TCH-012 DIFFERENCES IN PURITY BETWEEN BIOSIMILAR FILGRASTIMS AND COPY BIOLOGICAL FILGRASTIMS

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Background Biosimilars are follow-on versions of peptide biological drugs, and differences in manufacturing and formulation can result in variations in physicochemical and clinical profiles. The European Medicines Agency (EMA) has set stringent standards (Ph Eur) that must be met for the approval of a biosimilar.
Purpose Standards of manufacture may differ between biosimilars approved via EMA pathways, and copy biologicals that lack approval pathways. Therefore, we undertook comparative characterisation tests of a range of biosimilar products from different global regions to determine if variations exist. This study is the first of its kind.
Materials and Methods Samples of Nivestim (Ni), Neupogen (Ne), Tevagrastim (T), Ratiograstim and Zarzio (Z) were obtained from the EU region; and Leucostim (L), GeSysin (G), Filgen (F) and Neukine (Nk) were obtained from the Middle East and Africa (MENA) region. All samples were within the expiry date. Samples were analysed for impurities using iso-electric focussing (IEF) to identify differences in charge, size-exclusion high-performance liquid chromatography (SEC-HPLC) to identify differences in higher molecular weight impurities, reverse phase HPLC (RP-HPLC) to identify differences in total and individual related impurities, and ion chromatography (IC) to detect differences in f-met filgrastim and related, more acidic, impurities.
Results All biosimilars met EMA standards for IEF and SEC-HPLC analysis. Total impurities (RP-HLPC) for the EU products were in the range 1.8–2.6% and within EMA requirements (≤3.5%); however, the MENA samples contained impurities in the range 5.9% (G) – 8.2% (L), which is beyond the Ph Eur range. IC analysis revealed f-met and acidic impurities to be <0.20% for most EU products (threshold 1.0%) and 0.4% for Ne. However, for MENA compounds, these impurities comprised 0.4% (Nk) – 1.7% (G) of the samples.
Conclusions Copy biologicals from MENA have higher levels of impurities than biosimilars from the EU and do not meet EMA standards for approval.

No conflict of interest.

TCH-013 DISINFECTANT EFFICACY OF ULTRAVIOLET LIGHT IRRADIATION IN AN AUTOMATED SYSTEMS FOR THE ASEPTIC COMPOUNDING.

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Background Ultraviolet (UV) light irradiation is used in a variety of applications, such as food, air and water purification. The mechanism of UV disinfection differs considerably from chemical disinfectants: UV is mutagenic to bacteria, viruses and other microorganisms by damaging nucleic acids and preventing replication. However, the effectiveness of UV disinfection depends on a number of factors: time of UV exposure; power of the UV source; presence of UV barriers like airborne particles; microorganism resistance.
Purpose To study the effectiveness of UV disinfection inside APOTECAchemo, the robot for preparing antitumour drugs in use at the University Hospital of Ancona. The Killing Rate (KR) and optimal exposure time were determined.
Materials and Methods 5 different microorganisms were chosen for the study in order to cover all the most common families of microbes: Candida albicans; Escherichia coli; Bacillus subtilis; Staphylococcus aureus; Pseudomonas aeruginosa. Different concentrations of each organism (from 107 CFU/ml to 0.5 CFU/ml) were subjected to UV radiation for different exposure times. The plates were located inside the APOTECAchemo compounding room, using the robot's UV equipment. The KR (logarithmic ratio of the concentration of microorganisms after and before irradiation) was plotted against the exposure time in order to chart the inactivation curves.
Results With a four-hour exposure, the UV irradiation kills all microorganisms at the highest concentrations. The location of the plates inside the system showed only a slight effect on the killing rate, probably thanks to the mirror effect of the stainless steel surfaces. Bacillus subtilis confirmed the strongest UV resistance, indeed 4-hour exposure was necessary to kill 107 CFU/ml. The least resistant microorganism was Escherichia coli, which required 2 hours of UV irradiation.
Conclusions UV radiation is a fundamental step in the sterilisation of workplaces. In fact, 4-hour exposure showed an effective sterilisation (KR < 7) outcome, even for very resistant microorganisms (Bacillus subtilis).

No conflict of interest.

TCH-014 EVALUATION OF LONG-TERM BIOLOGICAL ACTIVITY OF INFLIXIMAB 10 MG/ML AND 5 MG/ML IN NACL 0.9% BY ELISA

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Background Tumour necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine, the main mediator in inflammatory and autoimmune diseases, as well as during various attacks on cells such infections. It is therefore involved in the course of a large number of pathologies such as rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis and ulcerative colitis. Infliximab (Remicade) is a chimeric monoclonal antibody (75% human, 25% murine) which acts by binding to TNF-α and blocking its effect. The cost of treatment with infliximab is quite high and the stability indicated by the manufacturer once the vial is opened is 24 hours.
Purpose The purpose of this research has been to evaluate the biological activity of infliximab when reconstituted and diluted to 10.0 mg/ml and 5.0 mg/ml in NaCl 0.9% in a long term stability study up to 15 days. A study of the drug degradation has been also tackled to cheque any remaining activity.
Materials and Methods An indirect non-competitive ELISA immunoassay was developed based on the use of ELISA plates sensitised with TNF-α. The plates were incubated 'overnight' at 4°C using recombinant TNF-α from E. Coli at a concentration of 1 μg/ml. The immunoassay was validated in terms of calibration function (from 0.2 to 50.0 μg/ml), detection limit (0.06 μg/ml), precision as within-day reproducibility (relative standard deviation lesser than 10%), and accuracy as percentage of recovery (higher that 90%). The infliximab solutions of 10.0 mg/ml and 5.0 mg/ml in NaCl 0.9% were stored refrigerated at 4°C protected from daylight. The biological activity of these solutions was tested periodically up to 15 days by the ELISA method developed. The ELISA was also used to study the drug degradation in a stress study involving the exposure of samples of infliximab (50.0 mg/ml) for 24 hours to different stress conditions: basicity (NaOH 0.1M), acidity

(HCl 0.1M), oxidation (H_2O_2 1% and 10%), temperature (50°C) and ultraviolet light (250 w/m, 25°C).

Results All the samples analysed showed considerable biological activity; this biological activity was surprisingly even observed in those samples subjected to strongly stressed conditions. For the reconstituted sample of 10.0 mg/ml, a remaining activity of 52% was observed. In the case of the 5.0 mg/ml sample, the remaining activity decreased to 35%.

The biological activity measured using the samples submitted to stress conditions indicated a remaining activity at least equal to the upper concentration studied in the calibration function, i.e, 50 μg/ml. These samples were analysed directly, without dilution, because they had been expected to lose their biological activity totally.

Conclusions The biological activity of infliximab solutions of 10.0 mg/ml and 5.0 mg/ml in NaCl 0.9% when stored refrigerated at 4°C protected from the daylight was maintained at 52% and 35% respectively up to 7 days. The biological activity was also shown in infliximab samples submitted to stress conditions. More experiments are currently being conducted to confirm these results.

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No conflict of interest.

TCH-015 EVALUATION OF THE CHEMICAL AND PHYSICAL STABILITY OF SODIUM DICHLOROACETATE, AN ORPHAN DRUG FOR RARE METABOLIC DISEASES

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Background Sodium dichloroacetate (Na-DCA), not a patented substance, which is used in the treatment of rare diseases with congenital defects of the pyruvate-dehydrogenase complex (PDHC), produces a marked reduction in acid-base imbalance and lactic acid levels toxic to the brain parenchyma.

Purpose To evaluate the physical-chemical stability of sodium dichloroacetate in aqueous solution.

Materials and Methods Six grammes of sodium dichloroacetate were dissolved in 60 ml of water for injections (WFI). The exact concentration of the solution obtained was calculated by extrapolation from a calibration curve, recording the absorbance value at the wavelength of 198 nm of suitable standard solutions (5–50 μg/ml) of sodium dichloroacetate dissolved in water for injections (WFI). The solution was divided between 3 dark glass containers. The first container was kept at room temperature (r.t.), the second one in a refrigerator at +4°C, the third one in a freezer at –20°C. The stability of the samples, kept at different temperatures, was checked at 31, 45, 54 and 60 days; for each sample, using appropriate dilution, absorbance values were recorded (λ = 198 nm) and through the sodium dichloroacetate calibration curve made daily, the concentrations of the substance being analysed were calculated. The results were expressed as percentages of sodium dichloroacetate in solution.

Results Samples kept at +4°C were stable throughout the observation period. Samples kept at r.t. were stable until 30 days from preparation, while afterwards a slow and gradual decay could be observed. Samples kept at –20°C showed a progressive increase in concentration.

Conclusions The observed increase in samples at –20°C can be explained by the formation of a secondary species with a higher extinction coefficient than sodium dichloroacetate. Data suggest that sodium dichloroacetate solutions should not be stored at –20°C or at r.t. for more than 30 days.

No conflict of interest.

TCH-016 EXTENDED CHEMICAL-PHYSICAL STABILITY OF 25 mg/ml AZACITIDINE SUSPENSION

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Background Azacitidine is used for haematological pathologies. The summary of product characteristics (Vidaza) indicates stability of 45 minutes at room temperature and 22 hours if prepared with water for injections (WFI) at 2–8°C at reconstitution and refrigerated.

Purpose To assess the chemical-physical stability of azacitidine suspension 25 mg/ml.

Materials and Methods Analysis fofllowed an approved protocol.

The validity of the reference material (azacitidine–Sigma Aldrich-batch-SLBD1299V) was checked before starting the analysis.

100 mg of drug was reconstituted with 4 ml of refrigerated (2–8°C) WFI. The sample and standard suspension were stored at 5°C in a temperature-controlled refrigerator.

For International Conference Harmonization guideline the solution can be considered stable if the % assay of azacitidine with respect to the initial value is reduced by less than 5%.

Azacitidine concentrations were determined by a stability-indicating HPLC method under the following conditions: X-Terra RP18 column (150 × 4.6 mm, 5 μm); 4°C autosampler temperature; phosphate buffer pH = 6.5 and acetonitrile/water = 40/60 as mobile phase; 0.8 ml/min flow rate; 230 nm UV detection; 20 μl injection volume.

At these conditions the sample and a standard suspension were analysed at 0/22/24/48/72/96/168 hours.

The % assay of azacitidine was calculated at each cheque point and the results were compared with the assessed 100% values for assay at t_0.

Results The azacitidine assay (%) determined by HPLC is reported in the table below.

Average values obtained by triplicate injections at each cheque point are reported.

Conclusions The variation of the % assay of azacitidine with respect to the initial value is less than 5% for at least 48 hours.

A microbiological study on azacitidine suspension is ongoing at our hospital. Positive results will allow us to use unused azacitidine suspension within 48 hours of reconstitution with considerable cost savings.

Abstract TCH-016 Table 1

Time(hour)	% Azacitidine assay	% Azacitidine assay vs. t initial value
0 h	110.73	102.62
22 h	109.97	101.92
24 h	107.90	100.00
48 h	103.87	96.27
72 h	96.01	88.98
96 h	101.04	93.64
168 h	87.18	80.80

No conflict of interest.