

EVALUATION RADIOCHEMICAL PURITY OF

¹⁷⁷Lu-LABELLED RITUXIMAB CONJUGATES USING HPLC METHOD

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In the field of radiolabelled molecules, Rituximab appear as promising molecules for radiopharmaceutical design, because it can target specifically to CD20 antigens in non-Hodgkin lymphoma.

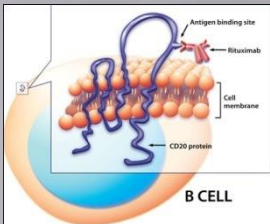


Fig. 1 Rituximab

The radiochemical purity of the labeled conjugates was determined using SE-HPLC:

- column BioSep-SEC-s3000 (300 x7.5 mm; Phenomenex)
- flow rate 1ml/min
- isocratic elution – eluent 0.1 M phosphate buffer pH 5.8
- UV detection at 220 and 280 nm
- analysis time 20 min
- sample volume: 20µl



Fig. 2 HPLC

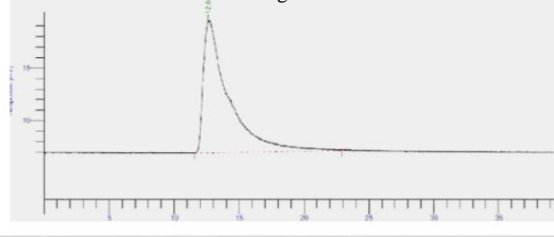
Results:

¹⁷⁷Lu-Rituximab radioimmunoconjugates with high radiolabelling yield and average of radiochemical purity (above 94.7%) and was obtained specific activity up to 1.5GBq/mg.

- Rituximab was conjugated with DTPA-, DOTA- and 1B4M - freeze dried kit
- labelled with ¹⁷⁷Lu used was 565 MBq (in 5 µL) per kit. the reaction mixture was incubated at 38°C for 1 hour
- 10 µl of 10 mM DTPA solution was added in 10 µl of radiolabelled conjugate to bind non-reacted ¹⁷⁷Lu
- HPLC analysis was performed 5 min after DTPA addition

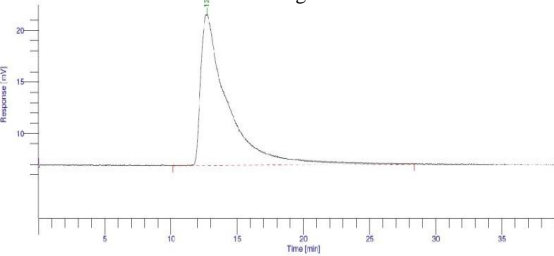
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interface Serial #	4305280096	A/D mV Range	1000
delay Time	0.00 min	End Time	40.00 min
sampling Rate	1.0000 pts/s	Area Reject	0.000000
sample Volume	1.000000 µl	Dilution Factor	1.00
sample Amount	1.0000	Cycle	5
data Acquisition Time	29-Jul-14 19:16:02		

Radiochromatogram of ¹⁷⁷Lu-1B4M-Rituximab



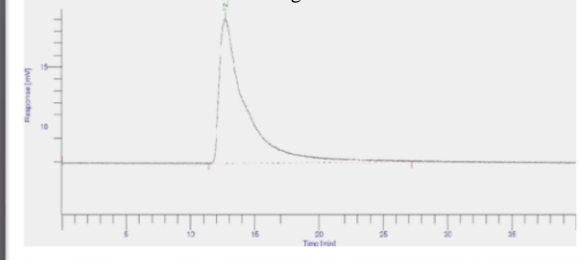
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Sample Volume	1.000000 µl	Dilution Factor	1.00
Sample Amount	1.0000	Cycle	3
Data Acquisition Time	29-Jul-14 17:53:06		

Radiochromatogram of ¹⁷⁷Lu-DTPA-Rituximab



operator	208604	Sample Name	177Lu-Ab-DTPA
sample Number	006	Study	PRC/VE IAEA
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instrument Name	Radomatic-150TR	Channel	A
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delay Time	0.00 min	End Time	40.00 min
sampling Rate	1.0000 pts/s	Area Reject	0.000000
sample Volume	1.000000 µl	Dilution Factor	1.00
sample Amount	1.0000	Cycle	4
data Acquisition Time	29-Jul-14 18:34:34		

Radiochromatogram of ¹⁷⁷Lu-DTPA-Rituximab



Conclusion:

With the obtained results we can conclude that ¹⁷⁷Lu- Rituximab radioimmunoconjugates can be used for development of the preclinical studies in experimental animal model.