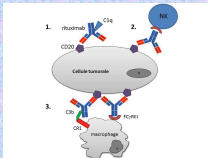


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INTRODUCTION



What is Rixathon® (Rituximab. 10mg/ml) ?

- Chimeric monoclonal antibody against the protein CD-20 of immune system B cells.
- Prescribe to treat Non-Hodgkin's Lymphoma (NHL), Chronic lymphoid leucemia (CLL) and Rheumatoid arthritis (RA).
- Currently, the known stability is 30 days after dilution in NaCl 0.9% polyolefin bags at 2-8°C, and additional 24h at 25°C

➔ Can Rixathon® bags at 1 and 4 mg/mL be kept more than 30 days at 4°C ?

➔ In case of prolonged temperature excursion at 25°C, is Rixathon® stability preserved ?

MATERIAL AND METHODS

Physical and chemical stability analysis:

- pH / Osmolality / density
- Turbidimetry : Calculation of Aggregation Index (AI) by UV Spectroscopy at 350 and 280 nm
- Dynamic Light Scattering (DLS)
- Size Exclusion Chromatography (SEC)
- Ionic Chromatography

AI = A350 / (A280 - A350) \* 100



Structural stability analysis :

- Primary structure analysis by peptide mapping
- Secondary structure analysis by Infrared Spectroscopy (FTIR-ATR)
- Tertiary structure analysis by fluorescence and UV Spectroscopy

➔ Analysis have been realised in triplicate. All methods used have been demonstrated as an indicator of stability for antibodies (Ph. Eur. 10.0. « Monoclonal Antibodies for Human Use »).

➔ Bags and vials have been stored at 4°C for 12 months. with a prolonged temperature excursion to 25°C for 28 days.

RESULTS & DISCUSSION

Physical and chemical stability

- ✓ Osmolality was between 287 ± 1 and 365 ± 2 mOsm/kg. There was any modification of osmolality values over time (variations < 10 mOsm/kg).
- ✓ pH remained constant over time with an average value of 6.5 ± 0.05 at day 1. Variations are lower than the fixed standard of 0.5 pH unit.
- ✓ Density variations didn't exceed 0.01 g/cm<sup>3</sup> whatever the tested conditions, with average value of 1.004 ± 0.0002 g/cm<sup>3</sup>.

✓ Agregation index (AI) remained lower than 10 during the study, confirming the absence of visible and sub-visible aggregate formation.

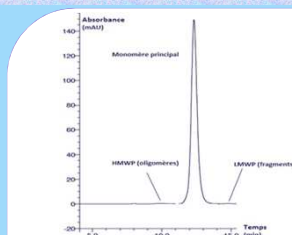
Average Agregation index	D0	M12 4°C	D28 25°C
1mg/mL bags	0.24 ± 0.12	0.79 ± 0.48	0.34 ± 0.09
4mg/mL bags	1.73 ± 0.98	1.26 ± 0.07	0.58 ± 0.07
Vials (10mg/mL)	4.18 ± 0.73	1.27 ± 0.09	0.41 ± 0.19

Table 1 : Average Agregation Index over time

	Diameter (nm)	pdi
1mg/mL bags	11.95 ± 0.34	0.07 ± 0.026
4mg/mL bags	12.07 ± 0.24	0.06 ± 0.01
Vials (10mg/mL)	12.73 ± 0.08	0.06 ± 0.02

Table 2 : Hydrodynamic diameter and average pdi.

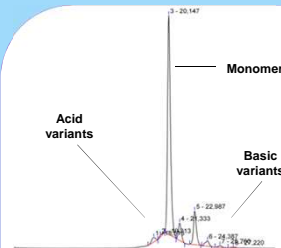
✓ No modification hydrodynamic diameter has been mesured by DLS during the study. The distribution remained monodisperse (pdi ≤ 0.1). No sub-populations of submicronics aggregate. micronics aggregate or particles over time (0.3 nm to 10 µm)



✓ No chromatographic SEC profiles modification happened during the study: no monomeric peak retention time modification, nor HMWP peaks (High Molecular Weight Proteins) nor LMWP peaks increasing (Low Molecular Weight Proteins) occurred.

Table 3 : SEC isoforms distribution percentage over time.

Peak ratio (%)	J0			M12. 4°C			J28. 25°C		
	HMWP	Monomer	LMWP	HMWP	Monomer	LMWP	HMWP	Monomer	LMWP
1mg/mL bags	0.48 ± 0.03	99.5 ± 0.03	0.02 ± 0.01	0.44 ± 0.02	99.5 ± 0.02	0.06 ± 0.01	0.45 ± 0.01	99.5 ± 0.01	0.05 ± 0.01
4mg/mL bags	0.49 ± 0.02	99.5 ± 0.02	0.02 ± 0.01	0.43 ± 0.02	99.5 ± 0.02	0.06 ± 0.01	0.45 ± 0.01	99.5 ± 0.01	0.05 ± 0.01
Vials (10mg/mL)	0.49 ± 0.03	99.5 ± 0.03	0.03 ± 0.01	0.44 ± 0.02	99.5 ± 0.02	0.07 ± 0.01	0.45 ± 0.01	99.5 ± 0.01	0.05 ± 0.01



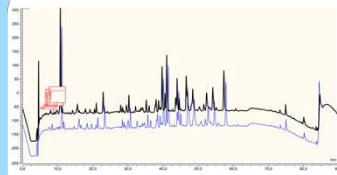
✓ Ionic chromatography showed no modification of ionic variants distribution, especially the absence of deamidation phenomenon.

➔ Absence of chemical instability.

Table 4 : Ionics variants distribution percentage over time.

Peak ratio (%)	J0			M12. 4°C			J28. 25°C		
	Acid variants	Monomer	Basic variants	Acid variants	Monomer	Basic variants	Acid variants	Monomer	Basic variants
1mg/mL bags	2.58 ± 0.25	79.16 ± 1.9	18.26 ± 2.1	2.96 ± 0.14	78.84 ± 2.01	18.2 ± 0.36	4.56 ± 0.36	78.96 ± 1.6	16.49 ± 1.92
4mg/mL bags	2.69 ± 0.25	78.36 ± 1.8	18.93 ± 1.99	2.9 ± 0.19	79.6 ± 1.73	17.5 ± 0.52	4.34 ± 0.52	80.11 ± 1.73	15.55 ± 1.78
Vials (10mg/mL)	2.61 ± 0.23	78.26 ± 2.1	19.12 ± 2.24	2.61 ± 0.22	80.67 ± 1.74	16.73 ± 1.92	4.73 ± 0.22	81.34 ± 1.39	13.93 ± 1.57

Structural stability



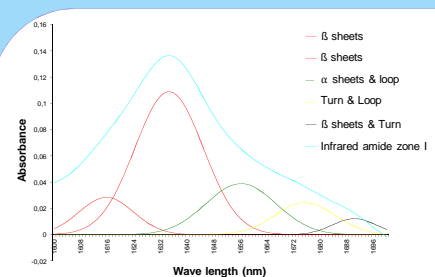
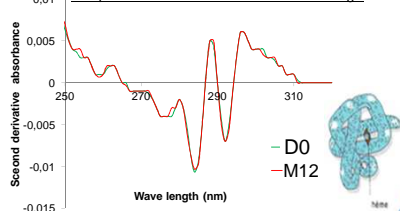
✓ The spectra obtained by peptide mapping didn't show any primary structure of the antibody modification over study time at 215 et 280 nm.



✓ Second derivative spectra remained stackable over time. There is no tertiary structure modification of the antibody during the study.

This is confirmed by fluorescence spectroscopy analysis. The same results are obtained after 28 days at 25°C.

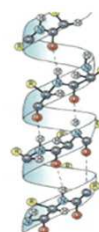
Rixathon UV Second derivative spectra comparison at D0 and M12 after 4°C storage



✓ Infrared spectroscopy analysis didn't show any modification of the secondary structure of the antibody over time.

	D0	M12. 4°C	D28. 25°C
β sheets	67.1 ± 1.4%	66.8 ± 0.1%	65.6 ± 1.1%
α sheets & Loop	15.7 ± 0.6%	15.7 ± 0.3%	15.7 ± 0.8%
Sheets & Loop	9.5 ± 1.2%	10.2 ± 0.9%	11.5 ± 1.9%
β sheets & Turn	7.7 ± 0.4%	7.2 ± 0.4%	7.2 ± 0.2%

Table 5 : Secondary structure turns and sheets percentage.



CONCLUSION

- ➔ Rixathon® is stable at least 12 months at 4°C and 28 days at 25°C after dilution in NaCl 0.9% polyolefin bags. Moreover, sterility study didn't show any microbial growth during the study.
- ➔ These datas will allow the early preparation of Rixathon® bags, under the hospital preparation status, and the return of unused bags.
- ➔ The early preparation of Rixathon® will permit to anticipate needs, improve the fluidity of the preparation and administration circuit, and finally generate financial savings.