Determination of biotin number linked to monoclonal antibodies

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Introduction

Monoclonal antibodies (Mab) are used in enzyme-linked immunosorbent Assay (ELISA) tests. This is an immunologic test used to detect or quantify a protein from a biological fluid. Biotins are grafted on Mab, in order to detect them using streptavidin conjugated to an enzyme such as Phosphatase Alkalin. A MALDI-TOF mass spectrometry is used (Matrix Assisted Laser Desorption Ionisation - Time Of Flight). The mass of both the Mab, and the biotinylated Mab is measured from the m/z value of the monocharged ion and the dicharged ion. The Mab mass is substracted from biotinylated Mab mass. As a result, the average number of biotin linked to Mab is deduced. Once method parameters have been optimized and automated, parameters that can affect the quality of measurement were determined. Drift of external calibration, as well as concentration effect, have been tested. A comparison between external and internal calibration, through the use of biotinylated antibody (anti-HSA) is presented here.

Experimental Conditions

MALDI-TOF spectra were acquired using the Ultraflex II (Bruker Daltonics, Bremen, Germany). Samples were prepared using the dry droplet technique and HCCA matrix (α-Cyano-4-hydroxycinnamic acid diluted at saturation in a mixture of H_2O/CH_3CN/formic acid). For higher sensitivity, the MALDI-TOF was used in the linear detection mode. 1µL of sample was homogenized on the target with 1µL of matrix. External calibration was carried out using both BSA dimer and monomer. Internal calibration was performed by the addition of a BSA monomer into the sample.

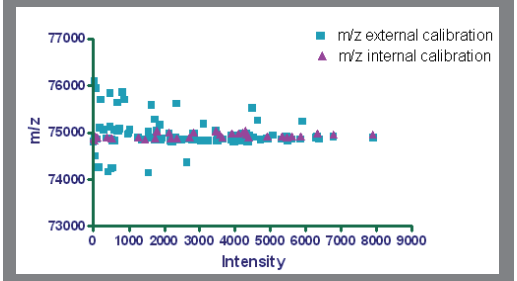
Results and discussion

The variance of the Mab mass was shown to depend on the signal intensity, using an external calibration. This phenomenon seems to be less accute using internal calibration (Graph A). The mass average (Student test) and variances (Fisher test) were compared for two experiments; no statistical difference (risk α equal to 5%) was observed, neither for the external calibration, nor the internal calibration. However when external and internal calibrations were compared (Graph B), the mean (p value=0.0011) and the variance (p value=0.0187) were statistically different. The variance was lower for an internal calibration. These results are therefore in favor of the systematic use of an internal calibration.

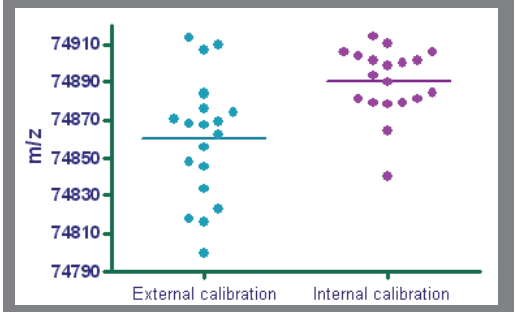
Conclusion

The average mass was estimated at 74860+/-15.5 m/z using external calibration and at 74890+/-8.5 m/z using internal calibration. These results are predictions, and will accordingly have to be confirmed by real validation.

A)



B)



Graph showing distribution of masses of dicharged biotinylated antibody anti HSA
A) Comparison including several experiments
B) Comparison including two experiments

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