PP-022

EVALUATION OF LONG-TERM BIOLOGICAL ACTIVITY OF CETUXIMAB 5.0 mg/mL (ERBITUX®) BY AN AD HOC ELISA METHOD

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COMPLEJO HOSPITALARIO UNIVERSITARIO DE GRANADA

Servicio Andaluz de Salud
CONSEJERÍA DE IGUALDAD, SALUD Y POLÍTICAS SOCIALES

20th Congress of the eahp. Hamburg, Germany – 25-27 March, 2015.The Hospital pharmacist´s agenda – patient safety first

BACKGROUND

Cetuximab (CTX) (Erbitux®) is a chimeric mouse-human monoclonal antibody IgG1 targeting epidermal growth factor receptor (EGFR). It is approved for use as treatment for metastatic colorectal cancer and squamous cell carcinoma of the head and neck.

PURPOSE AND OBJECTIVE

To evaluate the post-biological activity that remains in Erbitux® after opening single-use vials in a long term study. It was also evaluate the remaining activity when exposing the open medicine to different stress conditions to test risk to accidental exposure to light, heat, etc.

EXPERIMENTAL

❖ **COST OF TREATMENT:**

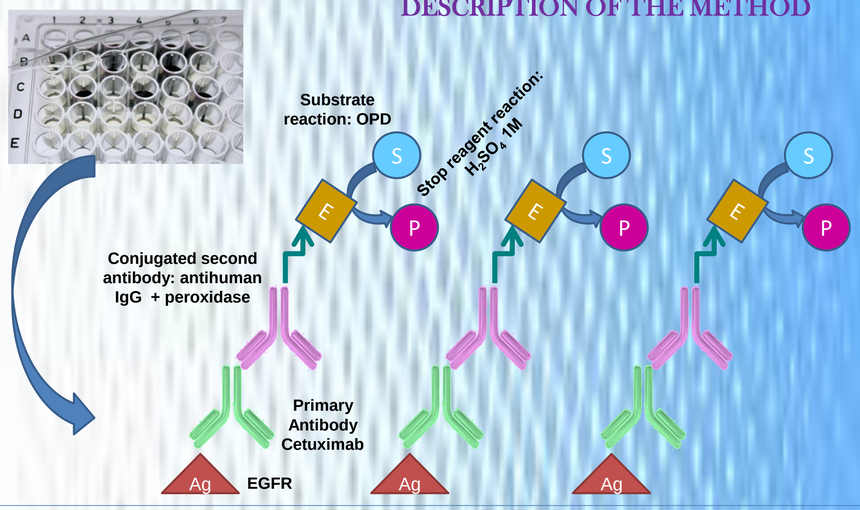
❑ **PHARMACEUTICAL FORM: 500 mg 100 mL^{-1} / Vial**

PSP: **184.896 €/vial** (5 mg / mL → 20 mL)

❑ **Administration: once a week intravenous use**

It was developed an *ad hoc* indirect non competitive ELISA based in the use of EGFR human to test biological activity of Cetuximab.

INDIRECT ELISA. DESCRIPTION OF THE METHOD

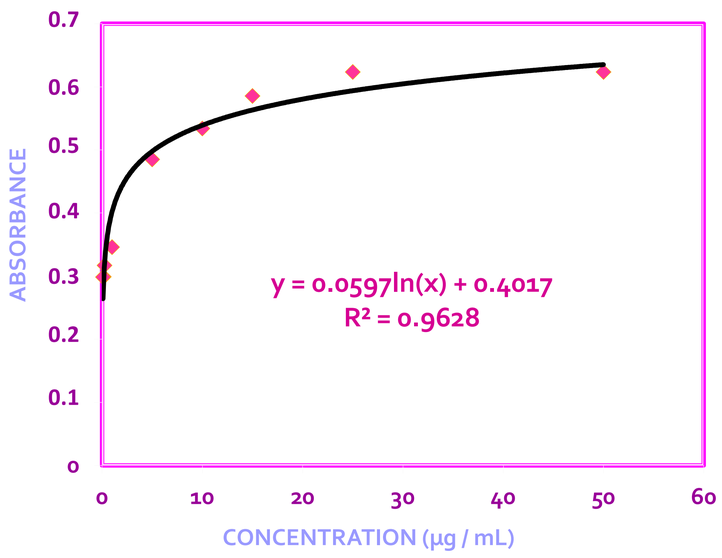


VALIDATION OF IMMUNOASSAY

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).

CALIBRATION FUNCTION

CETUXIMAB



SENSITIVITY

DETECTION LIMIT	0.10 µg/mL
QUANTITATION LIMIT	0.40 µg/mL
SENSING RANGE	0.40-50.0 µg/mL
DETECTION INTERVAL	0.10-0.40 µg/mL

PRECISION

The precision was **determined** as intraday and interday reproducibility (% RSD) and in all case was < 10%

REPEATABILITY			
CONCENTRATION (µg/mL)	STANDARD DEVIATION	AVERAGE ABSORBANCE (450-620 nm)	COEFFICIENT OF VARIATION (% RSD)
25.0	0.0088	0.4496	**3.97 %**
5.0	0.0075	0.2616	**2.86 %**
0.1	0.0062	0.1256	**4.96 %**
REPRODUCIBILITY			
25.0	0.06961	0.4657	**5.94 %**
5.0	0.07279	0.4430	**7.27 %**
0.1	0.08515	0.2013	**8.51 %**

ACCURACY

CONCENTRATION (µg /ml) n= 3	% RECOVERY
25.0	80-95%
5.0	
0.1	

ELISA method developed is simple, fast, precise and accurate.

RESULTS

DRUG DEGRADATION STUDY

CONCENTRATION	5.0 mg / mL →Abs. Reference: 0.6235
STRESS CONDITIONS (24h.)	**AVERAGE ABSORBANCE**
NaOH 0.1 M	0.1547
HCl 0.1 M	0.1090
NaCl 1 M	0.6553
H_2O_2 1% (v/v)	0.4077
H_2O_2 10% (v/v)	0.2423
50°C	0.0113
70 °C	0.0010
UV 50°C 250 w/m	0.7840

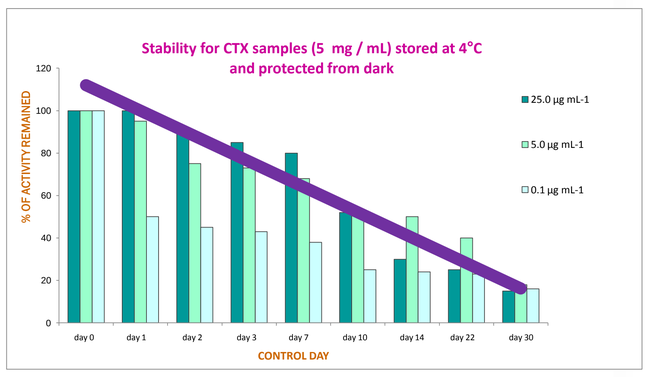
Residual biological activity remained in all samples submitted to the stress except in samples heated at 70°C.

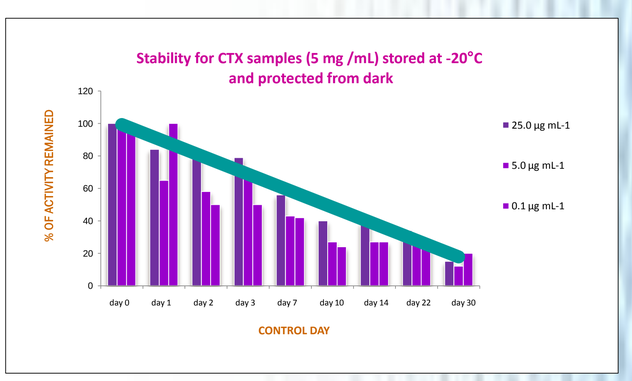
CROSS REACTIONS STUDY		
CTX ANTIGEN	BIOPHARMACEUTICAL	AVERAGE ABSORBANCE
EGFR 1.0 µg /mL	TRZ 5 µg / mL	0.0907
EGFR 1.0 µg /mL	RTX 5 µg / mL	0.0813
EGFR 1.0 µg /mL	IFX 5 µg / mL	0.0763
EGFR 1.0 µg /mL	BVZ 5 µg / mL	0.0930
EGFR 1.0 µg /mL	CTX 5 µg / mL	0.2663

There were not cross reactions with the rest of biopharmaceuticals analyzed.

Stabilty Study

Surplus samples of Erbitux® from the daily use of the Hospital Pharmacy Unit were stored at 4°C and -20°C protected from dark. Biological activity was tested up to 30 days.





The biological activity of Erbitux® decreased 5% when stored for 24 hours at 4°C. The decrease was 14% after 3 days, 20% after 7 days and 85% the last checked day. For CTX samples stored frozen at -20°C, the biological activity decreased from 16% (24 hours) to 85% the last checked day.

CONCLUSIONS

Regarding the biological activity of Erbitux®, it is stable within the 24 first hours after opening of the vial when stored at 4°C. These results will be further investigated by flow cytometry.

No conflicts of interest

ACKNOWLEDGEMENTS

Financial support: PI10/00201 (MICINN, Government of Spain)