STABILITY OF PROTEINS ADMINISTERED BY INTRAVENOUS ROUTE
THE USER PERSPECTIVE

Prof. Alain Astier. UPREC, Pole de pharmacie ; GHU Henri Mondor, School of Medicine , 94010 Créteil

The speaker declares no conflict of interest for this presentation

Background

- For hospital pharmacists, it is critical to have well-documented data about the stability of injectable drugs in practical situations: opened drug formulation, after reconstitution of a lyophilized product or after dilution in various vehicles.
- Unfortunately, these data are seldom (if never) available.

Background (2)

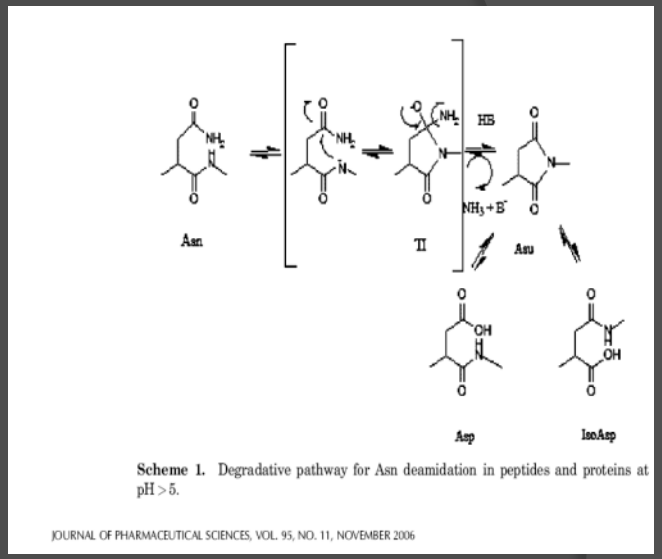
- The manufacturer's stability data after reconstitution or dilution were quite always quoted as "*stable for 8 or 12 hours at 4°C*", not for true reasons but only by the application of the "care principle" considering possible bacterial contamination or by the fact that stability tests were only conducted during a very short period.

Background (3)

- This problem is of paramount important for therapeutic proteins, mainly monoclonal antibodies (mAbs), which are very expensive drugs showing an explosive growth in all clinical fields, especially in oncology.
- Thus, It is unacceptable to loss, even small amounts, of these very expensive drugs for “non-scientifically based” reasons.

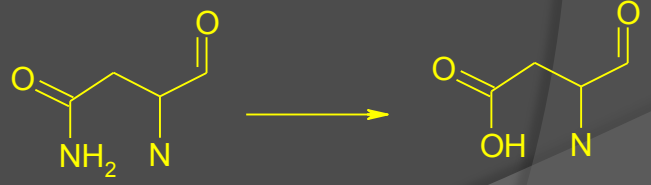
Protein instability

- Physical instability :
  - Aggregation
  - Denaturation
  - Adsorption on surfaces
- Chemical instability:
  - Desamidation
  - Disulfide bond breakage and formation
  - Oxydation
  - Hydrolysis
  - Isomerization
  - Non-disulfide crosslinking
  - Deglycosylation
  - Maillard reaction



Scheme 1. Degradative pathway for Asn deamidation in peptides and proteins at pH > 5.

JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 95, NO. 11, NOVEMBER 2006



Asparagine Aspartate

Aggregation of proteins

- Principal physical instability
- Non classical degradation
- General response of proteins to mechanical and thermal stresses
- Mainly underestimated: soluble or insoluble aggregates
- One of the most underestimated origin of aggregation is mechanical stresses:
  - Shaking or stirring
  - Shearing (rapid sampling by syringe)
  - Exposure to hydrophobic gas interface (bubbling or filtration)
- Major implications: induction of antibodies
  - Loss of efficacy (neutralizing Abs)
  - Apparition of toxicities = immunogenicicité (antigenic Abs)

Principal causes of instability

- Temperature
  - Elevation
  - Freezing
- Formulation pH
- Adsorption
- Salt effect
- Oxygen
  - Metal ions
  - Chelating agents
- Shaking and shearing
- Concentration

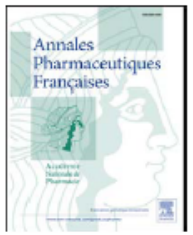
In-use or “practical” situations

- Temperature excursion
  - Cold-chain rupture
  - Bad storage practice in ward, non use, light exposure
- Extended storage times
  - Non or delayed administration
  - Residues
  - Dose-banding
- Preparation
  - Shaking, bubbling
  - Use of metallic needles
  - Use of various plastic devices
- Transportation
  - Pharmacy to ward
  - Pneumatic network
  - Long distances (between hospitals)

Daily problems

- May I kept opened vials for another patients ?
- What happen if a cold-chain rupture of my fridge occur during the WE ?
- May I use the patient's non-administered bag after one week ?
- May I transport safely the bag from Tours to Amboise…

Annales Pharmaceutiques Françaises (2011) 69, 221–231



ORIGINAL ARTICLE

Guidelines for the practical stability studies of anticancer drugs: A European consensus conference☆

Recommandations pour les essais de stabilité pratique des médicaments anticancéreux : une conférence de consensus européenne

C. Bardin[a,*], A. Astier[b], A. Vulto[c], G. Sewell[d], J. Vigneron[e], R. Trittler[f], M. Daouphars[g], M. Paul[b], M. Trojniak[h], F. Pinguet[i]

[a] *Hôtel-Dieu Hospital, 1, place du Parvis-Notre-Dame, 75181 Paris cedex 4, France*
[b] *UMR 7054, Henri-Mondor Hospital, School of Medicine, 94010 Créteil, France*
[c] *Erasmus Medical Center, Rotterdam, The Netherlands*
[d] *Plymouth University, Plymouth, United Kingdom*
[e] *INFOSTAB, 54000 Nancy, France*
[f] *University Hospital, Freiburg, Germany*
[g] *Centre Henri-Becquerel, 76038 Rouen, France*
[h] *Instituto Oncologico Veneto, Padova, Italy*
[i] *Val d'Aurelle Cancer Center, 34298 Montpellier, France*

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Available on Stabilis: www.stabilis.org

Stability studies of biologics requires several complementary analytical methods

- **Analytical methods**
  - Peptide mapping (I° structure)
  - 2th derivative FTIR or CD (II° structure)
  - 2th derivative UV or fluorescence (III °structure)
  - Turbidity or nephelometry (visible aggregation)
  - Dynamic ligth scattering (DLS; sub-visible aggregation)
  - IC and SEC chromatography (chemical modifications) native and after enzymatic treatments
  - Thermal denaturation curves

- **Determination of pharmacological activity**

Stability of biologics

- We have studied the stability of several biological drugs
  - Cetuximab
  - Trastuzumab
  - Rituximab
  - Bevacizumab
  - Ipilimumab
  - Infliximab
  - L-asparaginase

  submitted to thermal and/or mechanical stresses which can occur in practical situations

Stability of diluted rituximab

- To develop dose banding of rituximab in haematology (600 and 700 mg)
  - Batch preparation following GMPs = better quality
  - Money saving
- Need to have extended stability data

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Long-term stability of diluted solutions of the monoclonal antibody rituximab

Muriel Paul*, Victoire Vieillard, Emmanuel Jaccoulet, Alain Astier

APHP, GH Henri Mondor, Département de Pharmacie, Unité Pharmaceutique de Recherche en Essais Cliniques, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France

No dimerisation, oligomerisation or chain break

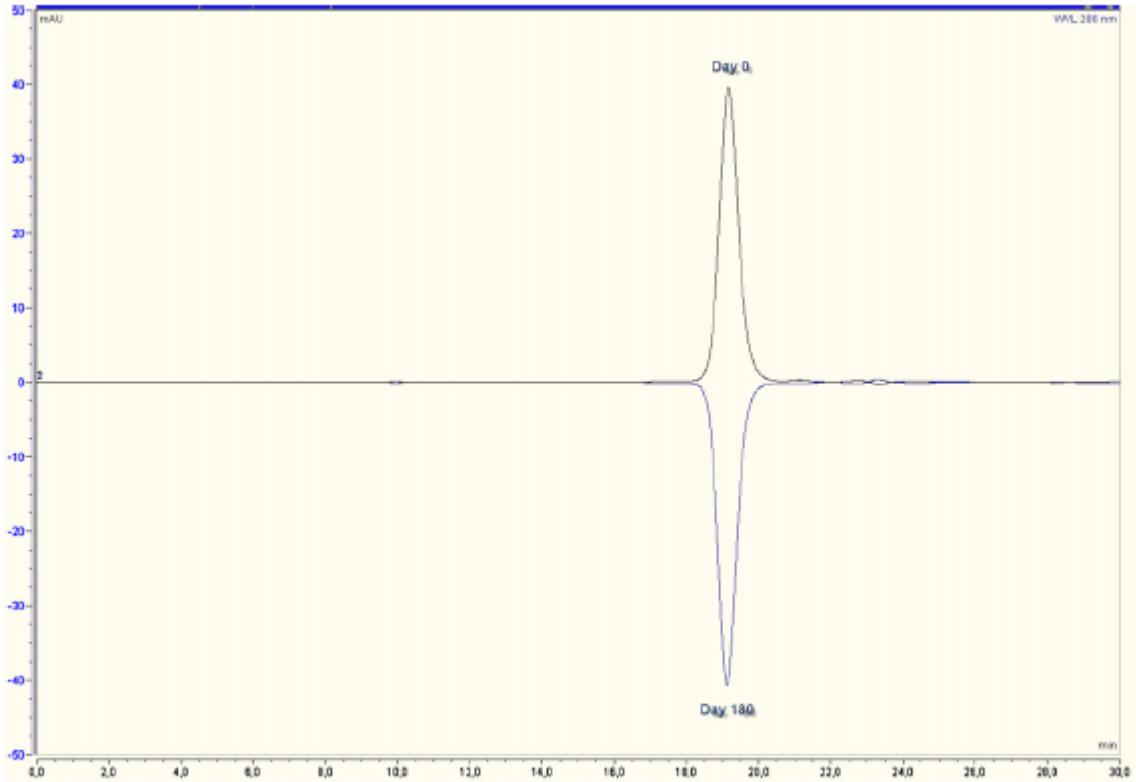


Fig. 2. Mirror comparison of size-exclusion chromatograms of diluted trastuzumab (0.8 mg ml^{-1} in saline) in polyolefin bags. Day 0: control; day 180: after 6 months of storage at 4°C. No high molecular weight peak was observed, demonstrating no sub-micronic aggregation during the long-term storage. No low molecular weight peak corresponding to mAb fragmentation was observed. Profiles were identical after storage at room temperature.

Size exclusion chromatography

Rituximab SEC

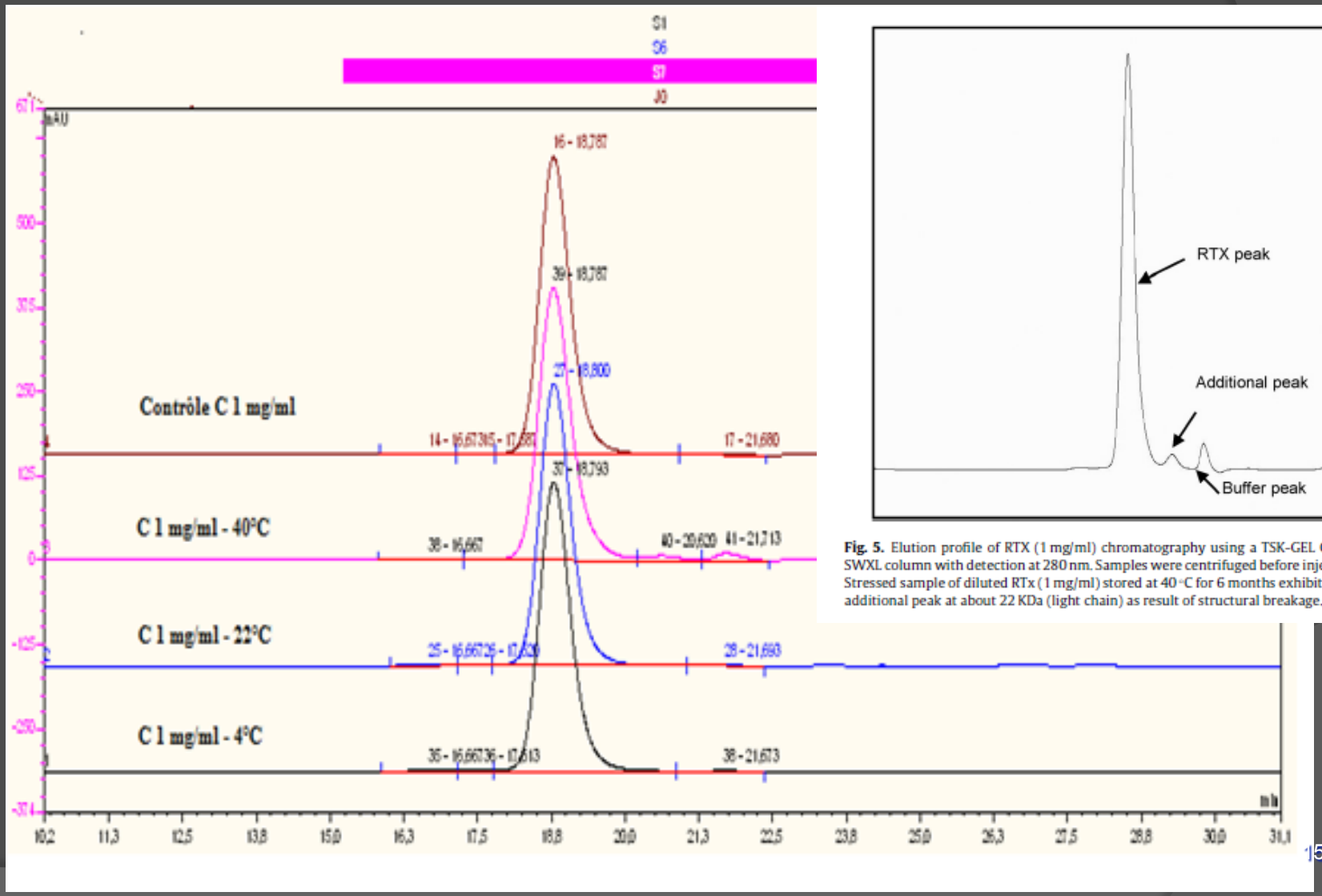
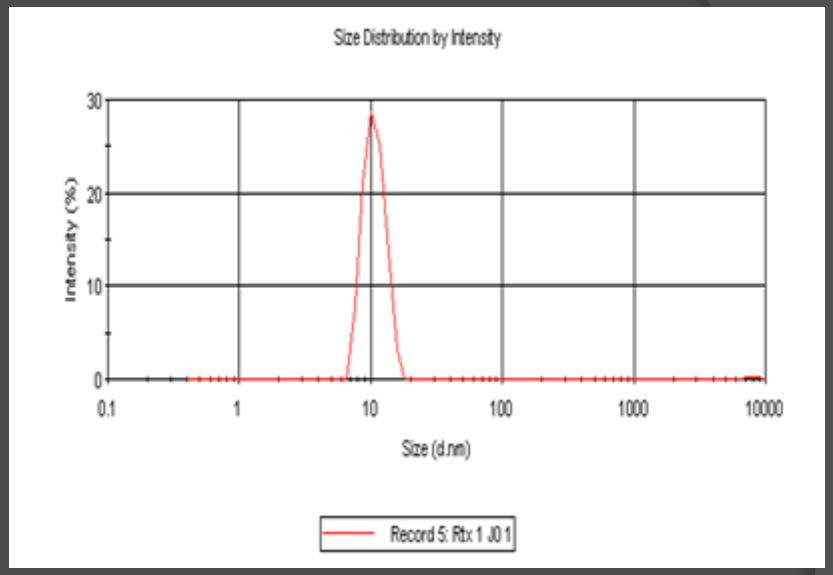
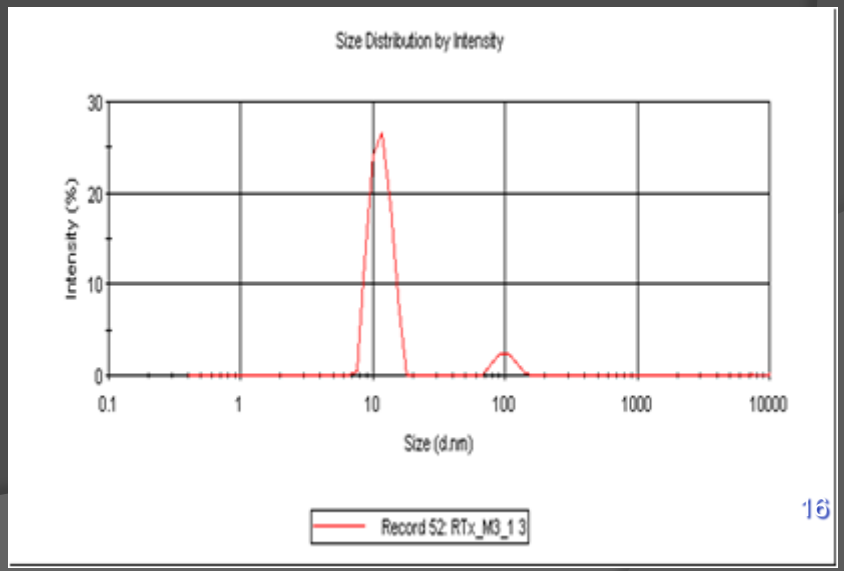


Fig. 5. Elution profile of RTX (1 mg/ml) chromatography using a TSK-GEL G4000 SWXL column with detection at 280 nm. Samples were centrifuged before injection. Stressed sample of diluted RTx (1 mg/ml) stored at 40 °C for 6 months exhibiting an additional peak at about 22 KDa (light chain) as result of structural breakage.

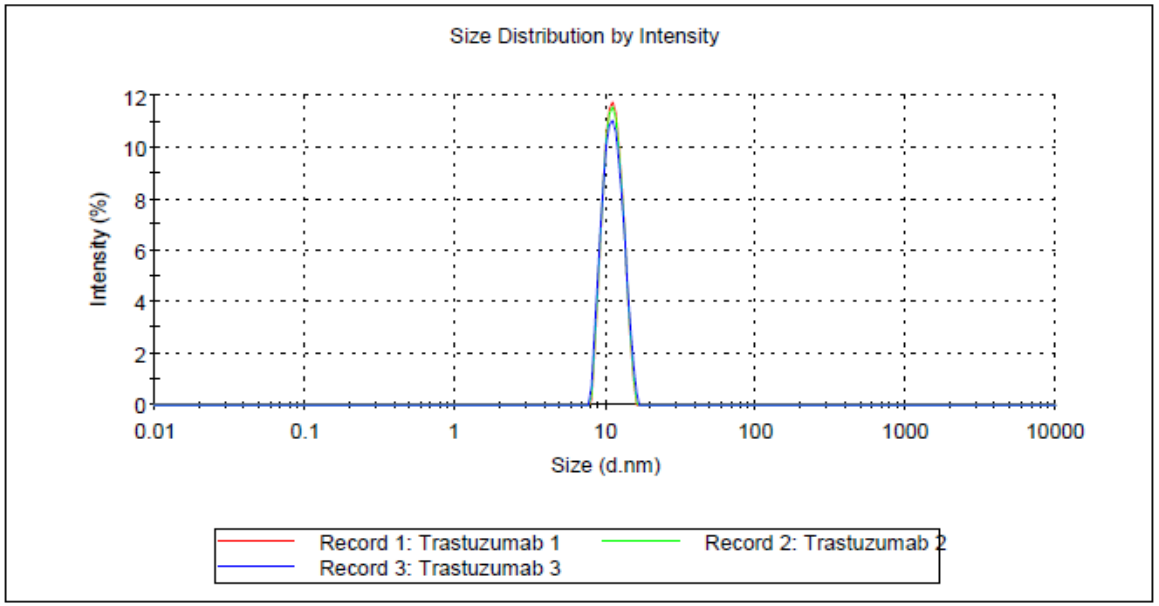
Rituximab Size analysis

- Diffraction laser spectroscopy DLS = molecular size and soluble aggregates
- After 3 months at 37°C: appearance of a 100 nm population





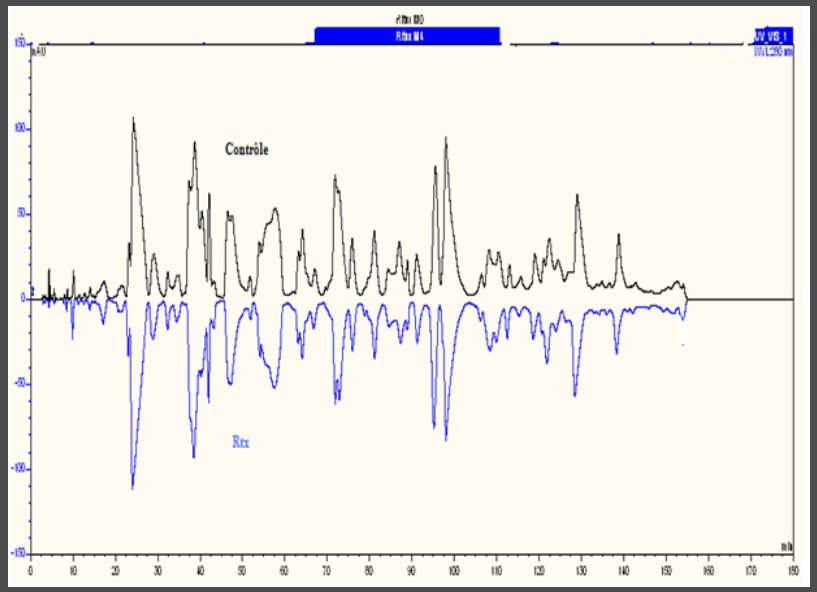
No submicronic aggregation



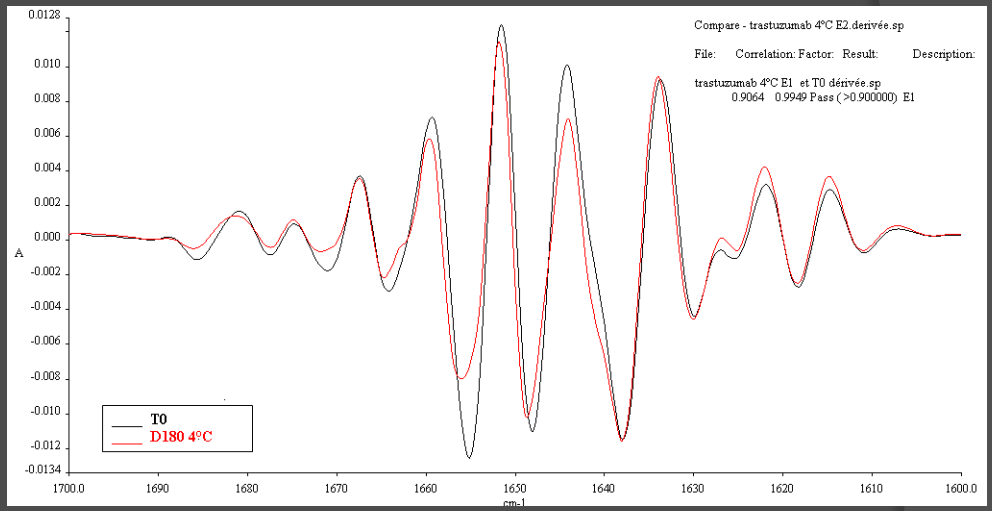
Storage (days)	Main Peak	2nd Peak	3th Peak	Pdl*
up to 6 months	11.395 +/- 0.045	-	-	0.0535

Dynamic light scattering

No alteration of I° and II ° structures

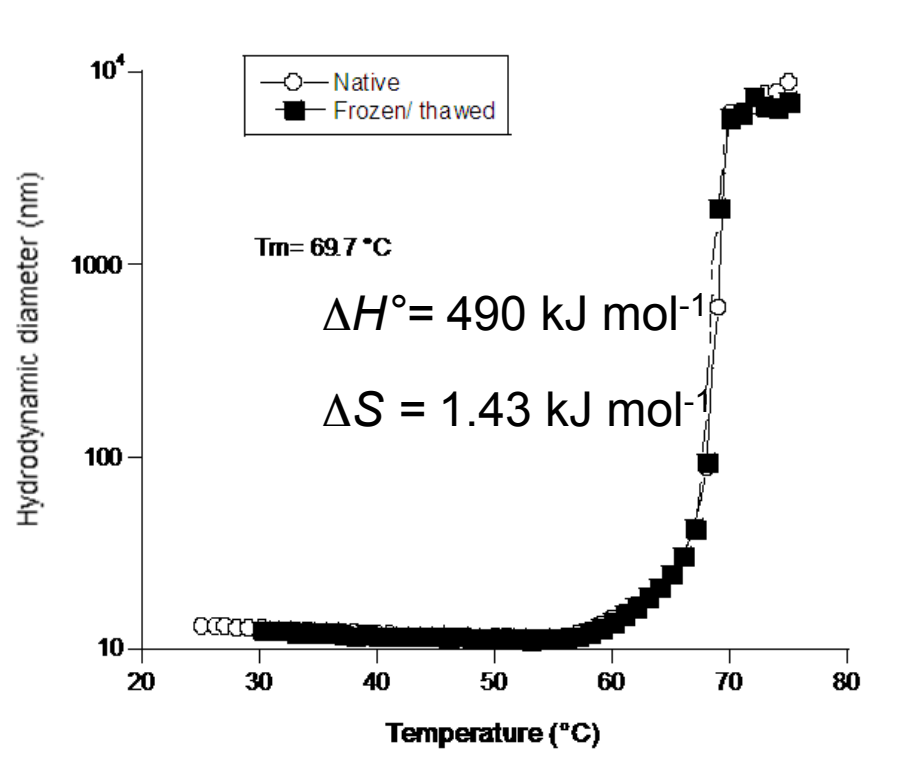


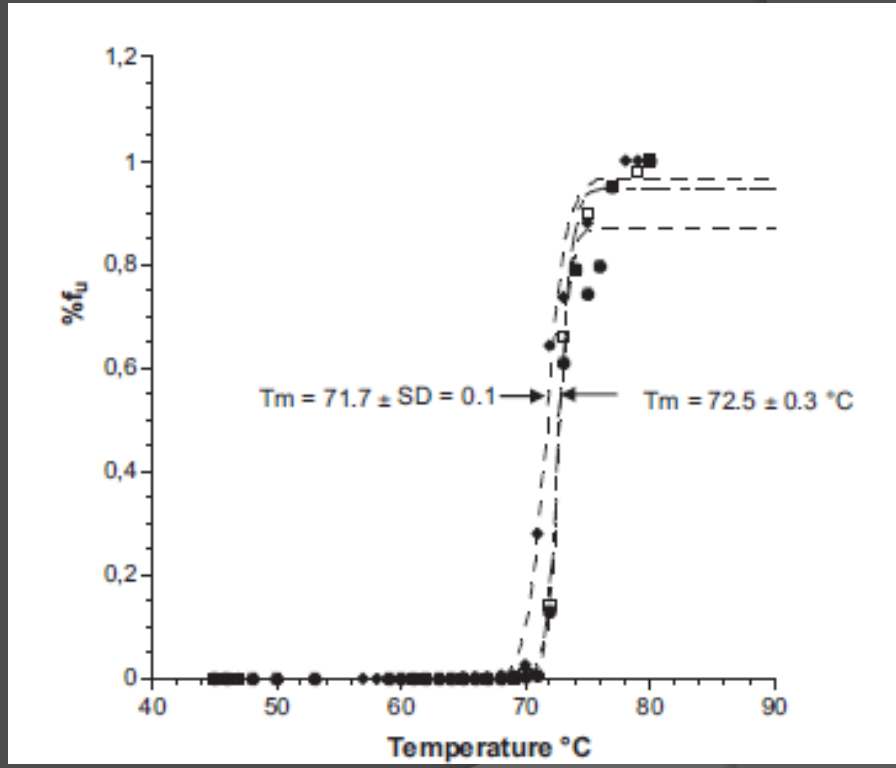
Peptide map



Second-derivative IR spectrometry

Thermodynamic stability curve





Biological activity was fully maintened

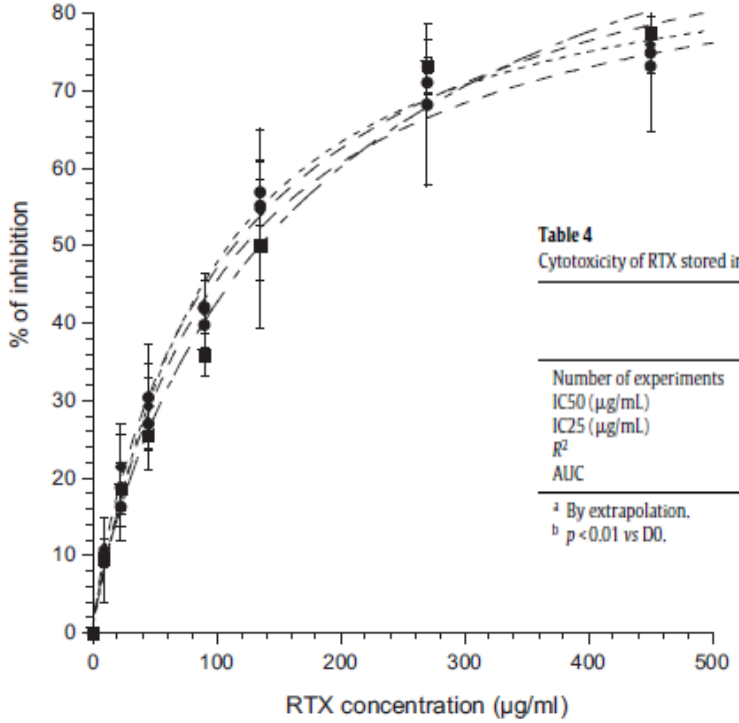


Fig. 7. RTX direct cytotoxic on CD20-expressing cells was determined through concentration-dependent cytotoxicity curves (concentration of Rtx: 0, 4.5, 9, 22.5, 45, 90, 135, 270 and 450 µg/mL) on a human lymphoma cell line (RAJI lymphoma cells (ATCC number: CCL-86)). Twelve wells were seeded for each concentration tested. The results were expressed as the percentage of inhibition in comparison to control (without RTX). The cytotoxic effects of RTX were not different after six months of storage at 4 °C.

Table 4
Cytotoxicity of RTX stored in different conditions on RAJI cells.

	D0	4 °C		40 °C	
		D30	D180	D30	D180
Number of experiments	10	2	5	3	2
IC50 (µg/mL)	137.2 ± 28.18	134.7 ± 21.6	125.8 ± 36.5	331.7 ± 32.2[b]	573.9[a] ± 181.1[b]
IC25 (µg/mL)	41.9 ± 8.3	45.6 ± 60.56	35.8 ± 11.9	83.5 ± 8.8[b]	119 ± 33.9[b]
R^2	0.994 ± 0.035	0.986 ± 0.014	0.993 ± 0.031	0.992 ± 0058[b]	0.996 ± 0.002[b]
AUC	25,334 ± 2316	25,785 ± 1284	25,077 ± 2451	17,910 ± 329[b]	14,301 ± 433.2[b]

[a] By extrapolation.
[b] $p < 0.01$ *vs* D0.

Conclusion

- Diluted rituximab is stable up to 6 months in polyolefine bags keept at 4°C or 22°C
- In-advance preparation and dose-banding are feasible

Methods are stability-indicating

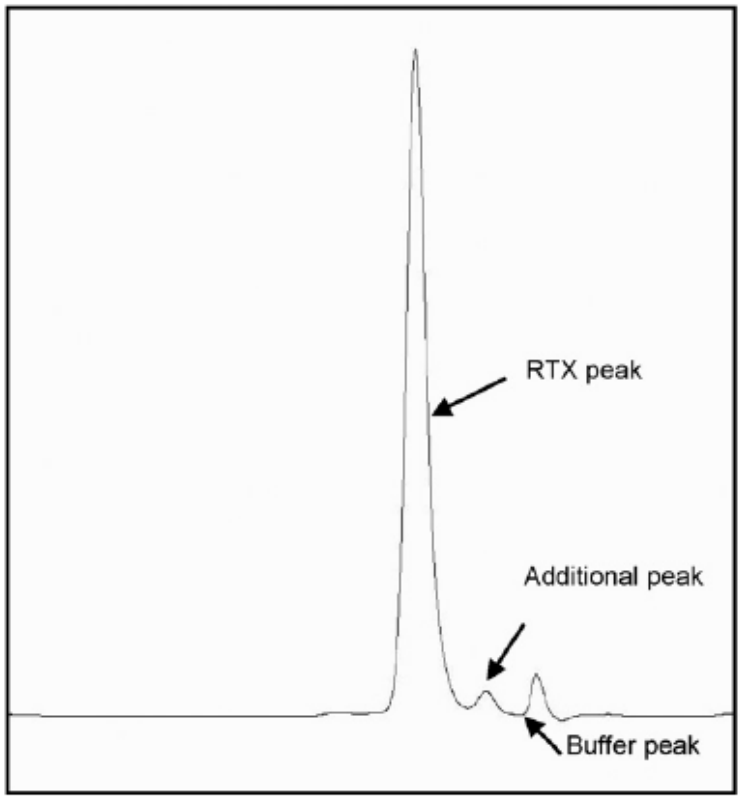


Fig. 5. Elution profile of RTX (1 mg/ml) chromatography using a TSK-GEL G4000 SWXL column with detection at 280 nm. Samples were centrifuged before injection. Stressed sample of diluted RTx (1 mg/ml) stored at 40 °C for 6 months exhibiting an additional peak at about 22 KDa (light chain) as result of structural breakage.

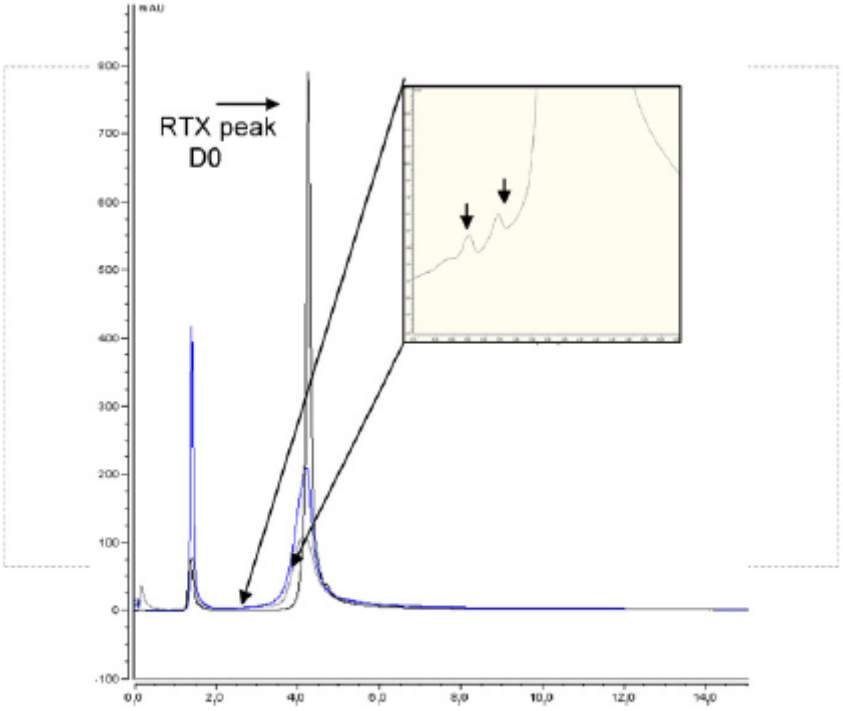
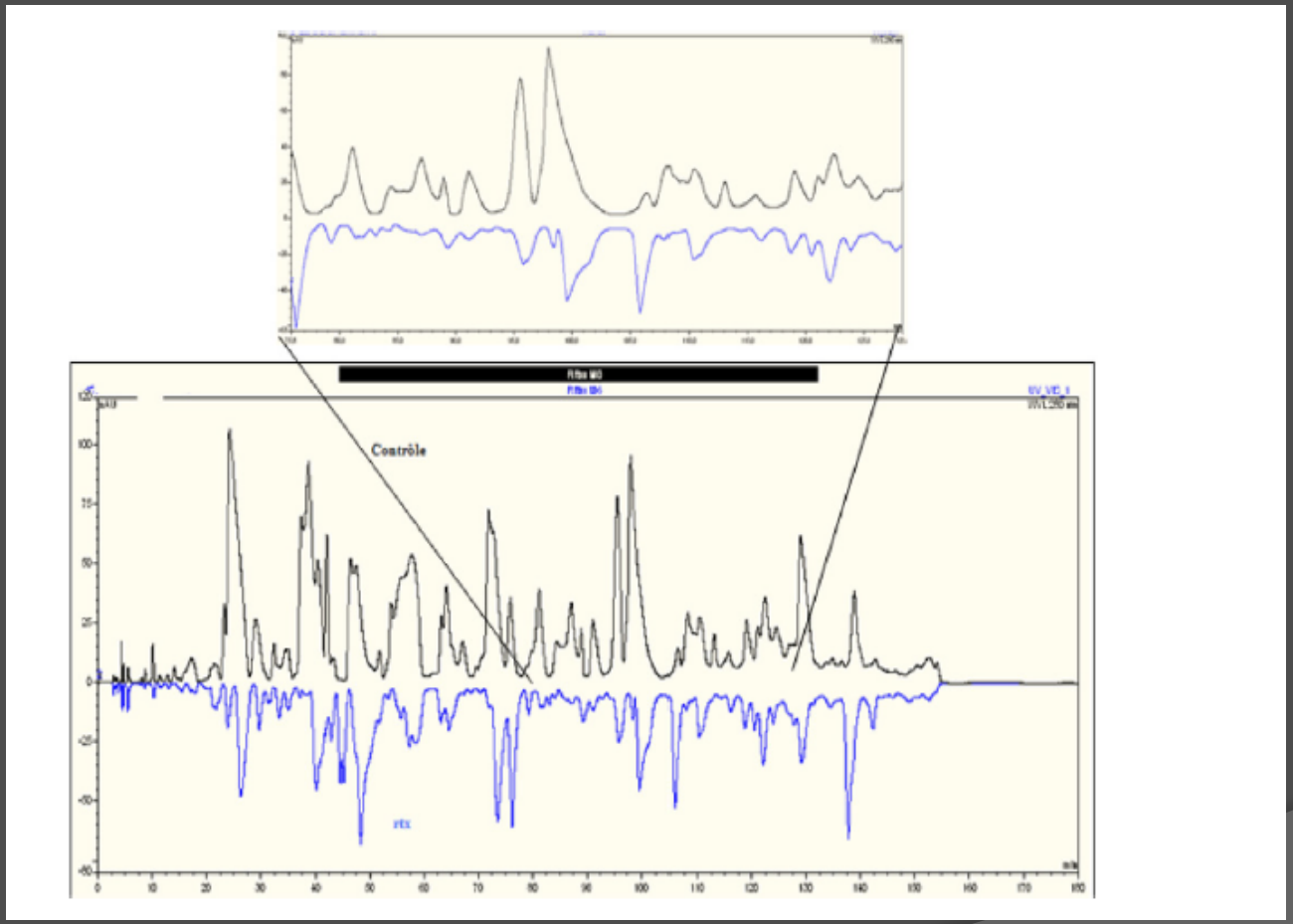


Fig. 4. Cation-exchange chromatographic profile of RTX (1 mg/ml) using a WCX-10 column at pH 6.0 eluted with a linear salt gradient from 0 to 50% of 1M NaCl and detection at 280 nm. Decrease in AUC was observed at 40 °C from three months and reaching 45% at six months. Changes of shape of main peak were also observed. Two new peaks were found just before the main peak after six months at 40 °C (inset).

Methods are stability-indicating



Peptide mapping

Stability of diluted trastuzumab

- One recent study on diluted trastuzumab (0.4 and 4 mg/ml) in bags: stable for 1 month

  Kaiser J, Kramer I. Physicochemical Stability of Diluted Trastuzumab Infusion Solutions in Polypropylene Infusion Bags. Int J Pharm Compound 2011 ; 15, 6 : 515-520.

- Limited estimation of the chemical stability, no estimation of physical stability

- The objective of our study was to fully assess the physical and chemical stability of diluted trastuzumab (0.8 and 2.4 mg/ml) after storage up to six months at 4°C in polyolefine bags.

Trastuzumab

- Identical methodologies

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journal homepage: www.elsevier.com/locate/ijpharm

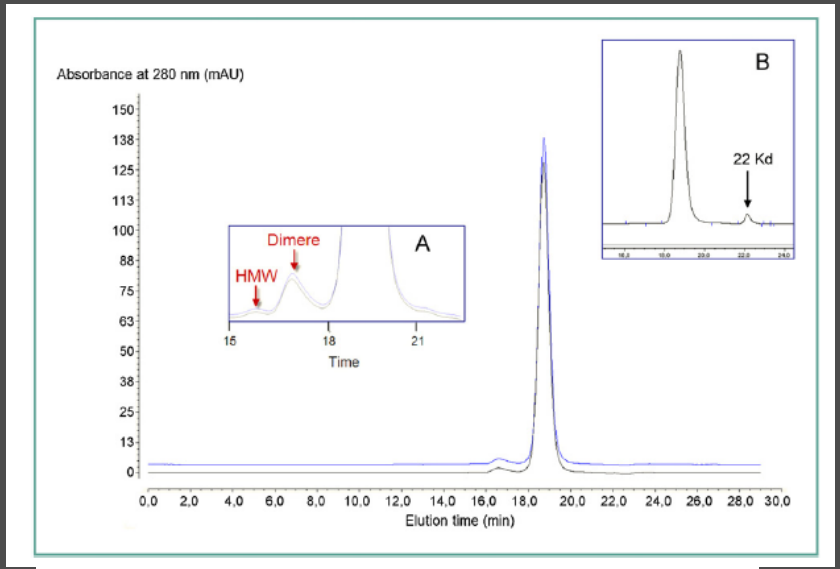
Note

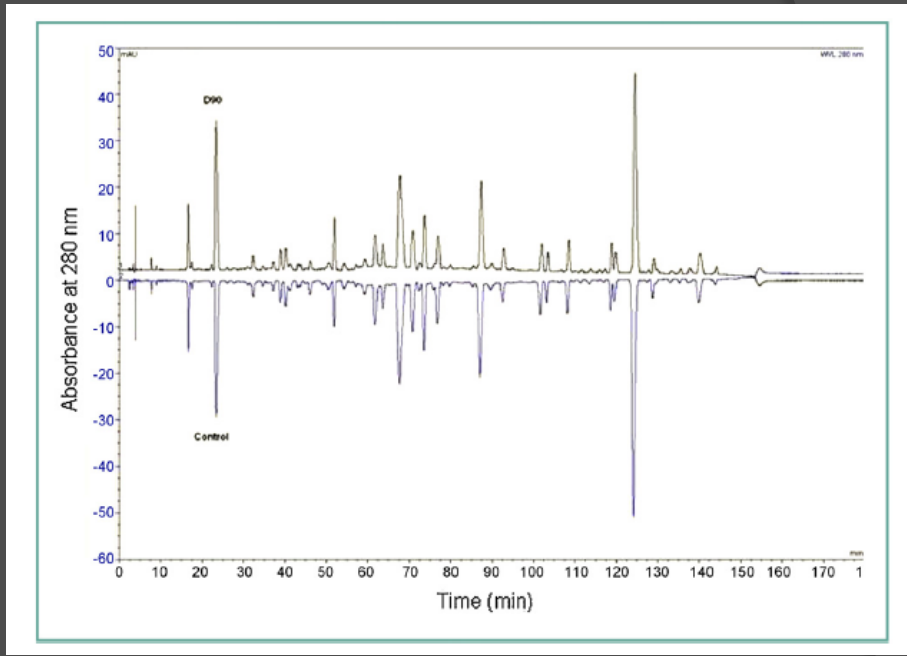
Long-term physico-chemical stability of diluted trastuzumab

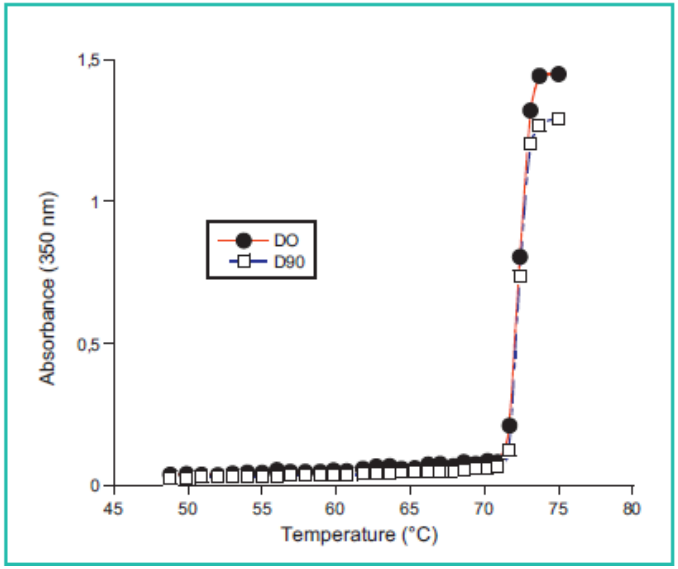
Muriel Paul[a,*], Victoire Vieillard[a], Ricardo Da Silva Lemos[a], Laurence Escalup[c], Alain Astier[a,b]

[a] Department of Pharmacy, Henri Mondor Hospital Group, Créteil, France
[b] UMR 7054, School of Medicine, Paris 12, University, Créteil, France
[c] Department of Pharmacy, Curie Institute, Paris, France

Stability of trastuzumab







No physico-chemical alteration

Stable for more than 6 months !

Conclusions

- Trastuzumab diluted at 0.8 to 2.4 mg/ml in saline, stored in polyethylene bag at 4°C, is stable up to 6 months.
- This excellent stability could authorize the safe anticipated preparation or by batch by pharmacy centralized units.
- No problem if temperature excursions of vials occur !!

Stability of Ipilimumab

- Ipilimumab : **YERVOY®** commercialized by BMS
  - **Human monoclonal antibody anti-CTLA-4** (IgG1kappa)
  - Advanced **melanoma** (non removable or metastatic)
  - 80 000€ per treatment …..
- Posology:
  - 3 mg/kg
  - Every 3 semaines → 4 doses
- IV Administration
  - **Diluted in NaCl 0,9%** or dextrose 5 % (from 1 mg/ml to 4 mg/ml)
  - Bags with or without PVC
- **Manufacturer claims : Stability of diluted solutions 24 hrs at 25 C and 2 - 8 °C**

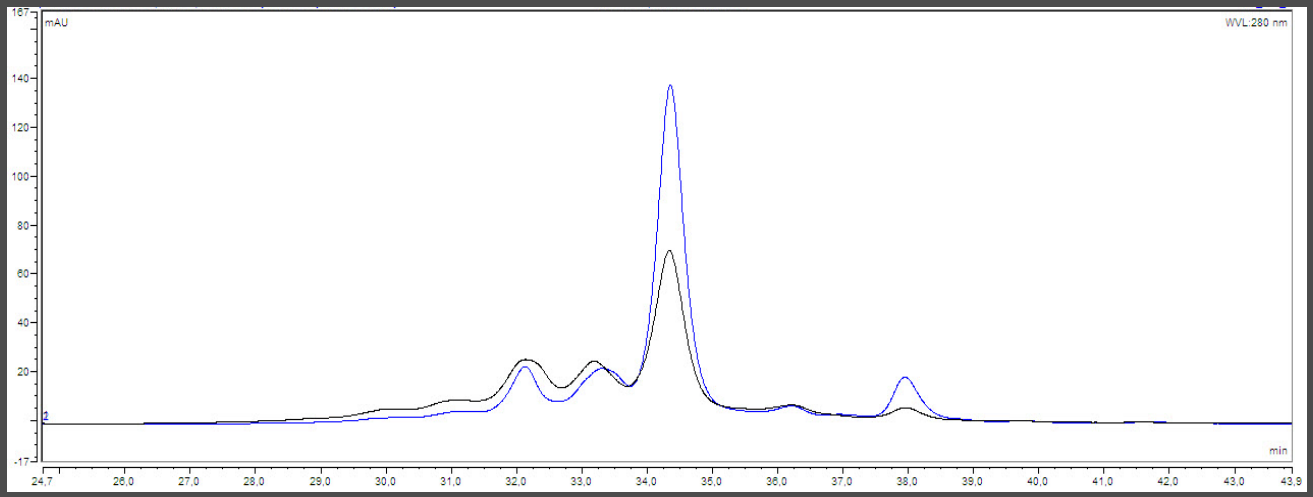
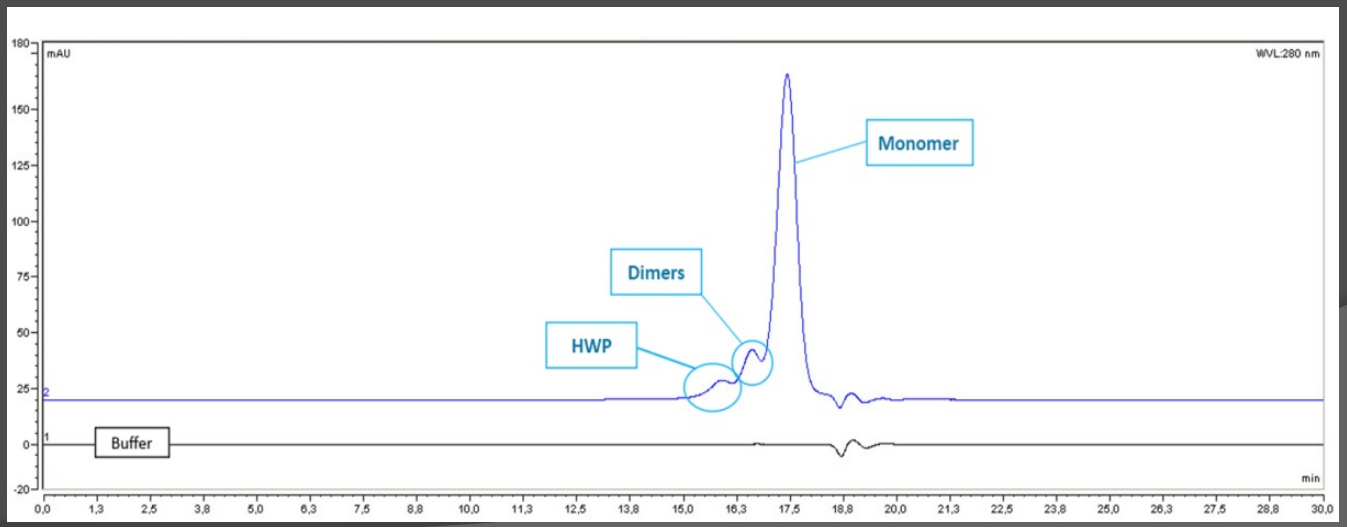
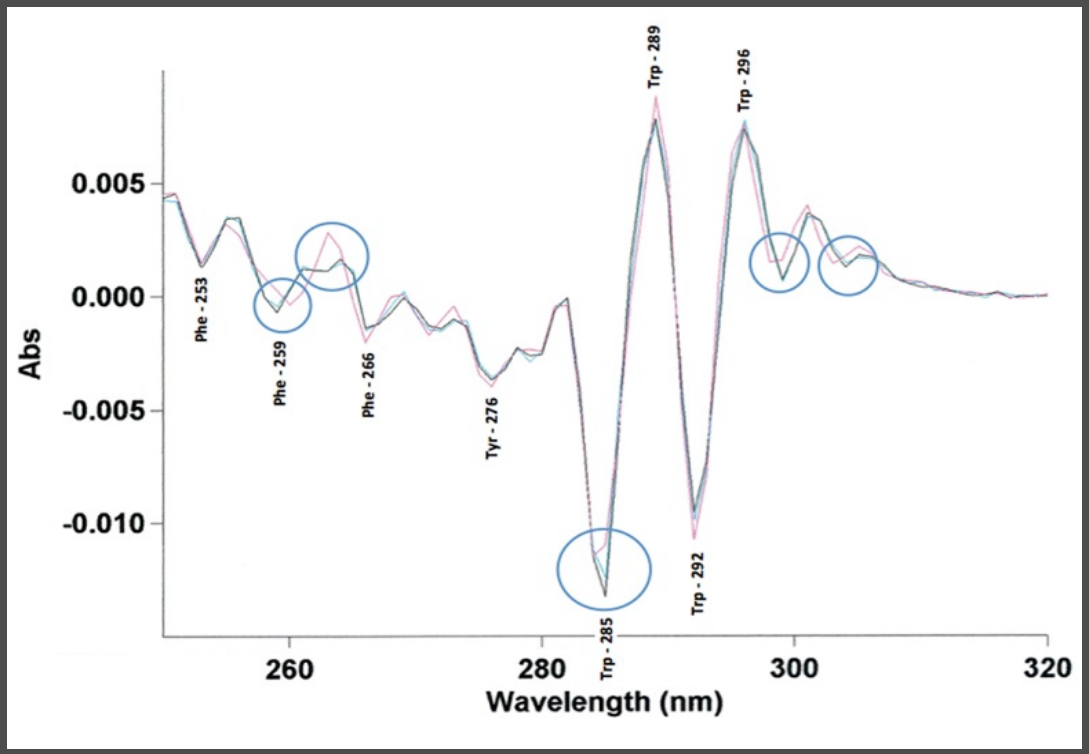


Fig. 4 : Cation-exchange chromatographic profile of IPI (1 mg/ml) using a WCX-10 column at pH 6.0 eluted with a linear salt gradient from 0 to 50% of 200 mM NaCl and detection at 280 nm. H0 profile (blue line) was compared to 4 days profile after heating at 60°C (black line). AUC of the different peaks were modified (+24.8%, -11.3%, -47.2%, -40.5% and -78.8% for peaks 1, 2, 3, 4 and 5 respectively).





Storage (days)	Main peak	2nd peak	3rd peak	PdI
D0	12.04 ± 0.07	-	-	0.075 ± 0.030
D28	11.77 ± 0.09	-	-	0.046 ± 0.010
D0	11.94 ± 0.26	-	-	0.053 ± 0.020
D28	11.89 ± 0.09	-	-	0.067 ± 0.015
H96	13.39 ± 0.41	56.62 ± 7.94	1539.25 ± 249.84	0.422 ± 0.024

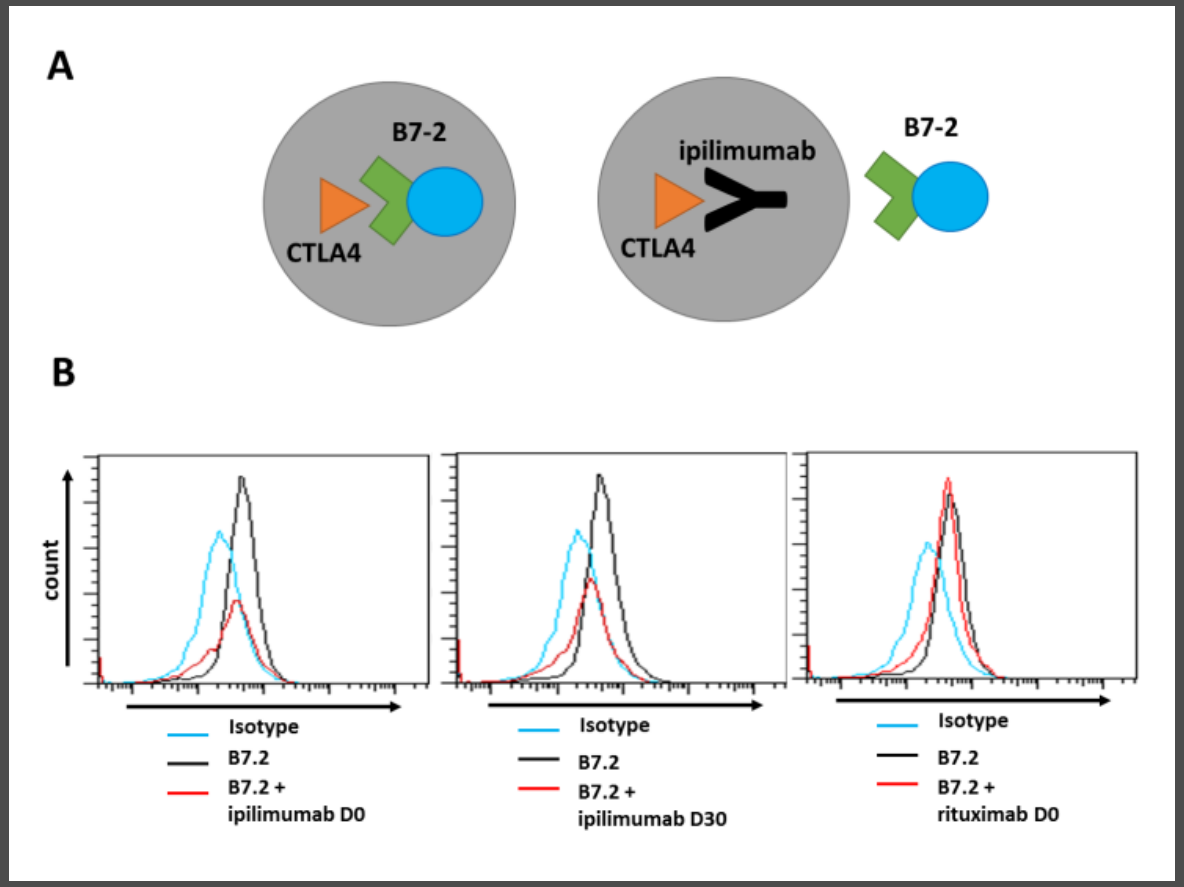
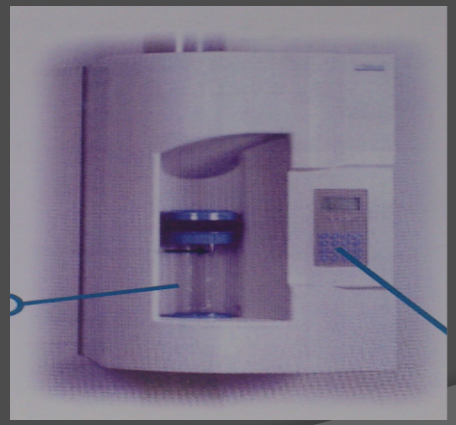


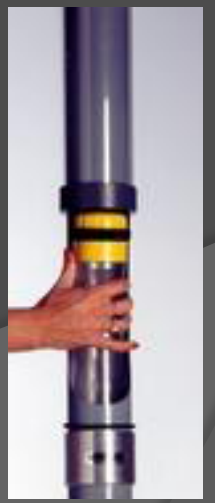
Fig. 5 : Functional activity of IPI determined using a cell-based competitive binding assay. A : inhibition of CTLA-4/B7.2 interaction by anti-CTLA-4 antibody IPI on a CTLA-4-Ig cell line. B : the mean fluorescence intensity was measured by flow cytometry. IPI upon opening (D0, left box) showed a major inhibition of B7.2 binding to CTLA-4 cells, compared to the negative control (rituximab, right box). After one month of storage of IPI vial at 4°C (D30, middle box), the binding inhibition profile remained similar to the reference sample (D0).

Submitted. Int J Pharm, 2015

Stability of mAbs in pneumatic conveying systems

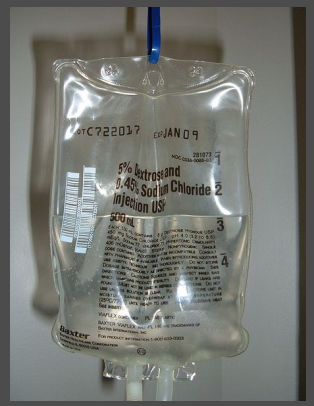
- mAbs are sensitive to mechanical stresses
- Pneumatic conveying systems (PCS) are curently used to send the final contenants of anticancer drugs from pharmacy to wards.
- Concerns about the stability of mAbs send by PCS





Experimental design

- Rituximab and cetuximab * were diluted in saline in polyolefine bags.
- Several conditions were tested:
  - presence of air (headspace) or no air
  - travel time
  - number of routes (1 to 8)
- Determination of physical stability.
  - size exclusion chromatography (SEC)
  - dynamic light scattering (DLS): submicronic populations
  - turbidity (350 nm): visible aggregates
  - infra-red spectroscopy: secondary structure

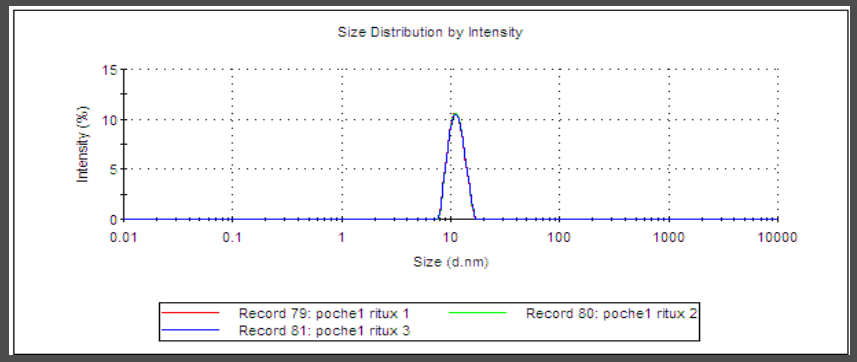


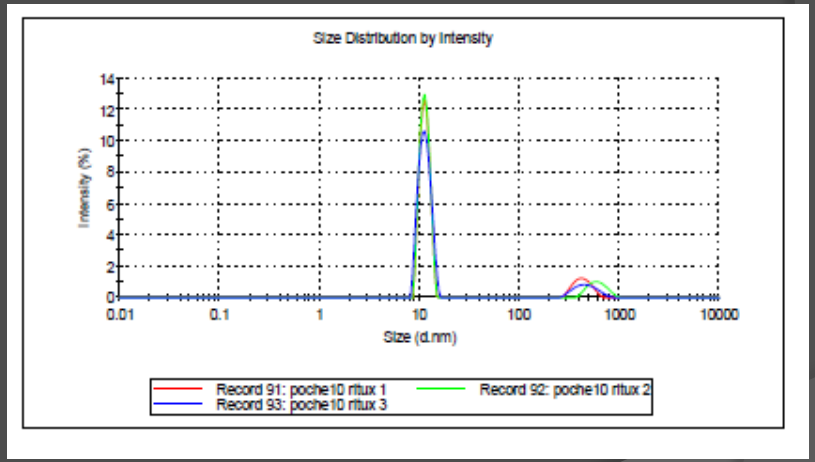
V. Vieillard, A. Ramssamy, K. Rilcy, A. Bellanger, A. Astier, M. Paul. Pneumatic conveying systems and physical stability of monoclonal antibodies: example of cetuximab. Poster P221, ECCO Congress, 2013

Role of air inside bags: aggregation

Without air

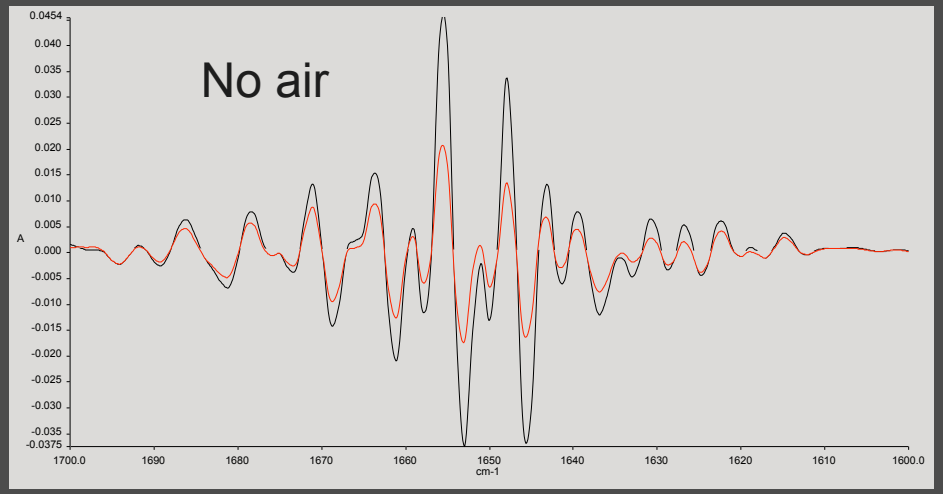
Up to 8 routes and without air into the bags, no modification was observed in comparison with the control (no route)

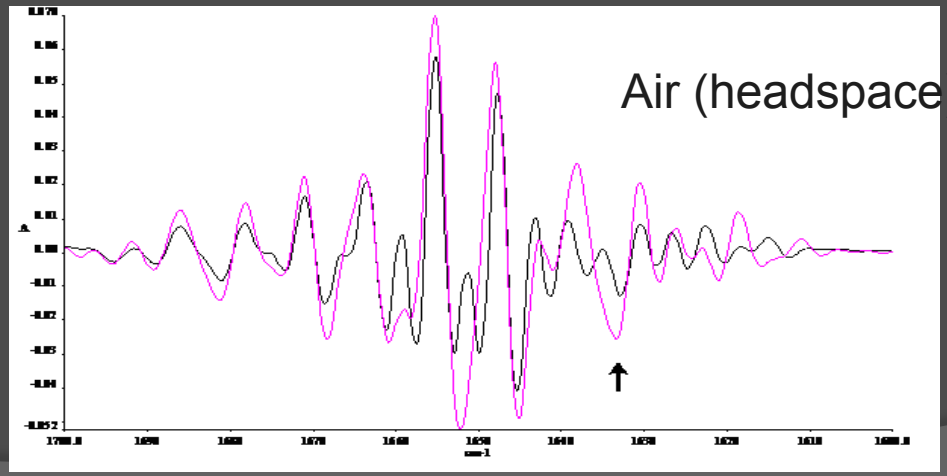


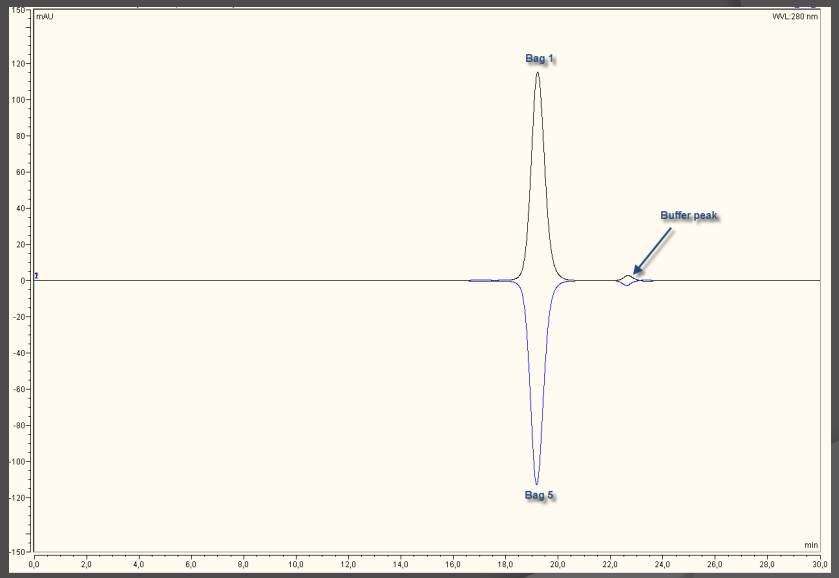


In presence of air, significant modifications were found after 4 cycles (1 cycle for cetuximab !)

Role of air inside bags : aggregation







Main results

- In practice, a pneumatic conveying system can be safely used for transport of diluted rituximab, and cetuximab (and probably for other monoclonal antibodies).
- However, the presence of air into the bags must be avoided because the aggregation induced by PCS is strongly dependant on the presence of air/liquid interfaces.

Conclusion (1)

- Antibodies are often much more stable indicated in the manufacturers recommendations (SPC)
- In particular, the resistance to the room temperature is very often sufficient to overcome the majority of temperature excursion problems in current situation.

Conclusion (2)

- It is more than critical to have real stability data for all common “in-use” situations
- If many “in-use” situations cannot be easily studied by the manufacturer, at the minimum, stabiliy data related to thermal excursion and realistic extended stability limits sould be furnished
- In the context of explosive growth of drug cost, our health system can no longer accept these unacceptable wastes.