PP-017

EVALUATION OF LONG-TERM BIOLOGICAL ACTIVITY OF BEVACIZUMAB 25.0 mg/mL (AVASTIN ®) BY AN AD HOC ELISA METHOD

Inmaculada Suárez González [1], Natalia Navas Iglesias [2], Antonio Salmerón García [3], José Cabeza Barrera [3], Luis Fermín Capitán Vallvey[1]

[1] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avda. Fuentenueva s/n ,18071, Spain.
[2] Department of Analytical Chemistry, Facultad of Sciences, Biomedical Research Institute ibs. Granada,University of Granada, Avda. Fuentenueva s/n,18071, Spain.
[3] Hospital Pharmacy Unit, Complejo Hospitalario Universitario de Granada, Biomedical Research Institute ibs. Granada, E-18012, Spain.

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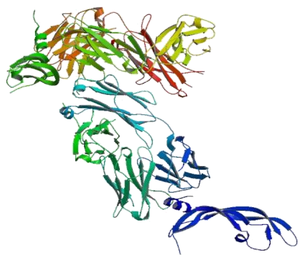
BACKGROUND

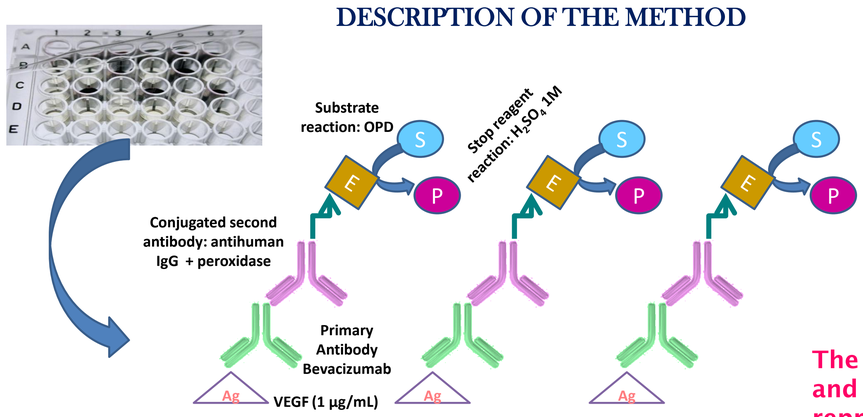


Bevacizumab (BVZ), the active substance of Avastin® (25 mg/mL BVZ), is a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody indicated in the treatment of several cancers.

PURPOSE AND OBJECTIVE

To evaluate the post-biological activity that remains in the medicine Avastin® after opening single-use vials in long term study.

EXPERIMENTAL

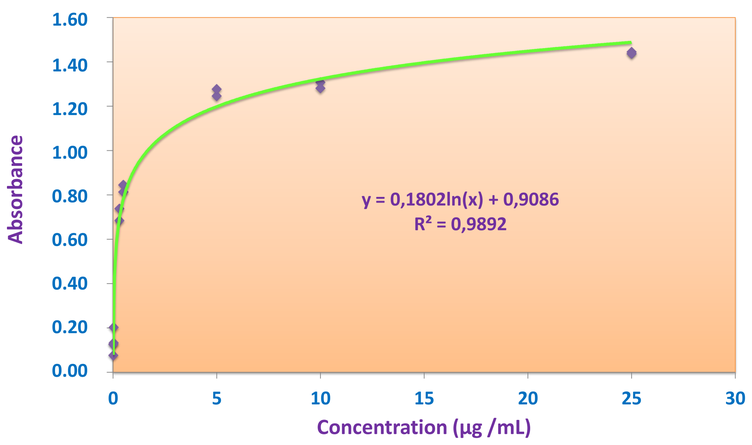


An *ad hoc* indirect non-competitive ELISA was developed and validated as stability indicating to test Biological Activity of BVZ.

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



DETECTION LIMIT	0.10 µg/mL
QUANTITATION LIMIT	0.35 µg/mL
SENSING RANGE	0.35-25.0 µg/mL
DETECTION INTERVAL	0.10-0.35 µg/mL

RECOVERY

CONCENTRATION (µg /ml) n= 8	AVERAGE ABSORBANCE (450-620 nm)	% RECOVERY
2.5	0.8921	96.68 %
0.5	0.3604	98.20 %
0.01	0.0529	94.46 %

REPEATABILITY & REPRODUCIBILITY

CONCENTRATION (µg/ml)	STANDARD DEVIATION	AVERAGE ABSORBANCE (450-620 nm)	COEFFICIENT OF VARIATION (%)
REPEATABILITY			
2.5	0.01089	0.7571	1.43 %
0.5	0.00856	0.3568	2.39 %
0.01	0.00454	0.0619	7.34%
REPRODUCIBILITY			
2.5	0.05538	0.8557	6.47 %
0.5	0.02898	0.3619	8.00 %
0.01	0.00716	0.1279	5.60 %

RESULTS

DRUG DEGRADATION STUDY

CONCENTRATION	25.0 mg /mL → Abs. reference: 0.7117
STRESS CONDITIONS (24 h)	AVERAGE ABSORBANCE
NaOH 0.1 M	0.2468
HCl 0.1 M	0.2860
H_2O_2 1% (v/v)	0.6493
H_2O_2 10% (v/v)	0.6450
NaCl 1.5 M	0.6815
50°C	0.6740
70 °C	0.0575
UV 50°C 250 w/n	0.5470

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

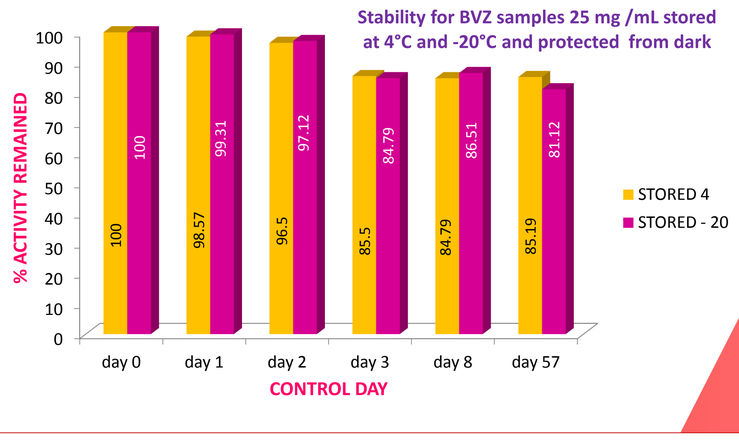
CROSS REACTIONS STUDY

BVZ ANTIGEN	BIOPHARMACEUTICAL	AVERAGE ABSORBANCE
VEGF 0.25 µg /mL	TRZ 0.5 µg /mL	0.0513
VEGF 0.25 µg /mL	RTX 0.5 µg /mL	0.0727
VEGF 0.25 µg /mL	IFX 0.5 µg / mL	0.0507
VEGF 0.25 µg /mL	CTX 0.5 µg / mL	0.0543

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

Stability Study

Surplus samples of Avastin® 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 for 57 days.



The biological activity of Avastin® was higher than 98% at day 1, and higher than 95% at day 2, but decreased 15% the initial biological activity at day 3. This value kept through the study (57 days) for the two storage conditions tested.

CONCLUSIONS

Regarding biological activity, the stability of Avastin® at the conditions used both refrigerated (4°C) and frozen (-20°C) was kept for two days. Considering the limit of +/-10% used in practical stability studies, it can not be considered stable from day 3 since the loss of biological activity was 15%. These results will be further investigated by flow cytometry.

No conflicts of interest.

ACKNOWLEDGEMENTS

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