



CONTEXT

Since several years, biosimilars of therapeutic proteins are available in EU, mainly growth factors such as erythropoietin and G-CSF. Very recently, the first biosimilar of RTX has been registered in EU one year after the mAb infliximab (CT-P10). Since a biosimilar was highly comparable to its princeps, it is likely to consider that its stability was also comparable.

MATERIAL&METHODS CT-P10, 50 ml vials at 10 mg/ml, was a gift from Biogaran (France).

- 3 different batches were used
- 2 concentrations in polyolefin bags : C1(1 mg/ml) and C2 (4 mg/ml)
- 2 storage temperature : +4°C and air conditioned room (21-25°C), in light-protecting bags.
- Times of analysis : T0 (control), Day 7, Day 14 and 1 month
- Physical stability analysis and chemical stability analysis
- Several orthogonal and complementary methods as recommended (ICH and SFPO recommendations)



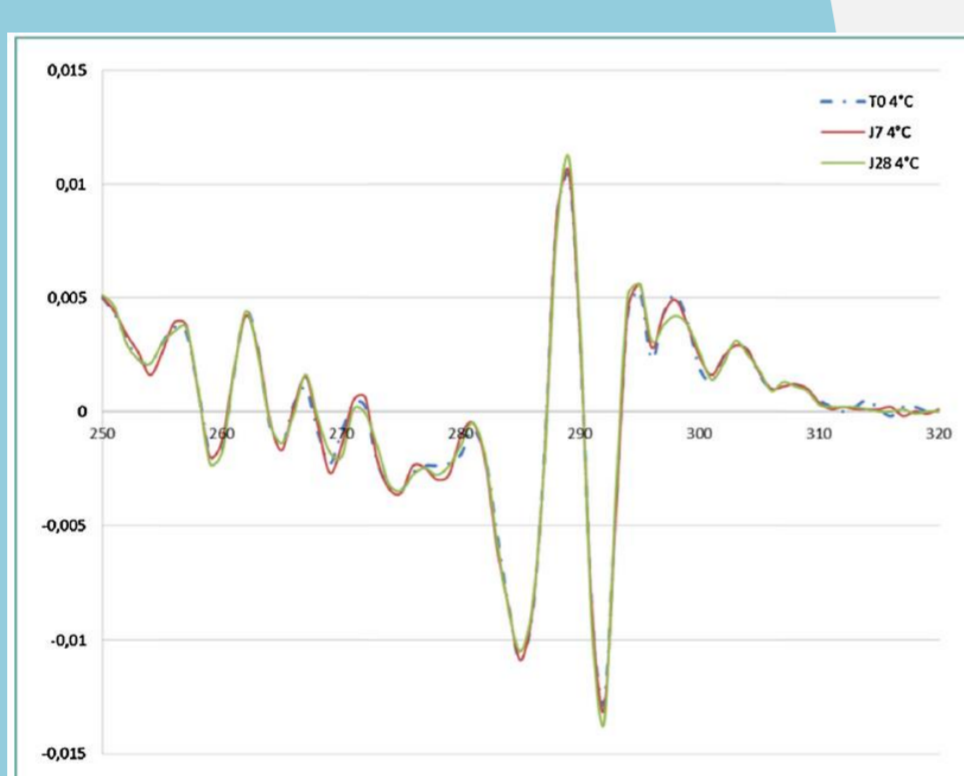
OBJECTIVE Determine the stability of the rituximab biosimilar CT-P10 after storage in polyolefin bags at 4°C and 25°C.

RESULTS& DISCUSSIONS

UV SPECTROMETRY: Absence of light diffracting aggregate formation during the 28 – day storage period whether at +4°C or at +25°C. For each concentration and storage temperature, the respective aggregation index at D28 was not different from T0.

	Batch 1	Batch 2	Batch 3
T0			
Mean	79.07	78.92	82.73
SD	1.08	0.28	0.98
D28 4°C			
Mean	75.70	78.38	78.90
SD	3.04	0.51	0.58
D28 25°C			
Mean	80.10	83.10	83.57
SD	0.07	0.05	0.03
Change from T0 to D28	+1.03	+4.18	+0.084

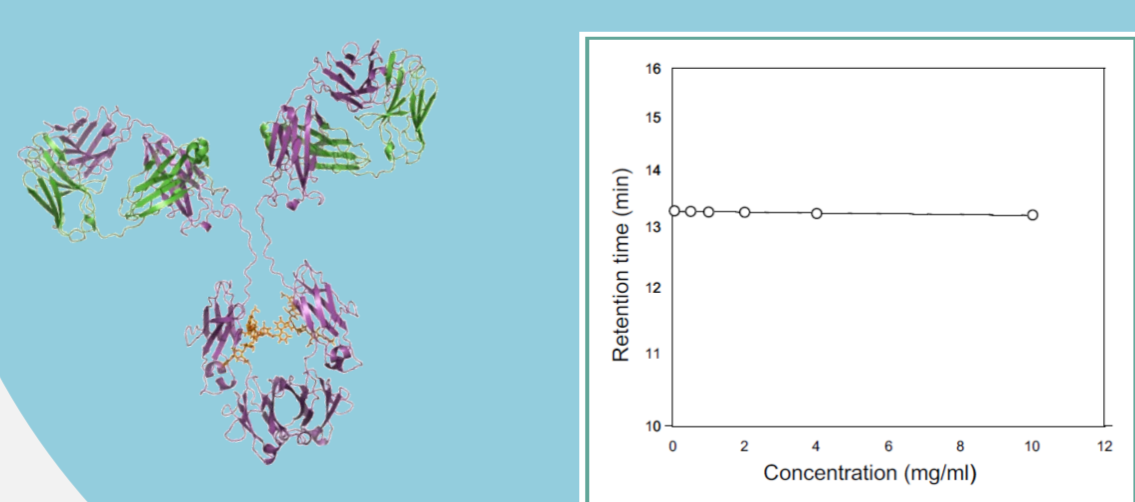
* P > 0.01.



The tertiary structure of the antibody remained completely unchanged after storage at 4°C for 28 days. After storage at 25 °C we observed small discrepancies.

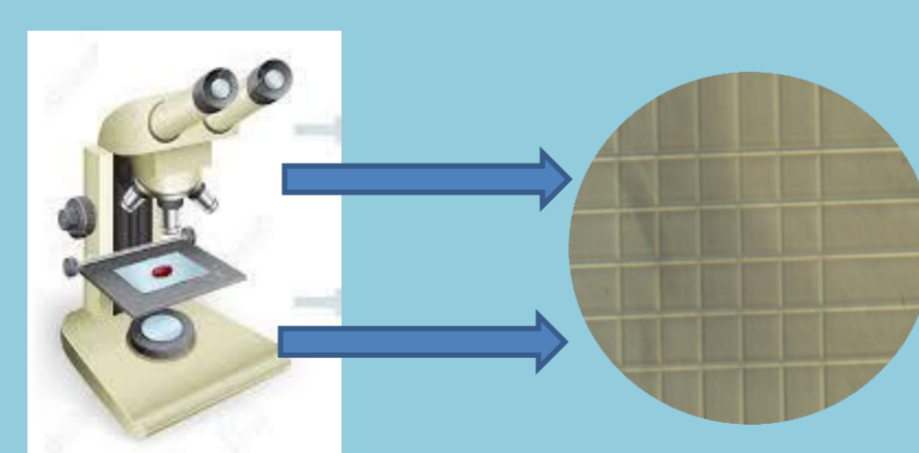
QUINARY STRUCTURE:

The results confirm the weak tendency of the biosimilar to form stable oligomers.



MICROSCOPIC IMAGE ANALYSIS:

The analysis showed no significant differences in particles levels after 28 days of storage



No significant changes in density and osmolarity were observed after 28 days of storage

Changes observed with pH remained very modest

No bacterial contamination was detected in any of the samples

CONCLUSION After dilution in sterile conditions with 0,9% NaCl in polyolefine bags, to the concentrations routinely used (1 and 4 mg/ml), the rituximab biosimilar CT-P10 appears to remain stable for at least one month at 4°C. After 28 days of storage at 25°C, only the tertiary structure appeared to be slightly. The same conclusions can be drawn for the vials of undiluted product.

DLS:

No change in hydrodynamic diameter or dispersity was observed during storage. A linear relationship was found between the hydrodynamic diameter and the protein concentration.

	C1 (1 mg/mL)						C2 (4 mg/mL)					
	4°C			25°C			4°C			25°C		
Batch no.	1	2	3	1	2	3	1	2	3	1	2	3
T0 Diameter (nm)	11.20	11.33	11.29	11.12	11.26	11.24	11.59	11.64	11.69	11.55	11.79	11.77
SD	0.13	0.10	0.03	0.03	0.03	0.03	0.05	0.09	0.05	0.06	0.02	0.06
DI	0.071	0.088	0.092	0.063	0.077	0.076	0.053	0.059	0.063	0.050	0.072	0.063
SD	0.010	0.021	0.003	0.009	0.012	0.011	0.004	0.016	0.004	0.009	0.008	0.010
D28 Diameter (nm)	11.25	11.29	11.49	11.34	11.30	11.24	11.47	11.46	11.52	11.39	11.43	11.50
SD	0.05	0.04	0.08	0.16	0.05	0.02	0.05	0.06	0.08	0.05	0.06	0.03
DI	0.084	0.088	0.123	0.099	0.091	0.095	0.056	0.055	0.061	0.059	0.040	0.081
SD	0.011	0.002	0.034	0.012	0.011	0.013	0.010	0.003	0.014	0.010	0.006	0.011

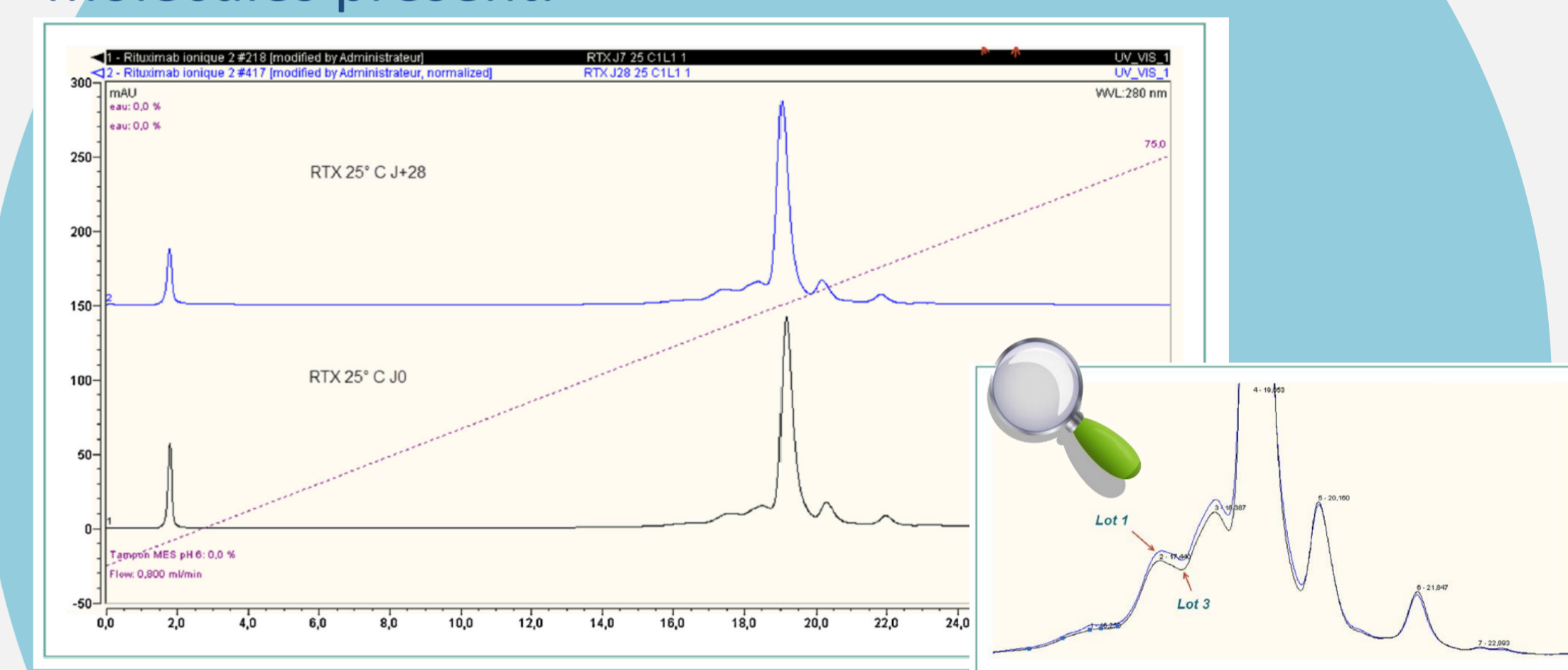
Tm: Thermodynamic stability does not seem to be affected by concentration, temperature or storage duration.

	4°C					
	C1			C2		
Batch no.	1	2	3	1	2	3
Mean	72.18	71.14	71.24	72.16	72.11	72.05
±SD	0.11	0.14	0.11	0.10	0.10	0.00

	25°C					
	C1			C2		
Batch no.	1	2	3	1	2	3
Mean	71.95	71.38	71.67	71.81	71.81	ND
±SD	0.22	0.12	0.29	0.22	0.04	ND

CEX:

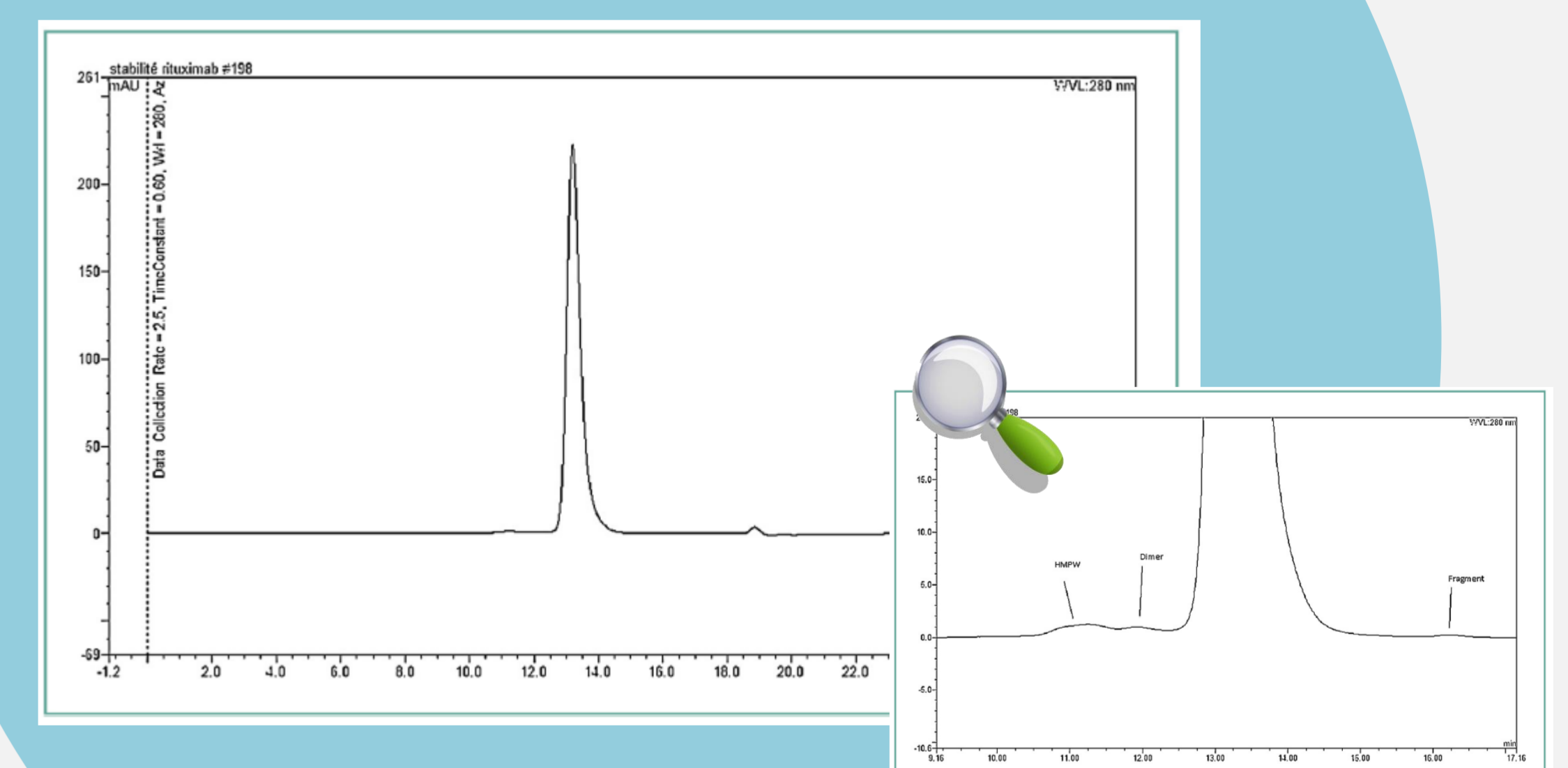
Seven rituximab charge variants were observed. The variant 4 predominated, accounting for a mean of about 86 % of the molecules present.



No new peak in either the acidic or basic part of the chromatograms, potentially corresponding to new degradation products, due to the deamidation of asparagine residues for example, was observed.

SEC:

No significant differences in AUC were observed regardless of concentration, batch, temperature or duration of storage.



No additional peaks were detected at day 28, demonstrating the absence of oligomer formation and modification of the molecule that might lead to fragmentation.