Split/double peaks in HPLC

The causes:

The causes usually lie in the fact that the pH of the mobile phase is too close to the pKa of a functional group of the compound(s) or, also, the buffer concentration that is too low. It is possible – although less likely – an obstruction by particles present in the matrix or dead volumes within the column, caused by the dissolution of the stationary phase itself.

The Solutions:

First of all, adjust the pH of the mobile phase to a value at least 2 units lower than the pKa of the most acidic compound or 2 units above the pKa of the most basic compound.

In other hand, the concentration of the buffer should be such that it guarantees an effectively constant pH throughout the entire column and that it "supplies" enough ions to neutralize the compound's molecules (about 10 times the sum of the maximum concentration of all compounds in the sample will be sufficient).

It is obvious that an adequate filtration of the samples and the use of guard columns prevents any obstructions by particles present in the matrix, so we strongly recommend its extensive use.

Finally, replacing the column – dead volumes or «channels» within the column are not «repairable» by any cleaning or regeneration process. However, if this is repeated frequently, consider revising the method used, changing the pH of the mobile phase or the type of bonded phase used.

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