



ZEOCHEM®

ZEOSphere® 100 C8 stability under CIP conditions

Cleaning in Place

During the chromatographic purification of proteins (e.g. insulin), proteinaceous impurities or aggregates can irreversibly bind to the stationary phase and thus decrease the separation performance. In order to remove the bound molecules, they have to be denaturated. This is commonly done by cleaning in place (CIP), where a solution of aqueous NaOH and an organic solvent is pumped through the column. CIP has to be performed every time the performance of the column is decreasing.

Test procedure

Two materials are investigated: ZEOSphere® 100Å C8 12 µm and one of the best competitor product present on the market. The stability of the two materials is evaluated by running different CIP cycles. Each CIP cycle is composed by different steps:

- (i) equilibration with EtOH (5 CV);
- (ii) CIP digest with a solution of NaOH 0.1 M aq. (pH 13)/EtOH: 50/50 v. (X CV);
- (iii) neutralization with a EtOH/H₂O/AcH: 10/90/0.2 w. buffer (5 CV);
- (iv) flushing with acetonitrile (6 CV).

CV: empty column volume [-].

After each CIP cycle, the retention time of uracile, toluene and amitriptyline are determined using a MeOH/25 mM KH₂PO₄ (pH 7): 80/20 v. buffer. Seven, respectively ten CIP cycles are run on the two columns. This corresponds to a total of almost 70 CV of CIP solution that is pumped through each column.

Results

The retention factor of toluene and amitriptyline is plotted as a function of the quantity of CIP solution pumped through the columns (expressed in CV).

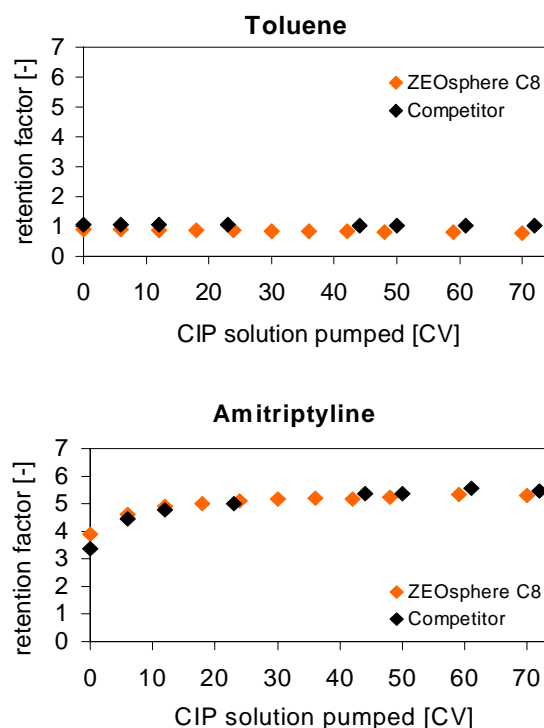


Figure 1: retention factors of toluene and amitriptyline as a function of CV of CIP solution pumped through the columns.

Conclusions

Both columns are affected by the CIP treatment. However the CIP process seems not to affect significantly the hydrophobicity of the two materials: the retention factor of toluene remains almost constant. The retention factor of amitriptyline is, instead, increasing with the amount of CIP solution pumped through the columns. This is due to the progressive increase of free silanol groups. The absolute increase of the retention factor of amitriptyline, after pumping 70 CV of CIP solution, is of 36% on ZEOSphere® 100 C8 and of 62 % on the competitor material.

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