Physicochemical stability of diluted trastuzumab solutions stored 6 months at 4°C

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Introduction

Trastuzumab (Tz) is a monoclonal antibody commercialized since 2000. It is used in different cancer treatments, such as those for breast cancer with an over expression of the receptor HER 2. Following the manufacturer recommendations, the stability of Tz reconstituted with bacteriostatic water is up to 1 month. However the same product reconstituted with water for injection is considered as stable only for 48 hours, suggesting that stability limits are only based on the possible risk of bacterial contamination and not on real physicochemical stability. Very limited independent studies concerning the stability of reconstituted Tz are currently available except a recent article published by Kaiser in 2011. A one-month stability after storage at 4°C was found*.

Therefore, the objective of this study was to fully assess the physical and chemical stability of diluted Tz (0.8 and 2.4 mg/ml) after storage up to six months at 4°C and room temperature.

Materials and methods

- Tz was diluted in NaCl 0,9% (Freeflex® polyethylene bags) to obtain 2 concentrations: 0,8 and 2,4 mg/ml.
- 2 batches were prepared for each tested conditions.
- Storage at 4°C during 6 months and room temperature.
- Samples were withdrawn and analysed at days D0, D14, D30, D90 and D180.
- Samples were centrifuged at 4000 rpm – 5 min before SEC, CEX and turbidimetry at 350 nm.
- Various protein characterization methods were used to determine changes in physicochemical properties of Tz including size exclusion HPLC (SEC), dynamic light scattering (DLS) and turbidity, cation exchange HPLC (CEX), UV spectrometry and peptide mapping.
- Results obtained at different times of storage were compared to those at day 0.

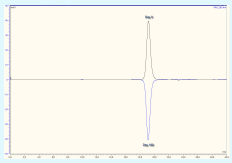
Bibliography

* Kaiser Jeanette, Kramer Irene IJPC Nov/Dec 2011 - Volume 15, Number 6 Physiochemical Stability of Diluted Trastuzumab Infusion Solutions in Polypropylene Infusion Bags

Results and Discussion

No modification of Tz characteristics was observed until 6 months of storage whatever the methods used.

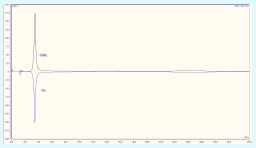
Chromatographic analysis



Time (days)	D0	D180
AUC (mAu*min)	25.55 +/- 0.19	24.89 +/-. 0.47
Tr (min)	18.84 +/- 0.054	19.125 +/- 0.0136

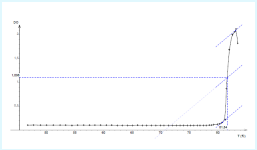
Figure 1 and Table 1: Only one peak in SEC was found. The retention time and AUC did not change after 6 months at 4°C.

Figure 2: A major peak was found at D180. There was no significant change in chromatogram profiles after 6 months.



Melting temperature analysis

Figure 3 and 4: The melting temperature remained around 77.3 °C +/- 0.517 with Malvern and 82.03 °C +/- 0.278 in UV method. These two methods indicate no structural destabilization of the protein.

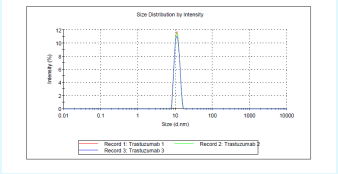


Dynamic light scattering

Figure 5 and Table 3: The size population stayed monodisperse with an unmodified mean hydrodyamic diameter (11,39 ± 0.045nm).

* Polydispersity Index

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



UV Spectrophotometric analysis

	J0	J30	J180
DO (350 nm)	0,00265± 0,0006	0,0048± 0,00021	0,0585± 0,00326

Table 4 and Table 5: After 6 months, a slight increase in optical density was found but remained low and ratio of absorbance stayed unchanged.

	J0	J30	J180
Ratio of absorbance	0,9972 ± 0,00028	0,9932± 0,0049	1,0161 ± 0,018

IR Spectrophotometric analysis

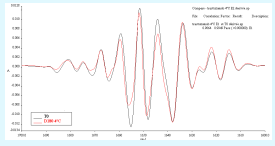


Figure 5: Second-derivative IR spectrometry
No modification of the secondary structure in FT-IR spectra was observed. Similarity coefficients were close to one.

Conclusion

On the opposite of the manufacturer recommendations, diluted Trastuzumab is strongly physico-chemically stable up to 6 months at 4°C. Indeed, no modification of its chemical, physical and structural characteristics and no aggregation were observed.

This excellent stability could authorize safe anticipated preparation by pharmaceutical centralized units to ensure maximal security in terms of sterile conditions and quality controls and to optimize hospital expenses due to this costly drug.