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TCH038

DEVELOPMENT OF AN ELISA ASSAY FOR THE DETERMINATION OF THE ANTIBODY INFLIXIMAB IN HOSPITAL CONDITIONS OF USE

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Infliximab (Remicade®) is a chimeric human–murine anti–human tumor necrosis factor (TNF) monoclonal antibody (75% human; 25% murine). It is approved for patients with rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis and ulcerative colitis. Thus, Infliximab blocks the damage caused by high levels of TNF–alpha by immune complex formation Infliximab–TNF–α. This complex is responsible for the reduction of serum levels of proinflammatory components as Interleukyn–6.

PURPOSE AND OBJECTIVE

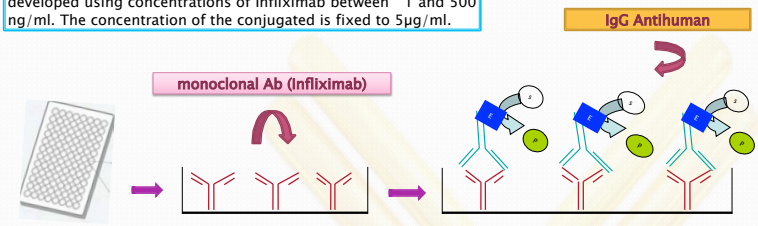
Development of an ELISA assay for the quantification of Infliximab when reconstituted in sterile water for injection (according to the manufacturer´s instructions) to obtain final concentrations of 10.0 mg/ml, 2.0 mg/ml and 0.5 mg/ml.

EXPERIMENTAL

1. Coated ELISA plates with Infiximab
2. Incubation at 4ºC 18h.
3. Washing
4. Addition of the blocker
5. Washing
6. Incubation at 37ºC 2h.
7. Addition of antihuman IgG peroxidase conjugated
8. Incubation at 37ºC 30 min.
9. Washing
10. Addition of the substrate OPD
11. Incubation at RT 20 min.
12. Addition 1.0 M sulphuric acid
13. Absorbance reading (450 – 620 nm)

A **direct and non competitive ELISA method** has been developed to determine Infliximab

After the optimization of several variables, immunoassay is developed using concentrations of Infliximab between 1 and 500 ng/ml. The concentration of the conjugated is fixed to 5µg/ml.



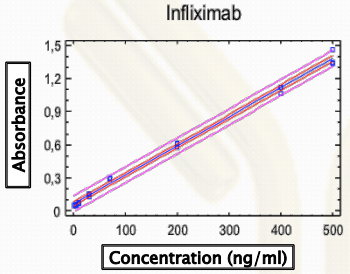
RESULTS

The quantification of Infliximab after reconstitution can be carried out by the **non–competitive direct ELISA** method developed and validated.

The proposed ELISA method shows satisfactory **accuracy and sensitivity values**. This makes it ideal for the quantitative determination of the drug Infliximab.

This ELISA is **simple, fast** and **easy to perform**.

The proposed ELISA method is **more sensitive when used anti–human IgG conjugated antibody instead of anti–mouse IgG antibody**.



$$y(Absorbance) = 0.062 + 0.00264 \cdot x\,(concentration)$$

Statistically significant relationship between absorbance and concentration for a confidence level of 99%, in the concentration range studied.

n	23
a	0.062
S_a	0.009
b (ml · ng^{-1})	0.00264
S_b (ml · ng^{-1})	0.00003
R^2 (%)	99.5
S_{RC}	0.03
DL	2.2
QL	7.5
Linear dynamic range (ng · ml^{-1})	7.5–500.0
Detection range (ng · ml^{-1})	2.2–7.5
Repeatability (%)	10.4
Reproducibility (%)	9.8
Recovery (%)	98.3

This study is part of a wider project that seeks to contribute to the establishment of practical stability studies of biotherapeutics based on monoclonal antibodies. Following several IHC guidelines, including ICHQ5C (stability testing of biotechnological/biological products), the chemical integrity of the IFX is currently being studied by size exclusion chromatography (SEC), and its biological stability by performing specific ELISA tests based on the TNF–α. The molecular integrity by MALDI–TOF Mass Spectrometry has been already studied and the results discussed in the current congress (Poster TCH034). By gathering all these results together, we can provide additional stability data covering practical uses of IFX. This also conforms to the recent recommendations of the "European conference consensus" sponsored by the French Society of Oncology Pharmacy (SFPO/ Annales Pharmaceutiques Françaises (2011) 69, 221—231).

ACKNOWLEDGMENT

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