

# LONG-TERM STABILITY OF DILUTED SOLUTIONS OF THE MONOCLONAL ANTIBODY INFLIXIMAB

PP-028

Award nominee



COMPLEJO HOSPITALARIO UNIVERSITARIO DE GRANADA

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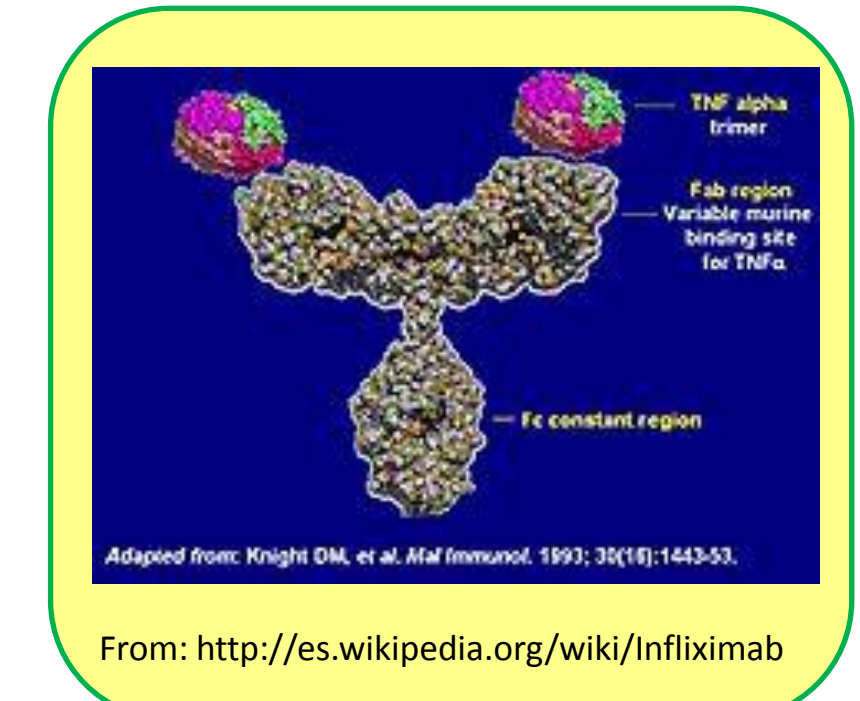


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**Objective:** To assess the long term stability of the therapeutic monoclonal antibody **Infliximab (Remicade®)** reconstituted in water for injection and in two diluted preparations in 0.9 % NaCl and stored at 4 °C and frozen at -20 °C.



## Background:

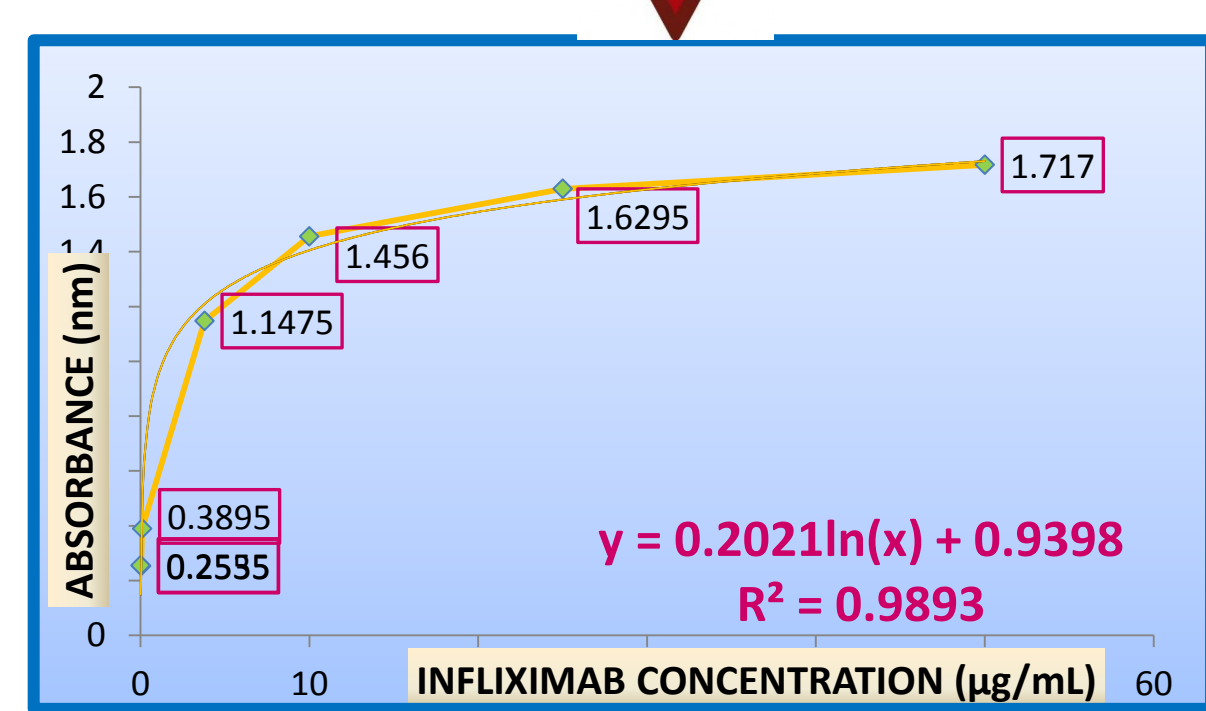
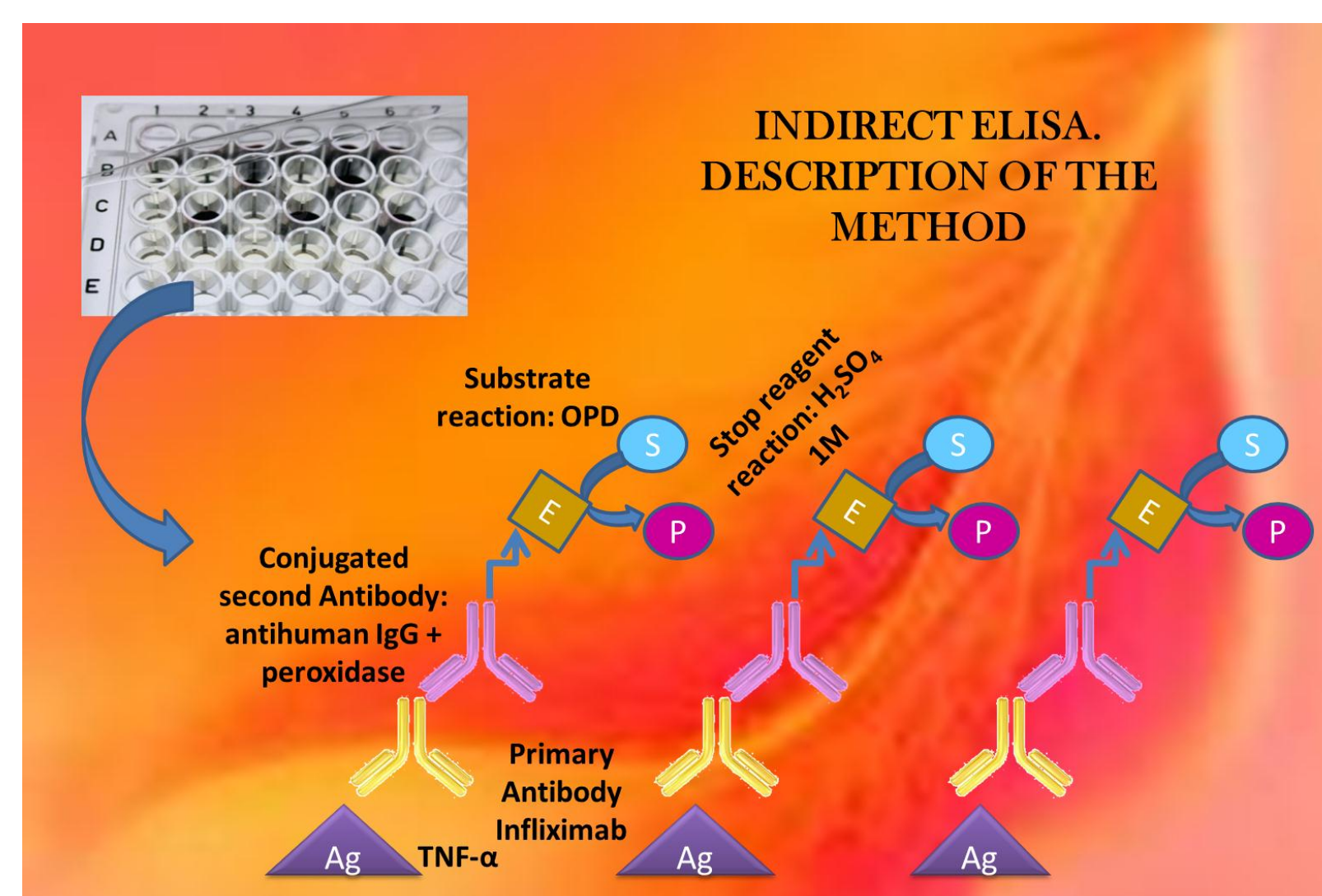
Infliximab (IFX) is a chimeric human-murine monoclonal antibody (mAb) directed against tumor necrosis factor alpha (TNF-α). It is indicated in the treatment of an important number of pathologies: rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis and ulcerative colitis. It blocks the damage caused by high levels of TNF-alpha by forming the Infliximab-TNF-α immune complex. This complex is responsible for the reduction of serum levels of proinflammatory components as Interleukin-6.

## Materials and methods:

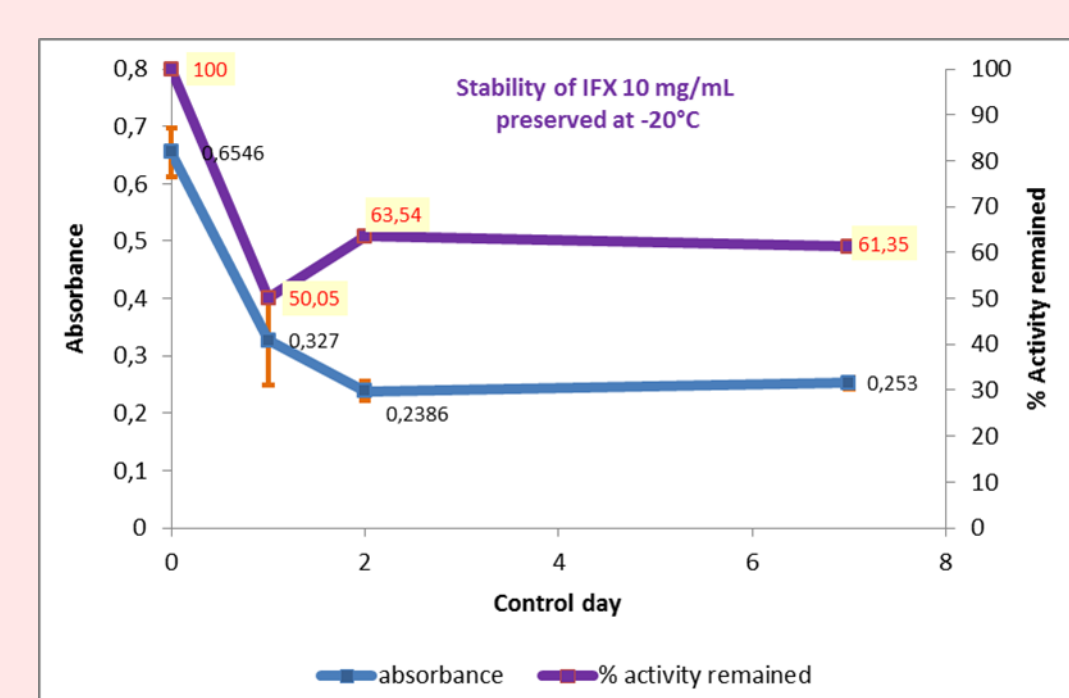
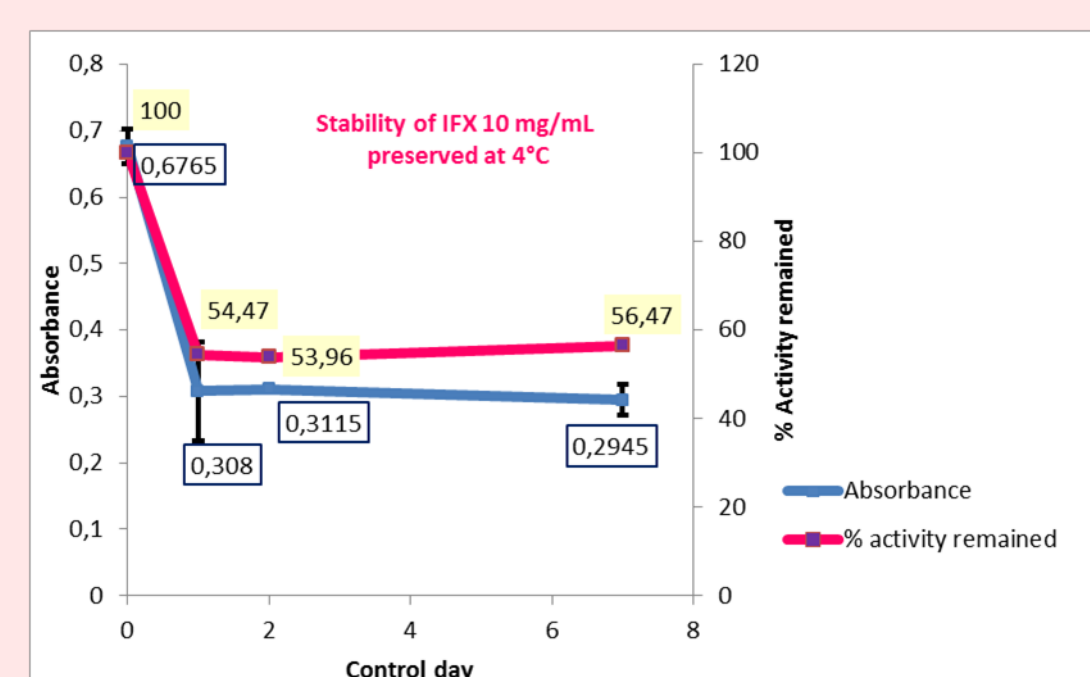
Ad hoc methods for assessing the **physicochemical properties** of Infliximab were developed and ICH validated: reverse phase high performance liquid chromatography with diode array detector [(RP)HPLC-DAD] for **quantification**; weak cation exchange high performance liquid chromatography [(WCE)HPLC-DAD] to track changes in the **isoforms profile**; size exclusion chromatography high performance liquid chromatography with diode array detector [(SE)HPLC-DAD] for **aggregates** detection; and matrix assisted laser desorption ionization mass spectrometry [(MALDI-TOF/MS)] to obtain peptide mass fingerprinting (PMF) in order to detect major **changes in the chemical structure**. **Biological activity** was assessed using a specific immunoassay based on the **ELISA** technique using plates sensitized with the **Tumor Necrosis Factor α (TNF α)**.

### Biological Activity

Specific Immunoassay  
ELISA based on TNF α

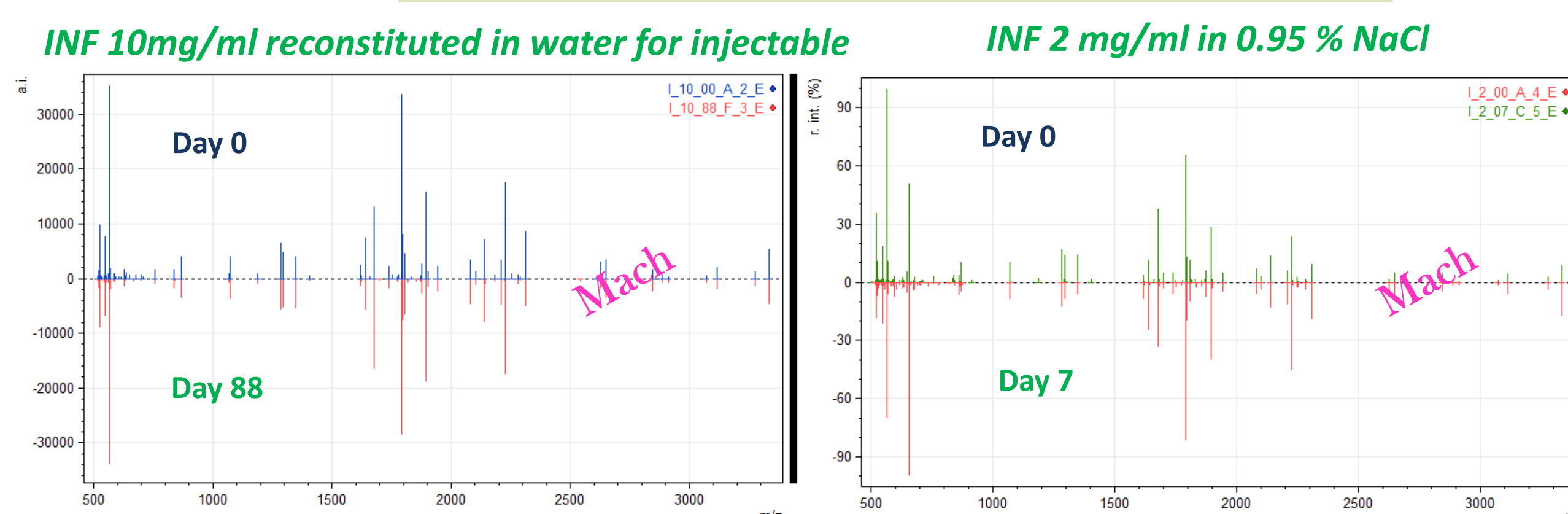


Biological activity was assessed using solutions IFX 10.0 mg/ml in water: stored refrigerated at 4°C and frozen at -20°C, protected from daylight up to 7 days.



## Infliximab Stability Study

MALDI-TOF/Mass spectrometry  
Peptide Mass Fingerprinting (PMF)  
10 mg/ml ; 2 mg/ml ; 0.5 mg/ml



**PMF STUDY**  
IFX solution of 10.0 mg/ml (in water for injectable preparation), 2.0 mg/ml (in sodium chloride 0.9 %) and 0.5 mg/ml (in sodium chloride 0.9 %) refrigerated at 4 °C or frozen at -20°C did not undergo dramatic chemical changes over the test period (three months for the solution of 10.0 mg/ml and a week for the more diluted solutions). The samples stored for 24 hours at room temperature showed similar behaviour.

### Accelerated degradation study ICHQ2(R1)

by ELISA - (RP)HPLC/DAD - (SE)HPLC/DAD - (WCE)HPLC/DAD

Temperature: 50 °C and 70 °C

UV-Light (ICH Q1B) for photostability testing

Ionic Strength: Mild (3.3 % v/v NaCl 1.5 M), Strong (25 % v/v NaCl 1.5M)

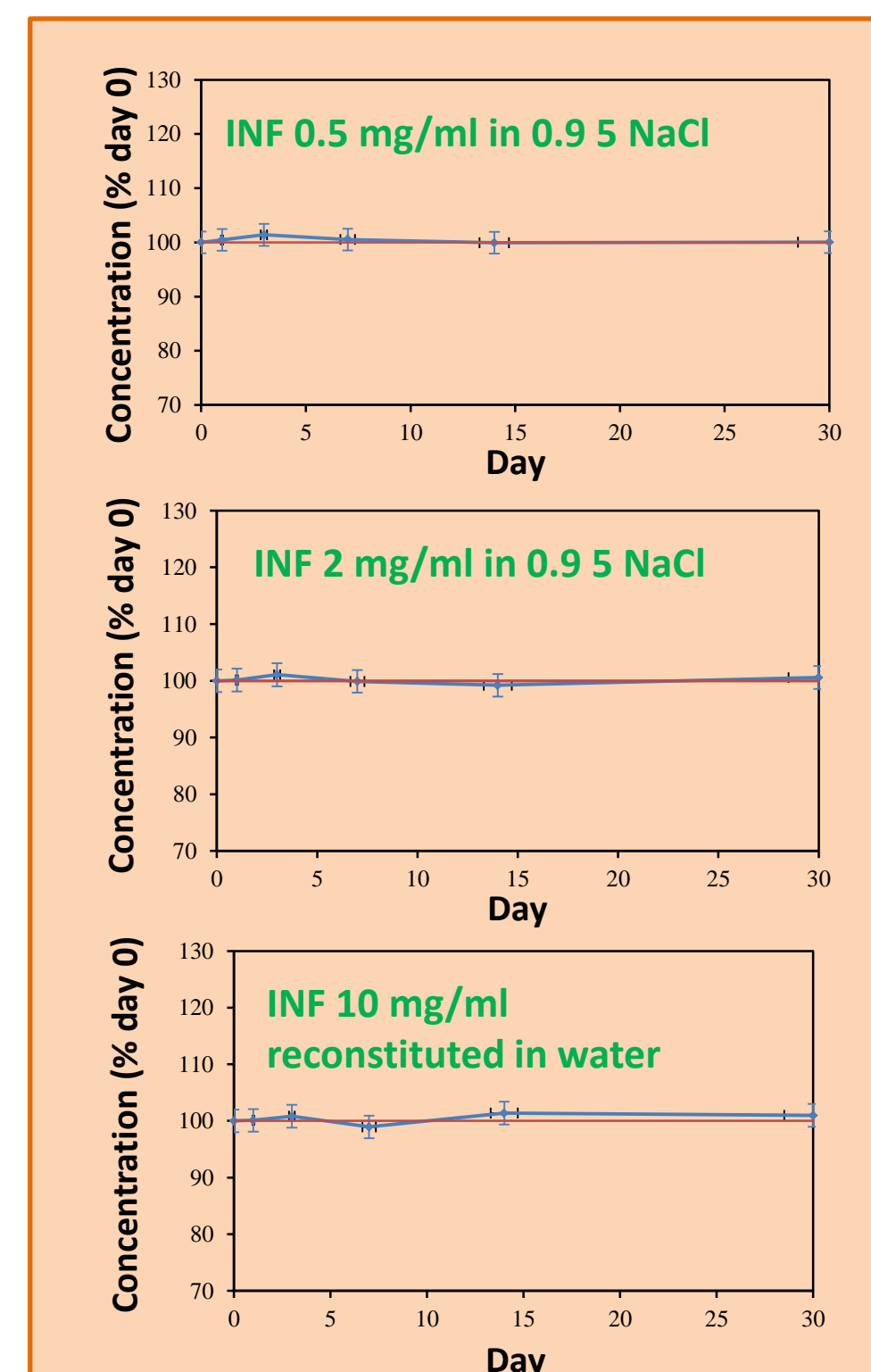
Oxidative conditions: Mild (3.3 % v/v H<sub>2</sub>O<sub>2</sub> 1 M), Strong (25 % v/v H<sub>2</sub>O<sub>2</sub> 1M)

Acidic and basic Conditions: Mild (3.3 % v/v HCl 1 M / NaOH 0.1 M), Strong (25 % v/v HCl 1M / NaOH 0.1 M)

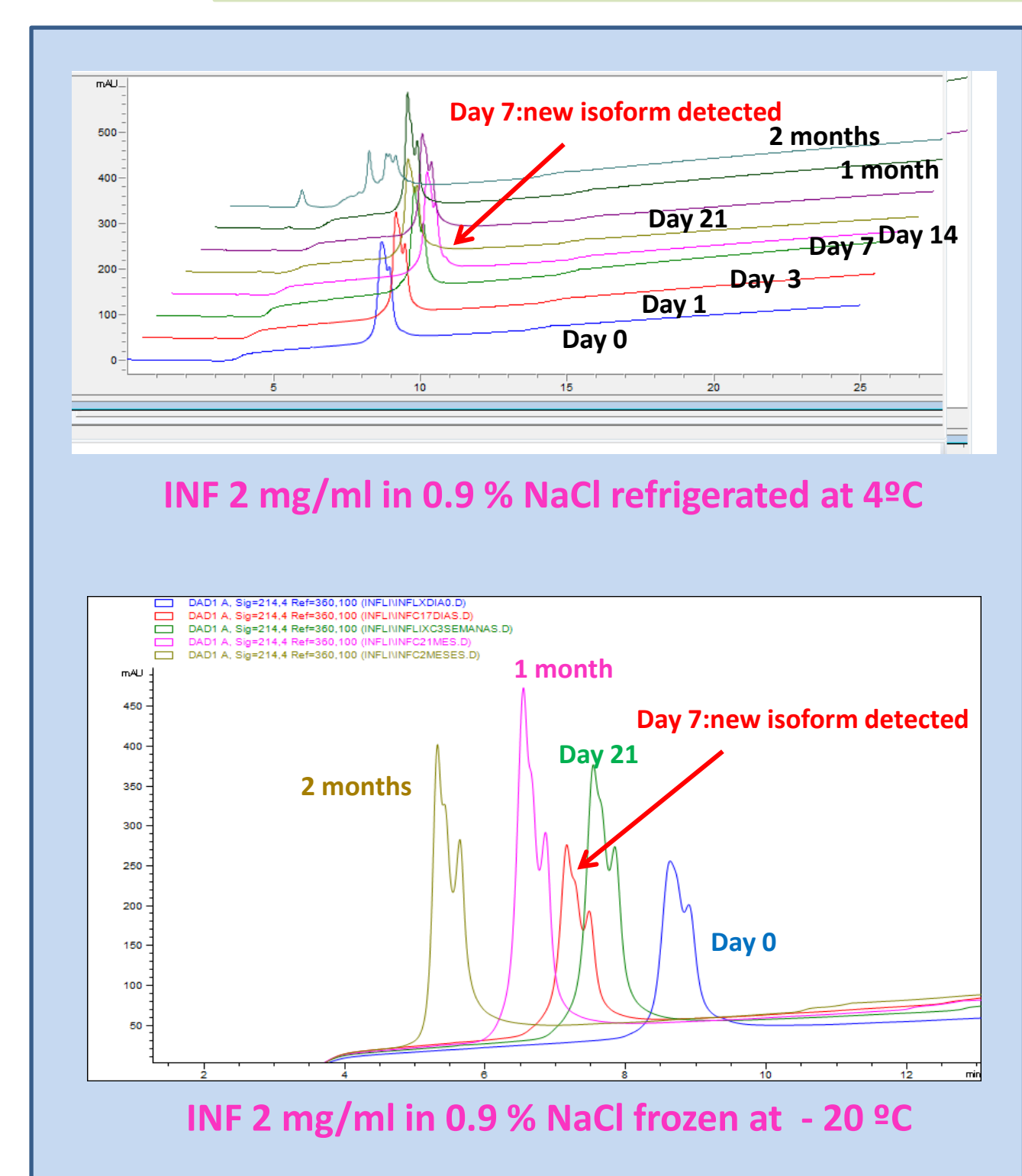
### Physicochemical properties

### Liquid Chromatography HPLC/DAD

### Reverse Phase (RP)HPLC/DAD Quantification

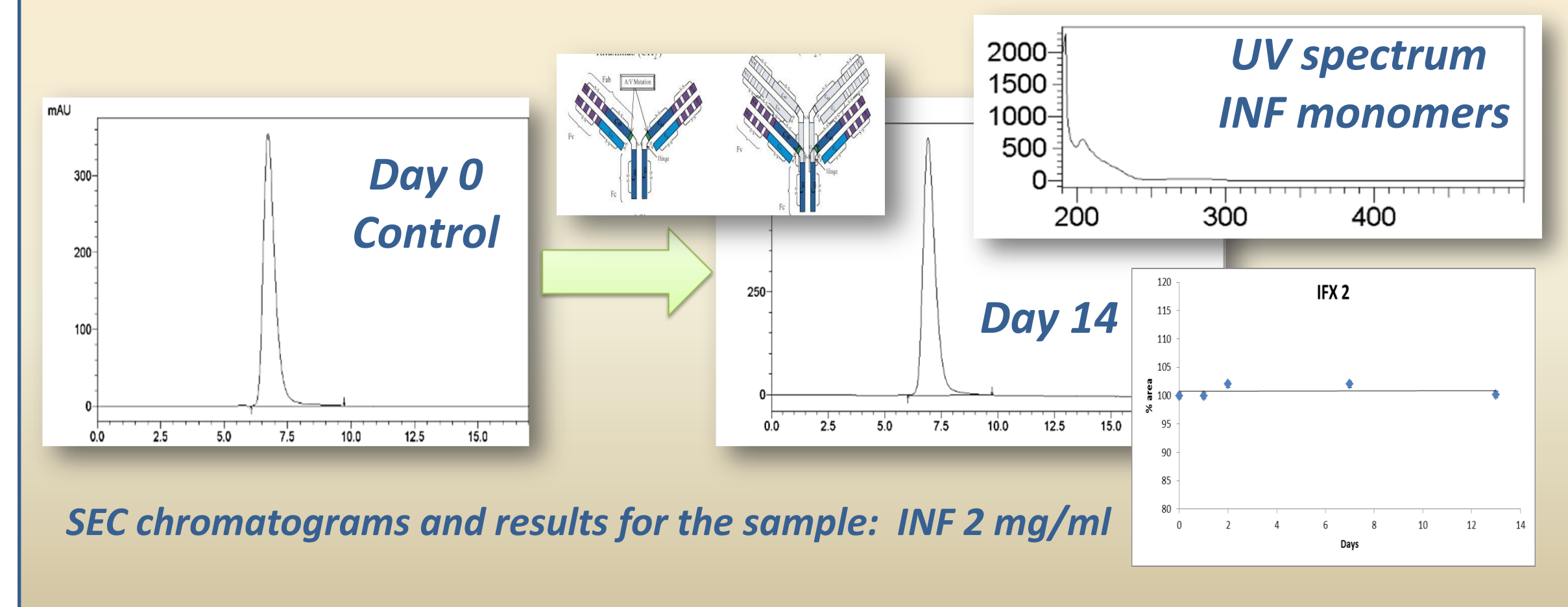


### Weak Cation Exchange (WCE)HPLC/DAD



### Size Exclusion [(SE)HPLC/DAD]

10 mg/ml in water ; 2 mg/ml and 0.5 mg/ml in 0.9 % NaCl  
Aggregates detection



## Results:

24 hours after preparation of the solutions the loss of biological activity was close to 50 %, rising to 63 % at day 2, a value that remained constant until the last control day (7). The overall quantity of IFX was assessed for a month during which it remained unchanged. No aggregate formation was detected in two weeks of testing. Slight changes in the chromatographic isoforms profile were detected after a week. Fingerprinting indicated minimal changes in the IFX structure (three months).

## Conclusion:

Despite the fact that no major changes were detected in the physicochemical properties of IFX after a week of testing (changes in the isoform profile at day 7), the ELISA results indicated an important decrease in the biological activity when the medicine was reconstituted 24 hours after preparation of the solutions. We have so far been unable to corroborate this result using a different method for evaluating biological activity -such as flow cytometry- due to technical problems which we are currently investigating.

Authors declare no conflict of interest.

## Acknowledgements:

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