Optimization of a dynamic coupling of ATIII and improvement of the efficiency of an affinity column

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Introduction

Nowadays, liquid chromatography is a very popular technique for pharmaceutical analyses. In the Development of Purification laboratory in Sanofi-Aventis Aramon, Affinity Chromatography (AC) is a professional analytical method. The protein, Antithrombin III (ATIII), is bonded to the affinity column. It is covalently coupled to the gel on the column. This column is used to quantify the relative percentage of the affine part in the hexasaccharide (F6) fraction of Ultra Low Molecular Weight Heparin (ULMWH). One objective was to compare the results, relative percentage of the F6 fraction, obtained with two different columns (the former column "A" and a new "B"). Several injections were realized. To improve the selectivity of column "A", as efficiency decreases with age, a dynamic grafting of ATIII on the gel column was realized, followed by analyses in order to obtain better and more accurate results.

Experimental method

• Analyses by Affinity Chromatography

For hexasaccharide separation, a chromatographic system AKTÄ Explorer was used. The column parameters are: "A" l= 28.2 cm; d=1.6 cm; Column volume (Cv) =56.7 ml and "B" l = 27.2 cm; d=1.6 cm; Cv=55.1 ml. The stationary phase is ATIII bonded to CNBr-activated Sepharose 4B (gel). Injections with defined concentrations were realized at 0.5; 1 and 2 mg /ml of F6.

After equilibration, the elution of non-affine F6 was realized with the first mobile phase and affine hexasaccharides is bonded to ATIII. Then affine F6 was eluted by a mobile phase with a higher Ionic strength.

• Coupling ATIII to Sepharose 4B of the column

A solution of ATIII, Enoxaparine, and a coupling solution was used to bond ATIII. After this grafting, analyses by AC were realized.

Results and discussion

Before ATIII bonding on the gel, the chromatogram obtained after analyses with column "A" (figure 1) shows a good resolution of the affine and the non-affine peaks. On the other hand, the shape of the peak reveals a preferential passage of molecules within the column. Furthermore, this phenomenon is more particularly observed on figure 2. As can be seen, firstly, the rate of affine F6 obtained with column B is compared with the value of standard affine F6 (18.1 ± 1 %). So, for column B, results are consistent whereas for column A, they are not: results for F6 fraction decreased. For this reason, a dynamic grafting of ATIII on the gel was tested. It was found that in this case, the affine F6 percentage increases compared with the first analyses before the bonding (figure 3).

Conclusion

According to the results obtained before the grafting, column "A" loses its efficiency. Bonding with ATIII was therefore realized, and was successful. The full preparation of the ATIII affinity stationary phase can be thus avoided, which allows time-saving and a considerable financial saving because ATIII is relatively expensive.

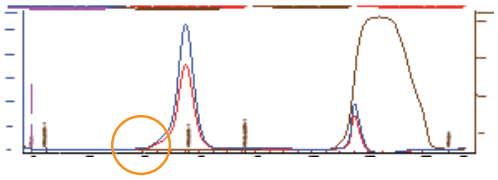

Figure 1 : Chromatogram obtained with the ATIII bonding to the gel on the column (the former "A") thanks to analyses by Affinity Chromatography.

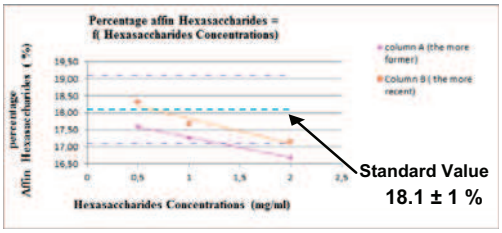

Figure 2 : Graph representing the affine F6 fraction according to the concentration in F6 injected on columns "A" and "B".

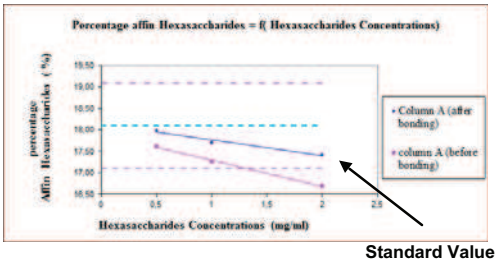

Figure 3 : Graph representing the affine F6 fraction according to the concentration in F6 injected on column "A" before and after grafting.

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