LONG TERM PHYSICO-CHEMICAL STABILITY OF IPILIMUMAB IN OPENED COMMERCIAL VIAL

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Ipilimumab (IPI) is a human monoclonal antibody indicated in the treatment of metastatic melanoma. This expensive medecine is generally prepared in cytotoxic preparation Pharmacy units. The stability is a major matter for pharmacist, and in this case, the manufacturer indicates a stability of only 24 hours after vial opening.

MATERIAL AND METHOD / OBJECTIVE

Physical stability analysis :
- UV spectroscopy 350 nm : turbidity
- Dynamic Light Scattering (DLS)
- Thermal denaturation curve
- Size Exclusion Chromatography (SEC)

Chemical stability analysis :
- Ionic Exchange Chromatography (IEC)
- UV second derivative

Practical conditions study
- 2 glasses : one stored at each temperature :
  - 4°C : traditional keeping conditions
  - 25°C : in case of out of the cold chain
- Ready to use solution in commercial vial (5 mg/mL)
- In light-protecting bags
- Samples withdrawn in aseptic conditions
- Times of analysis : : **T0** (control), **2 weeks, M1, M2 and M3**

In parallel, we studied the IPI stability in stressed conditions : heating at 60°C. The purpose was to prove that our analytical methods were stability indicating.

Objective

Study the physico-chemical stability of IPI in open commercial glass vial at 25°C and 4°C during 3 months

Expected answers :
→Use of vial residues ?
→Maintained stability after air inlet or metal ions ?

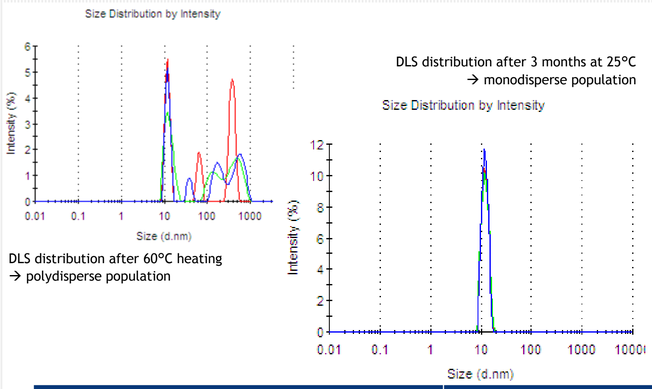
Results are presented as median +/- standard deviation (T0 versus 3 months) and measures were repeated 3 times. Statistical analysis was performed by non-parametric Wilcoxon tests (significance = $p < 0,05$).

RESULTS AND DISCUSSION

Mean OD at 350nm	T0	M3	T0 vs M3
4°C vial	0,0644 +/- 0,0003	0,0707 +/- 0,0006	p = 0,1 (NS)
25°C vial	0,0649 +/- 0,0005	0,0732 +/- 0,0009	p = 0,1 (NS)

No significant modification of **turbidity** was observed at 350 nm, unlike after heating.

60°C Heating	Mean OD at 350 nm
T0 not heated	0,0854 +/- 0,0009
T 8h heated	0,2477 +/- 0,0047
T 48h heated	0,3019 +/- 0,0054

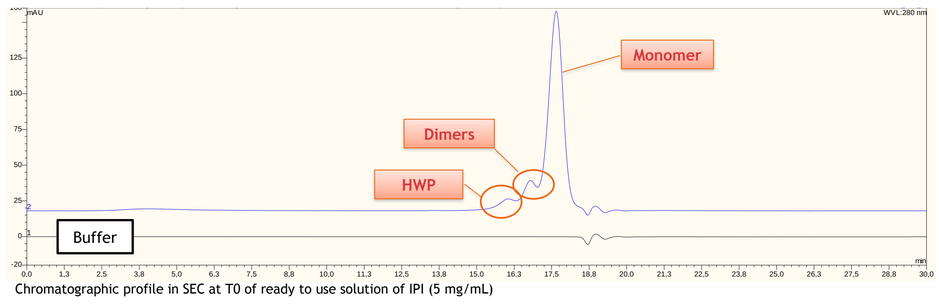


- No significant modification of mean **hydrodynamic diameters** mesured by DLS
- Low polydispersity index (< 0,1)

→ Monodisperse population

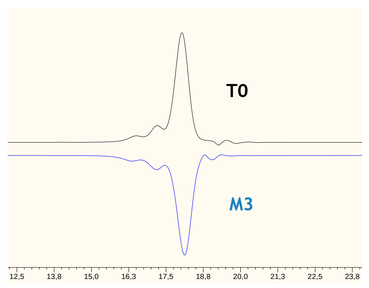
Mean diameter in DLS (nm)	T0	M3
4°C vial	12,04 +/- 0,07	11,82 +/- 0,06
25°C vial	11,94 +/- 0,26	11,94 +/- 0,26

We observed the pre-existence of High Weigh Particules (HWP) as dimers or tetramers in the commercial solution and a major peak which is the monomer.



Chromatographic profile in SEC at T0 of ready to use solution of IPI (5 mg/mL)

The chromatographic profile in **SEC** was not modified up to 3 months :



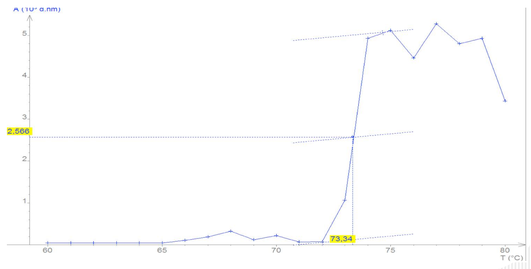
Chromatographic profile in SEC : T0 profile (black line) compared to 3 months profile after keeping at 25°C (blue line)

- 3 months SEC profiles were stackable to T0 at each temperature
- No chain scission was observed

	4°C vial		25°C vial		60 C heated solution	
	T0	M3	T0	M3	T0	H 96
% HWP	4,45	4,89	4,06	5,14	5,26	7,21
% Dimer	10,61	11,47	10,51	13,14	14,67	18,99
% Monomer	84,94	83,64	85,43	81,72	80,06	73,8

Temperature of aggregation (Tm) stayed around 73,4°C

Mean Tm (°C)	T0	M3
4°C vial	73,4°C	73,3°C
25°C vial	73,3°C	73,4°C

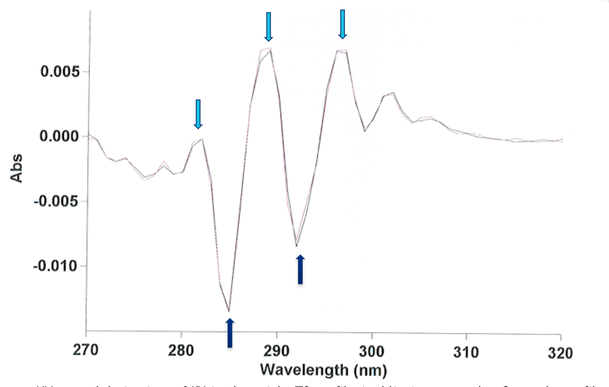


UV second derivatives were stackable to control T0 :

-at both temperatures

-at each analysis times

→ No change in tertiary structure



UV second derivatives of IPI in glass vial : T0 profile (red line) compared to 3 months profile after keeping at 25°C (black line)

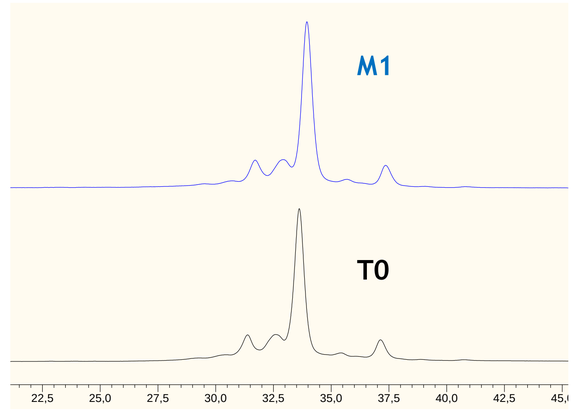
Ionic chromatography (IEC) :

-1 month chromatogram profile was not modified

-none new peak appeared

→ No desamidation

= no chemical instability



Chromatographic profile in ionic chromatography (IEC) : T0 profile (black line) compared to 1 months profile after keeping at 25°C (blue line)

IEC was not performed at M3 for technical reasons : we will perform it again.

News methods will be needed to confirm these results :
- **Peptide mapping :** primary structure
- **IR spectroscopy :** secondary structure
- **Biological activity**

Physical stability seemed to be maintained according to unchanged :
- turbidity → absence of aggregation
- hydrodynamic diameters
- Tm and SEC profiles.

And **chemically stability was ascertained** by :
- no chain scission in SEC
- no desamidation in IEC
- and UV second derivatives remained similar → absence of tertiary structure modification.

CONCLUSION

We demonstrated that **Ipilimumab stored in original vials at 4 and 25°C remained stable up to 3 months.** No significant physical or chemical instability was observed. Extended stability of unused vial residues, for a very expensive product like IPI, could permit an important cost saving in hospitals.