**Objective**

Long-term stability study of the molecular integrity of infliximab using peptide map fingerprinting when reconstituted and diluted in the typical hospital conditions.

**Analytical Method**

Trypsin digestion of protein. MALDI-TOF MS analysis of pancreatic aliquot 1 ml to end up with a 5 µl of protein.

**Results**

0.5 mg/mL infliximab solution

29% of the most representative peptides were lost, including 603 (M) and 735 (H). Nevertheless, protein identification in the taxonomy was the same for the IGHG1_HUMAN (average score 70), but different for rodentia. Results are shown at the end of the first period.

33% of the most representative peptides were lost, including 603 (M) and 735 (H/M). Nevertheless, protein identification in the same taxonomy was the same and with similar scores (69 for the IGHG1_HUMAN and 40 for MBP1_MOUSE).

22% of the most representative peptides were lost, including in this case only the peptide 603 (M). Nevertheless, protein identification in the taxononomy was the same as for the IGHG1_HUMAN (average score 70) but different for rodentia.

19% of the most representative peptides were lost. Identified peptides from IGHG1_Human and from RAB4B_Mouse were not lost. Nevertheless, protein identification in the taxonomy was the same for the IGHG1_HUMAN (average score 80) but different for rodentia.

17% of the most representative peptides were lost. Identified peptides from IGHG1_Human and from RAB4B_Mouse were not lost. Nevertheless, protein identification in the taxonomy was the same for the IGHG1_HUMAN (average score 70) and different for rodentia.

13% of the most representative peptides were lost. Identified peptides from IGHG1_Human and from RAB4B_Mouse were not lost. Nevertheless, protein identification in the taxonomy was the same for the IGHG1_HUMAN (average score 75) but different for rodentia.

**Discussion**

The mass peptide fingerprint of IFX obtained immediately after the preparation of the three different solutions studied (Table 1) showed similar patterns, but each solution had a different number of representative peptides with a variability of up to 26%. High degrees of variability are usual in these kinds of MALDI-TOF mass spectra due to the different processes that take place beforehand (enzyme digestion, yield of crystallization and ionization, etc.). Nevertheless, the same protein was always identified (with a high score) for infliximab (i.e. IGHG1_Human) (protein scores greater than 70 are significant). This result was expected since IFX is a chimeric IgG monoclonal antibody. The main part was not significant however in any of the cases, which means that no discussion could be based on it.

All the mass peptide fingerprints obtained during the test period for the three kinds of solution under the different storage conditions allowed to confirm the identity of the same proteins, the IGHG1_Human (scores greater than 70), in consequence, and despite the variability (up to 25%), it could be concluded that there were no dramatic changes in the main part of the IgG1 structure and the chimeric changes related with the chimeric sites. We also confirmed that the peptides identified were the same (shown with the same boxes in the table above).

**Stability Study Design**

IFX (2 mg/mL) is a monoclonal antibody against tumour necrosis factor alpha (TNF-α). It is used for the treatment of psoriasis, Crohn's disease, rheumatoid arthritis, spondyloarthropathies, and others. The stability of IFX is a critical requirement for maintaining its therapeutic and safety properties and for ensuring the quality of the final product. Commercially available solutions of IFX are supplied in a frozen state. When reconstituted, IFX solution has a shelf life of 8 weeks.

According to the manufacturer's instructions, IFX must be reconstituted by reconstituting the contents of the vial (20 mg/mL) by adding 1 mL of water for injectable preparations, or obtaining a solution of 10 mg/mL. The solution must be diluted with sodium chloride 0.9% up to 2 mL, obtaining varying concentrations depending on the dose required by the patient. The manufacturer recommends that reconstituted IFX be used in the preparation of infusions not to exceed 24 h and diluted between 0.1% and 1%.

On the basis of these instructions, we studied the chemical integrity of IFX at different concentrations and storage conditions. INFx concentration samples were stored in glass vials and at 4ºC and stored in polyethylene bags (polyethylene bags, each sample was modified in quadruplets. For storage conditions and the check times, as described in the table below.

**Table 1: IFX vial and storage conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time (h)</th>
<th>Storage</th>
<th>Mass Peptide Fingerprint</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2ºC, 8%</td>
<td>3</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
<tr>
<td>2ºC, 1%</td>
<td>3</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
<tr>
<td>0ºC, 0%</td>
<td>3</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
<tr>
<td>10ºC, 0%</td>
<td>3</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
<tr>
<td>10ºC, 0%</td>
<td>12</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
<tr>
<td>10ºC, 0%</td>
<td>24</td>
<td>0.5</td>
<td>No changes</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Conclusion**

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 ºC or frozen at -20ºC did not undergo dramatic chemical changes over the last 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 behavior.

This study is part of a wider project that seeks to contribute to the establishment of practical stability studies of biopharmaceuticals based on monoclonal antibodies. Following several ICH guidelines, including ICHQ9 (stability testing of biotechnological/biological products), the chemical integrity of the IFX is currently being studied by new exclusion chromatography (IESC) and the biological stability by means of specific ELISA tests based on TNF-α. 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 Pharmarcy (SPPF) and Annecy Pharmaceutiques Francaises (2011) 85, 221–231.

**Acknowledgment**

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