PHYSICOCHEMICAL AND BIOLOGICAL STABILITY OF DILUTED RITUXIMAB SOLUTIONS STORED 6 MONTHS AT 4°C

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BACKGROUND

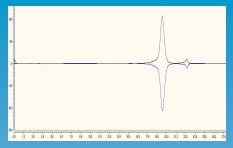
Rituximab (rtx) is the first IgG1 monoclonal antibody (mAb) successfully employed in onco-hematology. According to manufacturing instructions, rtx must be prepared by dilution in a 0.9% sodium chloride solution and must be administrated within 24 hours because of instability. As far as mAbs are concerned, surprisingly, little information on their stability upon storage is available except for therapeutic immunoglobulin that exhibit a long shelf life (>1 year) in liquid state. As it is an important challenge for pharmacist to assess the stability of biotechnology-issued anticancer drugs such as mAbs because of the widespread ready-to-use centralized preparation of these drugs by the pharmacy departments, the objective of this study was to assess physical, chemical and biological stabilities of diluted rtx (1 mg/ml) for six months under optimal storage temperature.

METHOD

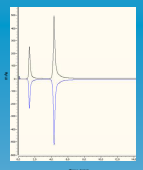
Rituximab (Mabthera ®) was diluted in NaCl 0.9% (Freeflex® polyolefin bags) to obtain a 1 mg / ml concentration.
Three batches were prepared under aseptic conditions.
Storage at 4°C during 6 months was applied.
Samples were withdrawn and analyzed at days D0, D14, D30, D90 and D180.
Samples were centrifuged at 4000 rpm- 5min before HPLC and UV Spectrophotometric analysis.
Various protein characterization methods were used to determine changes in physicochemical properties including size exclusion chromatography (SEC), cation exchange chromatography (CEX), dynamic light scattering and turbidity (analysis at 350 nm), second-derivative IR and UV spectrometry for the study of secondary and tertiary structure and peptide mapping. Cell culture with RAJI lymphoma cell was used to access biological stability.

RESULTS

Chromatographic analysis



Chromatogram in mirror for SEC



Chromatogram in mirror for CEX

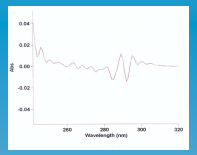
Time (days)	D0	D90	D180
AUC (mAu*min)	79.47+/- 3.93	76.75+/-. 1.18	75.5+/- 2.33
Tr (min)	19.02+/- 0.01	19.01+/- 0.02	19.01+/- 0.02
Molecular Weight* (kDa)	127.5 +/- 1.0	128.0 +/- 1.2	126.7 +/- 2.47

Different chromatographic parameters obtained in SEC for rtx stored at + 4°C

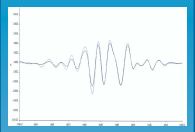
Time (days)	D0	D90	D180
AUC (mAu*min)	140.74 +/- 4.21	141.00 +/- 0.16	138.35 +/- 3.62
Tr (min)	4.27 +/- 0.021		

Different chromatographic parameters obtained in CEX for rtx stored at + 4°C

Spectrophotometric analysis

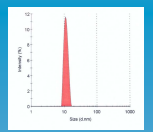


Second-derivative UV spectrometry
Tertiary structure



Second-derivative IR spectrometry
Secondary structure

Dynamic light scattering

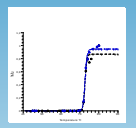


Storage (days)	Main Peak	PdI*
up to 6 months	11.18 +/- 0.24	0.07 +/- 0.030

Mean hydrodynamic (nm) diameter of rtx formulation (1 mg/ml)

* Polydispersity Index

Thermodynamic parameters

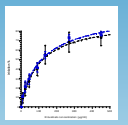


Determination of melting temperature

Storage (days)	T_m (°C)	m_i (cal.mol^{-1}.K)	ΔS(cal.mol^{-1}.K^{-1})	ΔH (Kcal.mol^{-1})
D0	72.25 +/- 0.31	-1385 +/- 12.7	- 139.3 +/- 3.15	51.7 +/- 3.15
D6 months 4°C	72.70 +/- 0.72	-1910 +/- 17.4	- 163.0 +/- 12.03	55.9 +/- 4.21

Biological activity

Time (days)	D0	D90	D180
Number of experiments	10	2	5
IC50 (µg/ml)	137.2 +/- 28.18	134.7 +/- 21.6	125,8 +/- 36,5
IC25 (µg/ml)	41.9 +/- 8,3	45,6+/6 0,56	35,8 +/- 11,9
R^2	0,994 +/- 0,035	0,986 +/- 0,014	0,993 +/- 0,031
AUC	25334 +/- 2316	25785 +/- 1284	25077 +/- 2451



Cell culture (RAJI lymphoma cell)

Black line for D0 and blue line for 6 months storage except second derivative UV spectrometry where DO is represented in red.

The optical densities at 350 nm are unchanged after a 6 month storage at 4°C demonstrating the absence of aggre gation. These results are corroborated by those of SEC (similar chromatographic profiles, AUC and retention time no modified) and DLS (Mean diameter unchanged) The thermodynamic parameters of thermal aggregation were similar traducing the absence of structural modification. The spectrometric methods also show that secondary and tertiary structures are conserved. The chromatographic profiles obtained in CEX and in peptide mapping (data non shown) are superimposable demonstrating the absence of chemical reaction like deamidation and modification of cleavage sites. The biological activity (cytotoxicity on CD20-expressing cells) are unchanged.

CONCLUSION

In the opposite of the manufacturer recommendations, the diluted Rtx is strongly stable (physico-chemically and biologically) up to 6 months at 4°C. Thes e stability data could authorize the anticipated preparation by pharmacy centralized units, improving the pharmacy workload and the quality of preparations and saving large amounts of money.