EVALUATION OF LONG-TERM BIOLOGICAL ACTIVITY OF TRASTUZUMAB 15.0 mg/mL (HERCEPTIN®) BY AN AD HOC ELISA METHOD

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BACKGROUND

Trastuzumab (TRZ) (Herceptin®) is a humanized monoclonal antibody IgG1 that acts against receptor 2 human epidermal growth factor (HER2). It is indicated in the treatment of early and metastatic breast cancer and metastatic gastric cancer.

PURPOSE AND OBJECTIVE

To evaluate the post-biological activity that remains in Herceptin® after opening single-use vials in long-term study. It was also evaluated the remaining activity when exposing TRZ to different stress conditions.

EXPERIMENTAL

It was developed an ad hoc indirect non-competitive ELISA based in the use of recombinant human HER2 to test Biological Activity of Trastuzumab.

VALIDATION OF IMMUNOASSAY

The developed ELISA test has been validated in terms of calibration function, sensitivity as detection and quantification limits, accuracy (as % of recovery), and precision (as intraday and interday reproducibility % RSD).

SENSITIVITY

DETECTION LIMIT 31.8 ng/mL
QUANTITATION LIMIT 100.0 ng/mL
SENSING RANGE 100.0-500.0 ng/mL
DETECTION INTERVAL 31.8-100 ng/mL

CONCENTRATION (µg/mL) ABSORBANCE AVERAGE (% RECOVERY)
0.4 0.6629 109.38%
0.1 0.2635 105.95%
0.01 0.1034 95.74%

RESULTS

Drug Degradation Study

Residual biological activity remained in all samples submitted to the stress except in samples heated at 70°C.

Surplus samples of Herceptin® from the daily use of the Hospital Pharmacy Unit were stored at 4°C, -20°C and -80°C protected from dark. Biological activity was tested up for 15 days.

Stability Study

The biological activity of Herceptin® decreased 25%, 30% and 47% the initial activity 24 hours after opening vials when stored at 4°C, 20°C and -80°C, respectively. The decrease was 50-60% after 2 days for the three storage conditions and it was maintained along the study (up to 15 days).

DRUG DEGRADATION STUDY

CONCENTRATION 15.0 mg/mL ... Abs. Reference: 0.4306

STRESS CONDITIONS (24 h.) AVERAGE ABSORBANCE
NaOH 0.1 M 0.2050
HCl 0.1 M 0.2420
H2O2 0.1% (v/v) 0.2300
H2O2 1% (v/v) 0.2305
NaCl 1 M 0.2330
50°C 0.2785
100°C 0.0763
UV 50°C 250 W/m 0.3823

CROSS REACTIONS STUDY

TRZ ANTIGEN BIOPHARMACEUTICAL AVERAGE ABSORBANCE
HER2 1.0 µg/mL 0.5490
HER2 1.0 µg/mL RTX 0.2 µg/mL 0.0480
HER2 1.0 µg/mL IFX 0.2 µg/mL 0.0360
HER2 1.0 µg/mL BV6 0.2 µg/mL 0.0497
HER2 1.0 µg/mL CTX 0.2 µg/mL 0.0453

No conflicts of interest.

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