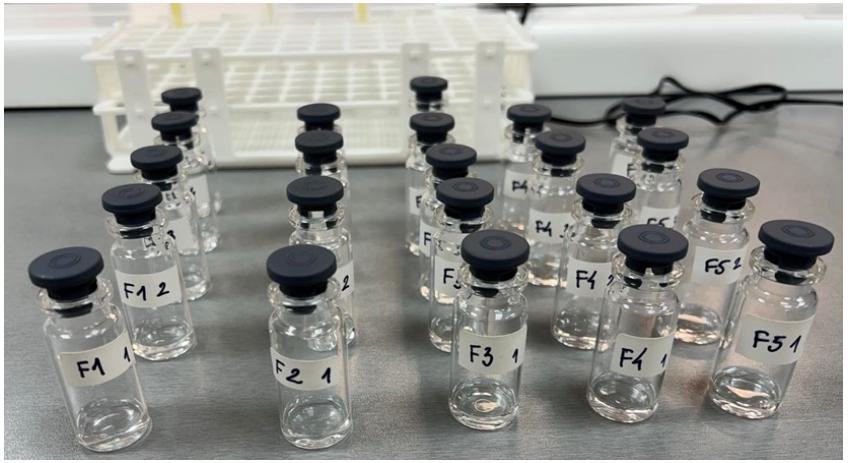
3b Dara 1B4M DTPA immunoconjugates in different molar excesses **prepared in Carbonate buffer**

Freeze drying process (Lyophilization)

Protocols and formulations of Daratumumab DOTA SCN x 30

Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
0,1M PBS pH=8.0 1% mannitol	0,01M PBS pH=8.0 1% mannitol	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 2:1	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 4:1	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 2:1 0,02% Polysorbate 20





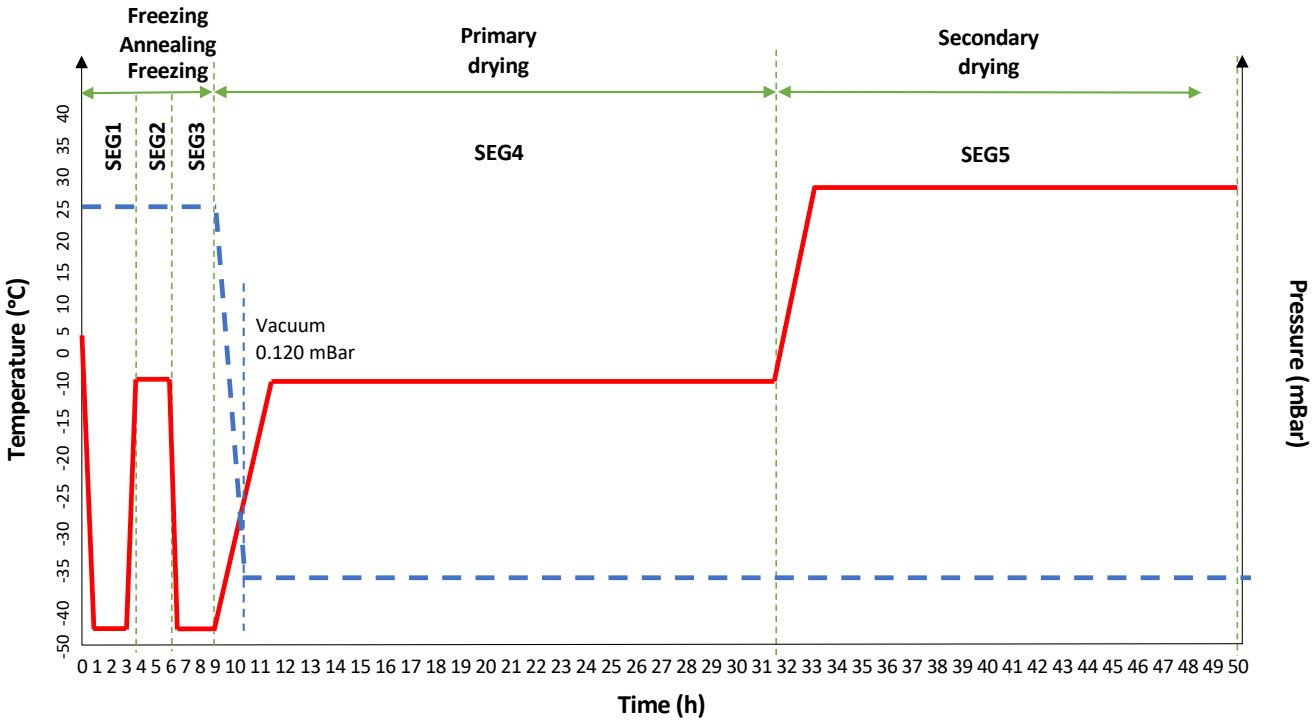
*Concentration of mAb in the form of immunoconjugate, in all formulations, is 1 mg/mL

** The first series were prepared in ultra pure water (W1-W5), and the second series in saline solution (F1-F5)

Freeze drying process (Lyophilization)

Protocols and formulations

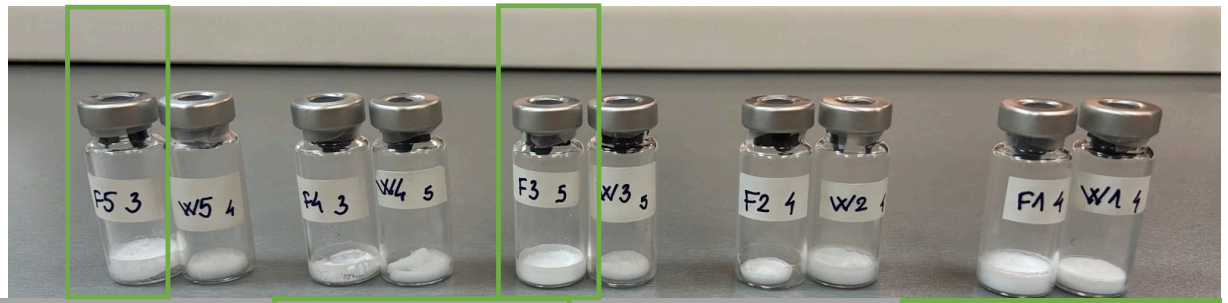
Freeze-drying protocol (I)	
SEG 1	Freezing: -40 °C
	Ramp 1 °C/min
	HOLD 3 h
SEG 2	Annealing: -10 °C
	Ramp 0.5 °C/min,
	HOLD 2 h
SEG 3	Freezing: -40 °C
	Ramp 1 °C/min
	HOLD 1.5 h
SEG 4	Primary drying: -10 °C
	Ramp 0.15 °C/min
	HOLD 18 h
SEG 5	Secondary drying: +25 °C
	Ramp 0.15 °C/min
	HOLD 8 h
	Total ~ 50 hours



Characterization of the freeze-dried formulation

Methods (1)

- Reconstitution time
- % of residual water (Karl Fisher)
- SE HPLC
- iTLC



Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
0,1M PBS pH=8.0 1% mannitol	0,01M PBS pH=8.0 1% mannitol	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 2:1	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 4:1	10 mM PBS 7.2 + sucrose : mAb = 1:450 + mannitol : sucrose = 2:1 0,02% Polysorbate 20

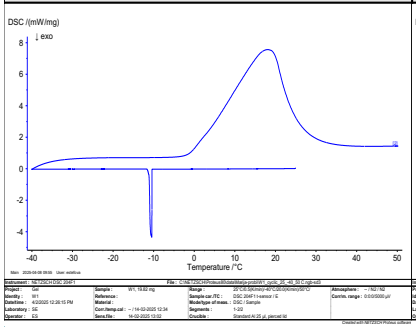
Parameter	W1	F1	W2	F2	W3	F3	W4	F4	W5	F5
Reconstitution time	<1 min in saline solution without visible particles									
Residual moisture (% of water, <3%) (Karl Fischer titration)	5.54	5.01	5.60	2.73	6.06	2.17	5.10	2.83	6.05	1.90
Purity (% of the main peak, >97%) (SE-HPLC)	93.86	94.98	93.01	91.23	99.76	99.75	99.73	98.90	99,76	97,95
Radiochemical purity (iTLC)	100 %									

Differential Scanning Colorimetry

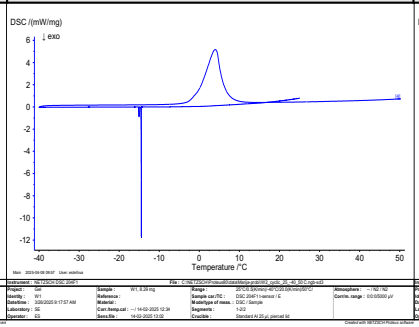
Method

- Cooling rate 0.5 ºC/min, 25 to -75 ºC
- Heating rate 20 ºC/min, to 50ºC

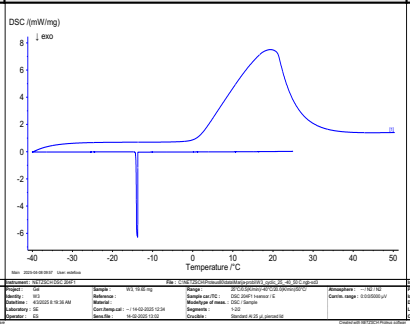
W1



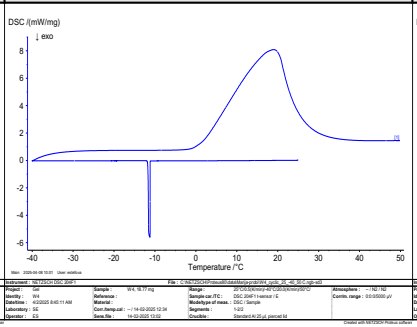
W2



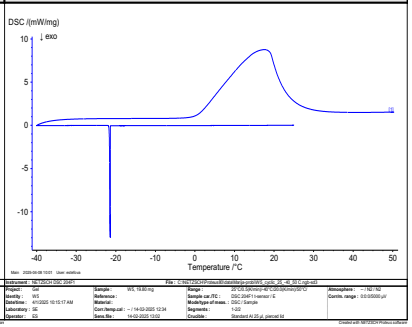
W3



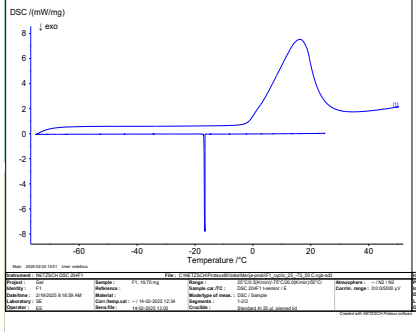
W4



W5



F1



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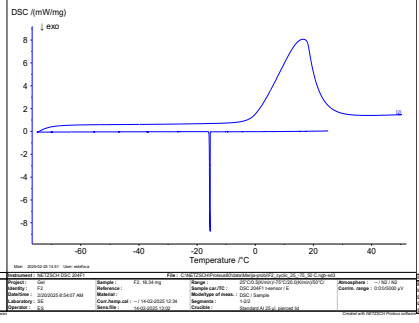

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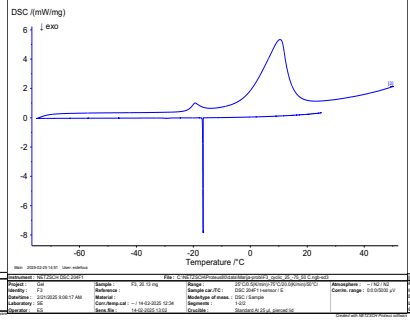

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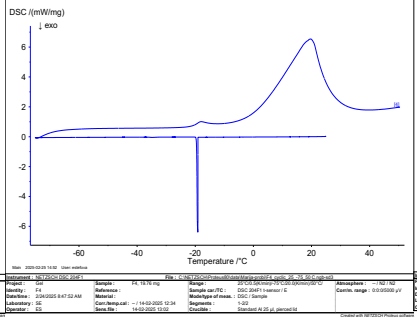

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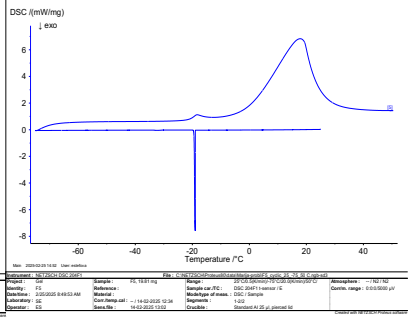
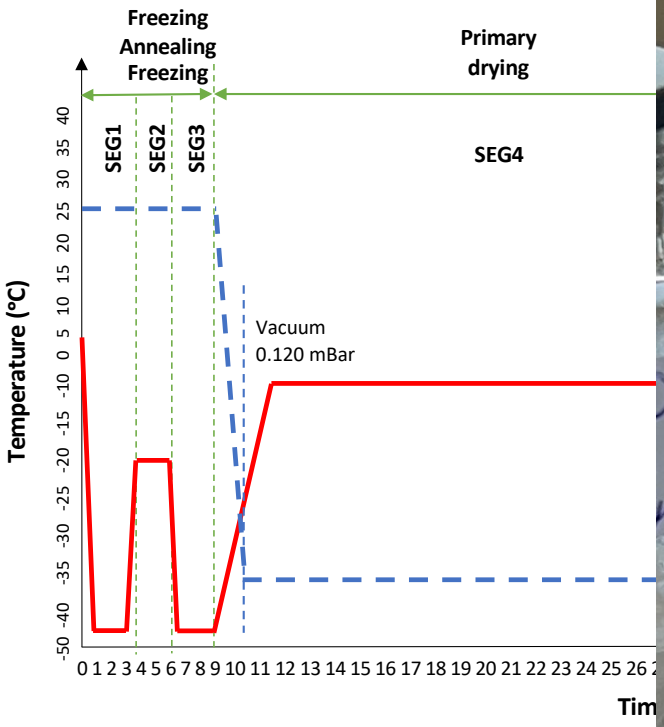
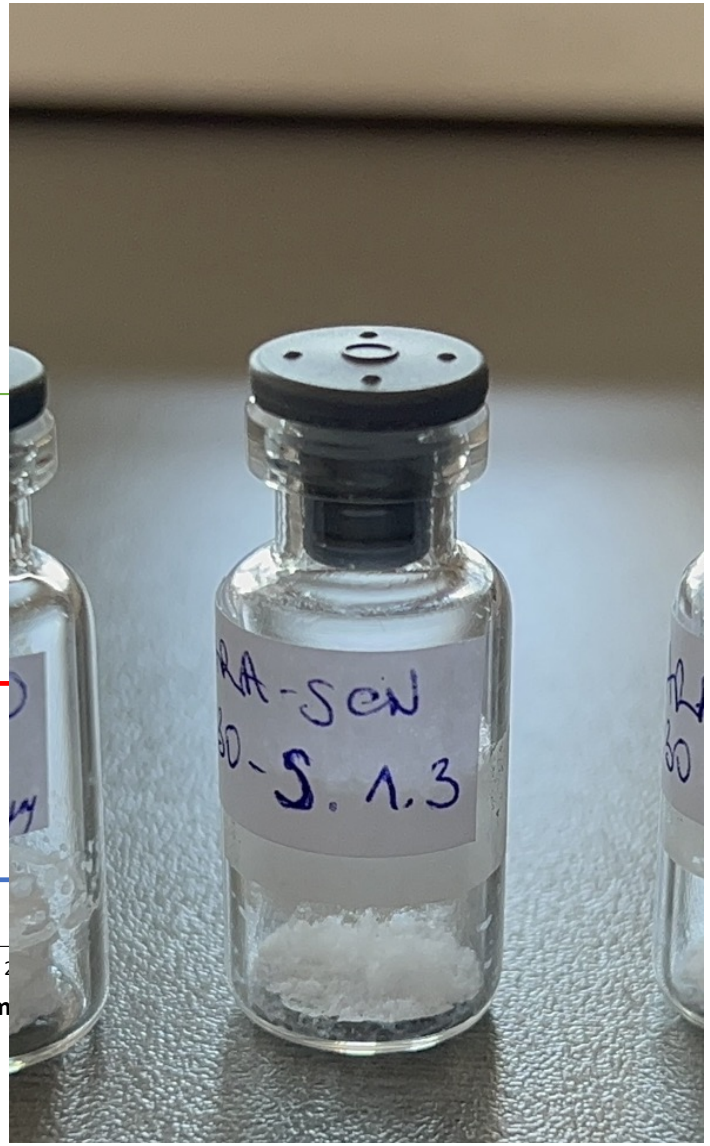


Freeze drying process (Lyophilization)

Protocols and formulations

Freeze-drying protocol (II)	
SEG 1	Freezing: -40°C
	Ramp 1°C/min
	HOLD 3 h
SEG 2	Annealing: -20°C
	Ramp 0.5°C/min,
	HOLD 2 h
SEG 3	Freezing: -40°C
	Ramp 1°C/min
	HOLD 1.5 h
SEG 4	Primary drying: -20°C
	Ramp 0.15°C/min
	HOLD 18 h
SEG 5	Secondary drying: +25°C
	Ramp 0.15°C/min
	HOLD 8 h
	Total ~ 50 hours

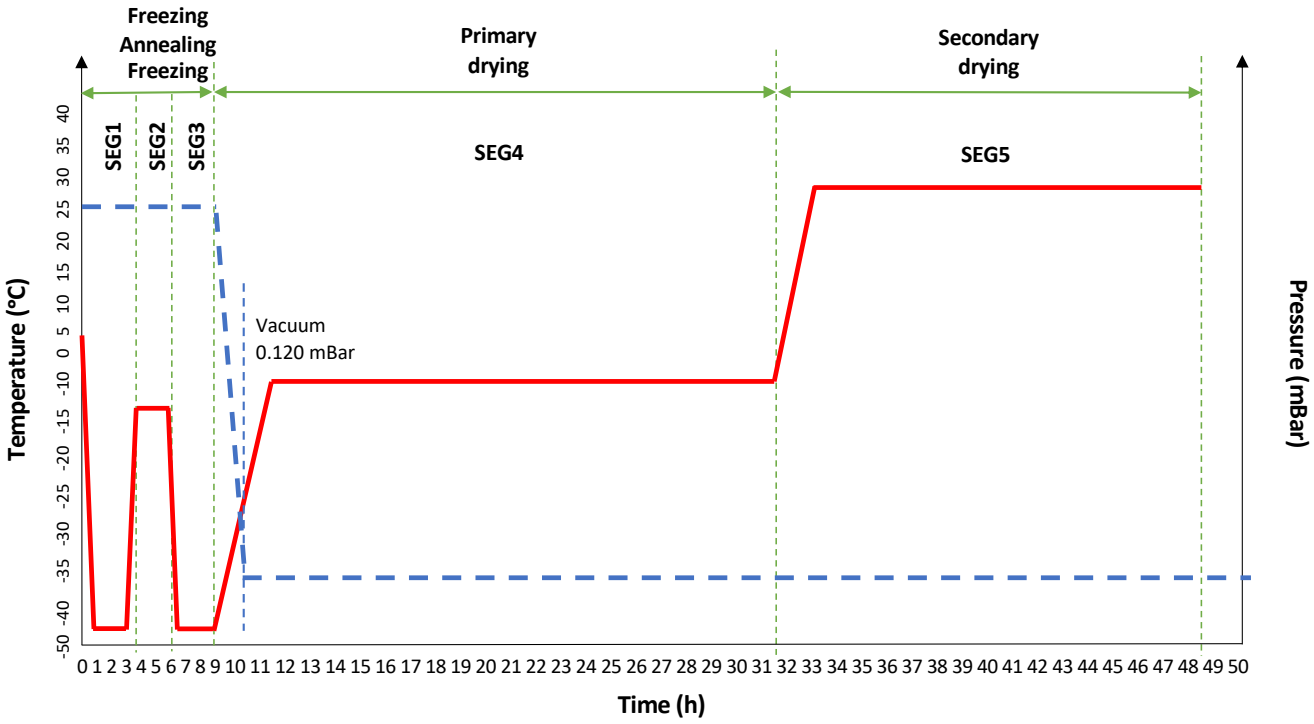




Freeze drying process (Lyophilization)

Protocols and formulations

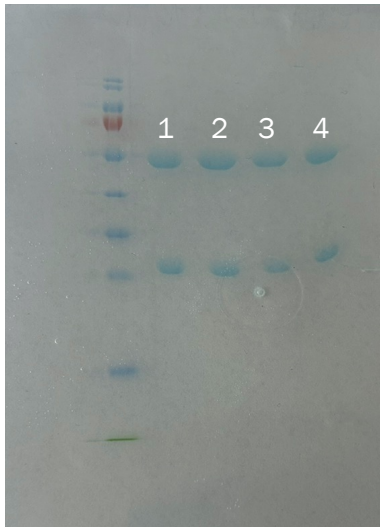
Freeze-drying protocol (III)	
SEG 1	Freezing: -40°C
	Ramp 1°C/min
	HOLD 3 h
SEG 2	Annealing: -15°C
	Ramp 0.5°C/min,
	HOLD 2 h
SEG 3	Freezing: -40°C
	Ramp 1°C/min
	HOLD 1.5 h
SEG 4	Primary drying: -15°C
	Ramp 0.15°C/min
	HOLD 18 h
SEG 5	Secondary drying: +25°C
	Ramp 0.15°C/min
	HOLD 11 h
Total ~ 50 hours	



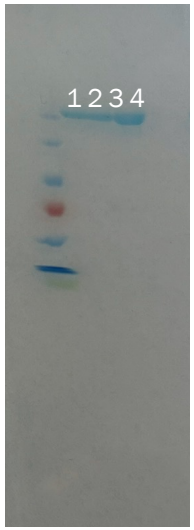
Characterization after freeze-drying

Methods (2)

- Structure with SDS PAGE
- iTLC after labeling with Lu-177

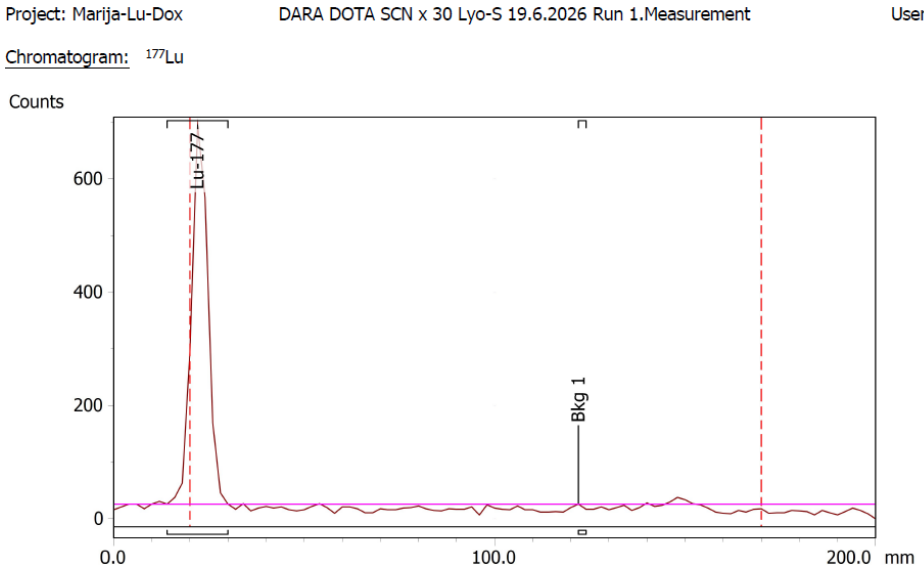


Reducing SDS PAGE



Non-reducing SDS PAGE

Samples by order
1. Purified Daratumumab
2. Purified freeze-dried Daratumumab
3. Purified Dara-DOTA SCNx30
4. Purified freeze-dried Dara-DOTA SCNx30



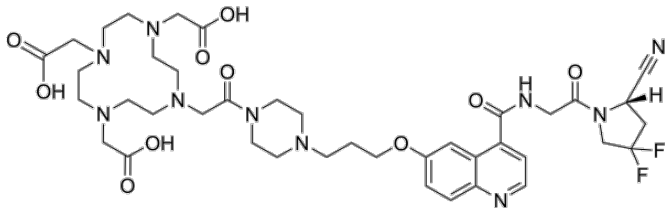
Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	14.0	30.0	0.013	1692	100.00	180.77
Bkg 1	122.0	124.0	0.680			
1 Peak				1692	100.00	180.77

FAPI - 04

Established Radiolabeling conditions

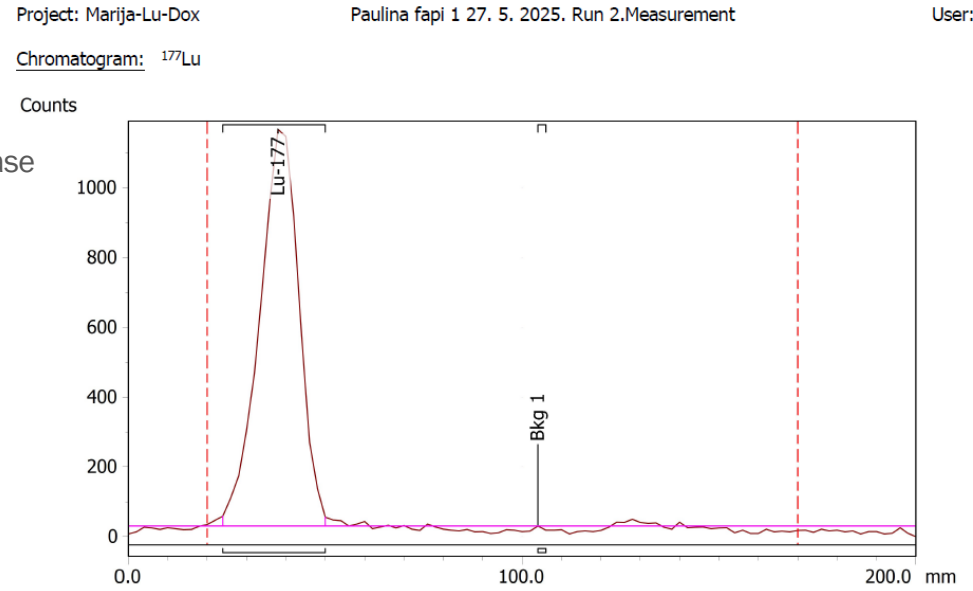
- Dissolved in DMSO (1 mg/mL)
- Adjusted pH with 0,5M Na-acetate pH 4.0
- Heating at 95°C for 15min
- iTLC for quality control in 0,1M Na-citrate as mobile phase



FAPI-04

CAS No. : **2374782-02-0**

(S)-2,2',2''-(10-(2-(4-(3-((4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	24.0	50.0	0.120	6611	100.00	111.75
Bkg 1	104.0	106.0	0.560			
1 Peak				6611	100.00	111.75

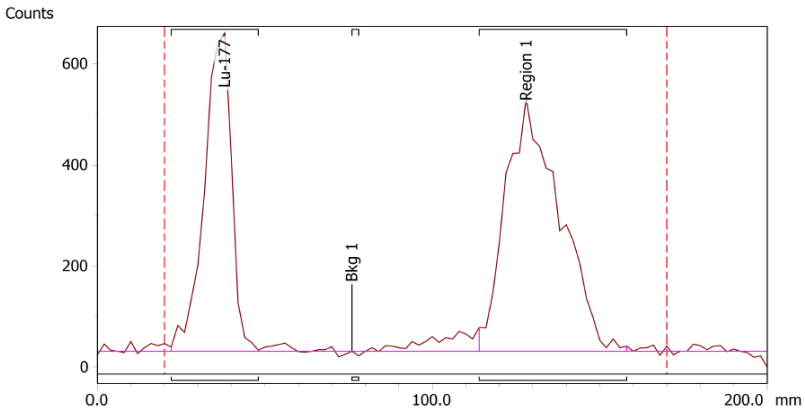
FAPI - 04

Formulation 1 X

- Dissolved in 0,9% NaCl (1 mg/mL)
- Formulated with Mannitol and Sucrose
- iTLC for quality control in 0,1M Na-citrate as mobile phase

Project: Marija-Lu-Dox FAPI-04 Lu-177 citrat Lyo 25.8.2025 Run 1.Measurement User: S

Chromatogram: ^{177}Lu



Regions: ^{177}Lu Detector: Front

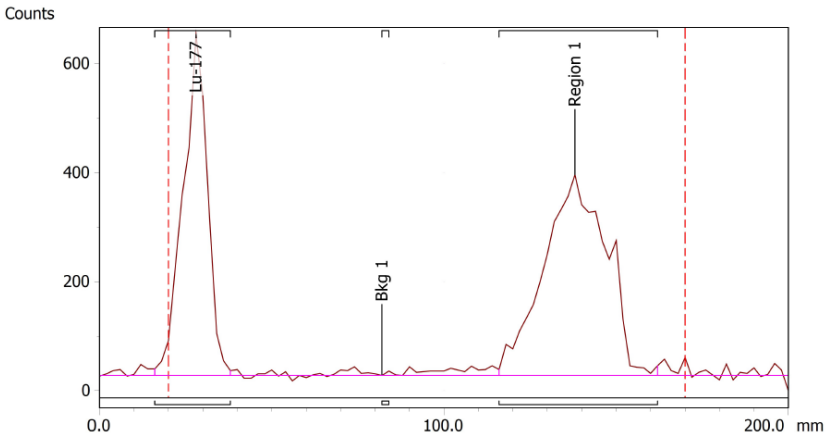
Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	22.0	48.0	0.120	2982	38.78	36.78
Bkg 1	76.0	78.0	0.373			
Region 1	114.0	158.0	0.720	4707	61.22	58.05
2 Peaks				7689	100.00	94.83

Formulation 2 ?

- Dissolved in 0,9% NaCl (1 mg/mL)
- Formulated with Sucrose
- iTLC for quality control in 0,1M Na-citrate as mobile phase

Project: Marija-Lu-Dox FAPI-04 Lu-177 citrat Lyo trehaloza kit 9.22.2025 Run 1.Measurement User:

Chromatogram: ^{177}Lu



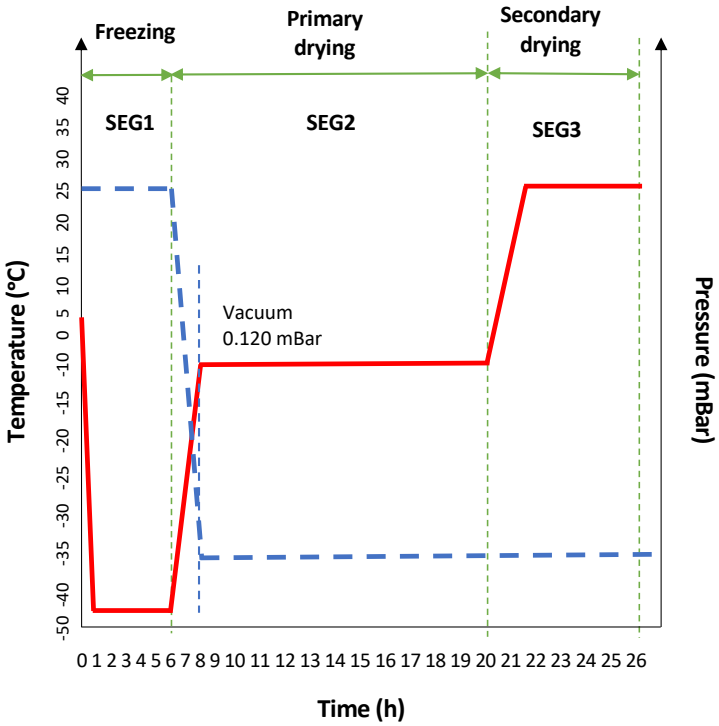
Regions: ^{177}Lu Detector: Front

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Lu-177	16.0	38.0	0.053	2577	39.87	37.47
Bkg 1	82.0	84.0	0.413			
Region 1	116.0	162.0	0.787	3887	60.13	56.51
2 Peaks				6464	100.00	93.98

FAPI - 04

Freeze-drying protocol

Freeze-drying protocol	
SEG 1	Freezing: -40°C
	Ramp 1°C/min
	HOLD 5 h
SEG 2	Primary drying: -10°C
	Ramp 0.15°C/min
	HOLD 12 h
SEG 3	Secondary drying: +25°C
	Ramp 0.15°C/min
	HOLD 4 h
	Total ~ 24 hours



Conclusions

Daratumumab-based radiopharmaceutical formulation
Promising results

FAPI-based kit formulations
Currently under development

Next Steps and Considerations

Daratumumab Kits

- Finalize **accelerated and long-term stability studies** of freeze-dried kits.
- Validate **reproducibility of radiolabeling** after extended storage.
- Prepare documentation and quality control protocols for **GMP-compliant production**.

FAPI Kits

Optimize the pre-lyophilization formulation
- Solubilization strategy
- Alternative buffers with low ionic strength
- Inclusion of surfactants or solubilizers

Reformulate excipients, exploring:
- Different cryo-/lyoprotectants
- Cake stabilizers

Adjust freeze-drying cycle parameters, including
- Slower freezing and extended annealing
- Modified primary drying temperatures
- Controlled secondary drying

Timeline

1. Develop, formulate, and characterize Lu-177 labelled: antibody - chelate conjugates based on the targeting properties of
   - **Daratumumab**
   - **FAP-based ligands**

2. To use commercially available chelators to compare their performance to identify the most suitable BFC that allows efficient radiolabelling with Lu-177, both for Daratumumab/FAPI

3. Establish and implement technology for ready-to-use preparation of freeze-dried kit formulations following GMP/ GMRP and related protocols applicable to antibody/ protein-based Lu-177 radiopharmaceuticals.

4. Establish protocols and standardize methods for identification and extensive quality control of the produced "ready to use" freeze-dried including stability study of the final formulation

5. Evaluate radiopharmaceutical's behaviour and effectiveness in veterinary patients (such as dogs) with cancers that correspond to those observed in human patients, such as Multiple Myeloma and FAP-expressing cancers - in vivo distribution, SPECT imaging, and dosimetry - proof-of-concept?

6. Implement *in silico* simulations to predict the pharmacokinetic (PK) and pharmacodynamic (PD) patterns of the developed radiopharmaceuticals-multi-compartmental model) for dose prediction?

Collaborative work and future directions

1. Supply of Ready-to-Use Freeze-dried Formulations (Daratumumab- or FAPI) for preclinical evaluation including In Vivo Studies in animal models

2. Development of New Freeze-dried Formulations upon Request - offering custom made formulation development, including excipient screening, process optimization, and Lu-177 radiolabeling compatibility

3. Freeze-drying and Preparation of ready-to use Kits – supporting the lyophilization of optimized formulations into stable, ready-to-use kits using validated protocols and quality control for in vivo testing

Institutional collaboration with:

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