LONG-TERM STABILITY OF DILUTED SOLUTIONS OF THE MONOCLONAL ANTIBODY INFILXIMAB

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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, psoriasis plaque psoriasis and chronic fistulising ulcers. It blocks the damage caused by high levels of TNF-α 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 Infliximabs 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 (WEXEX-HPLC/DAD) to track changes in the isomeric profile, size exclusion chromatography high performance liquid chromatography with diode array detector (SEC-HPLC/DAD) for aggregates detection, and matrix assisted laser desorption ionisation 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 alpha (TNF-α).

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).

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