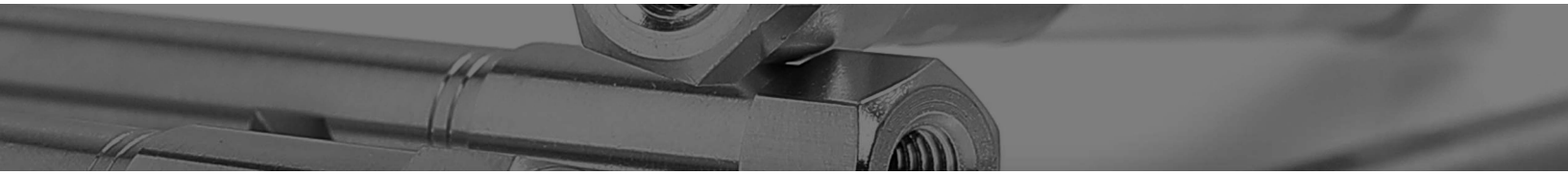

Q&A for Liquid Chromatography

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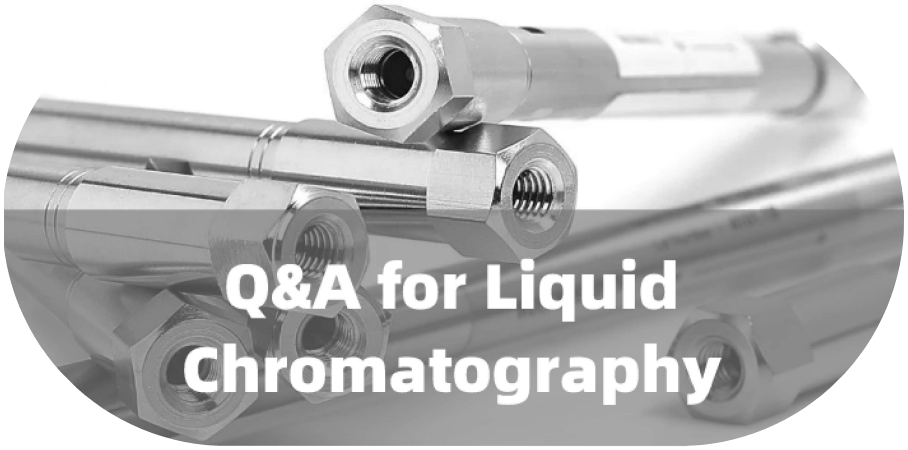

1

Q: What is the advantage of LC over GC?

A: LC has more advantages over GC as it has wider application range. GC is only limited to analyze low-molecular weight substances which are easily vaporized and it mainly targets at fundamental chemical raw materials. But any substance soluble in solvent, ranging from dozens to tens of thousands of molecular weight, can be analyzed by LC. In pharmaceutical, chemical, environmental protection, food and many other important fields, LC has become a leading analytical tool. There are also some circumstances in which both LC and GC can be applied, but sample preparation of LC is simpler. The appearance of LC compensates the defect that GC can not be directly used to analyze volatile, thermal instability and polymer compounds.

2

Q: Analysts often doubt whether the column can be flushed in the opposite direction. What kind of column can be flushed in the opposite direction? And what can't? After back flushing, should column be used in the normal direction?

A: Generally, both normal phase columns and reverse phase columns can be flushed in the opposite direction. But back flushing can't be used in columns with asymmetrical pore size of frits and this kind of column is rare to see now. Back flushing aims at washing off the contaminants of the column, so it is better to use the column in the normal direction to avoid contamination at both ends. Most of Welch columns can be flushed in the opposite direction, and we recommend to use columns in the normal direction and flush them in the opposite direction.

Q: Some manufacturers use a larger pore size (2~5μm) of the front frit to avoid blockage, back flushing will wash out packing materials under this situation. Will the pore size of the front and back frits be stated in the instructions?

A: If the pore size of the front and back frits are asymmetrical, the manufacturer will certainly mention it in the instructions. The pore size of the back and front frits of Welch columns is symmetrical.

4

Q: Should column be connected to detector during back flushing?

A: Back flushing means to connect the column to the system in the opposite direction. Theoretically, column can either connect or disconnect the detector. However, if the sample is filled with pollutants, it is recommended not to connect the detector and put waste collection bottle under the outlet. Because if there are pollutants being washed out, connecting to detector will cause extra pollution.

5

Q: When using ion exchange column to do method development, and acetonitrile and water were used as mobile phase, it was found that RT is 2.5 minutes with 60% acetonitrile and RT remains the same with 30% or 40% acetonitrile, but RT suddenly changed to about 13 minutes with 20% acetonitrile during the process of adjusting gradient program. What's the reason?

A: The retention mechanism of ion exchange columns is mainly electrostatic interaction. The main influencing factors include ionic strength, pH, secondary interaction of hydrogen bonds, and molecular exclusion. Therefore, in ion exchange chromatography, the retention mechanism is not as simple as reversed-phase chromatography, nor is it simply positively to the proportion of organic phase. It is necessary to consider whether the increase of aqueous phase proportion leads to the change of ionic strength of mobile phase or enhances the hydrogen bonding ability. In addition, whether the matrix of ion exchange column is silica gel or polymer also has different effects.

6

Q: For a commonly used C18 column, how to activate and maintain this new column? Why should I do that?

A: The activation of the new column is actually a process of equilibrium. During the transportation and storage of the column, solvent in the column is possible to volatilize, resulting in dried packing materials. As the bonded phase is not fully wetted, column needs to be activated. Welch columns can be activated according to the instructions. In addition to equilibrating with the mobile phase, sometimes it is necessary to equilibrate the new column with tested samples. The specific equilibrium method is also very simple, just increase the concentration of the injected sample or continue to inject when elution is not complete. The purpose of equilibrating a new column is to saturate the adsorption capacity of the sites of silica gel matrix packing materials surface with nonspecific adsorption.

7

Q: C18 column will be slowly activated by adding methanol. What is the purpose of this?

the injection volume. In this way, the active sites in the column can saturated with samples to avoid abnormal phenomenon.

8

Q: I had just installed a new C18 column the other day, and I flushed it with 100% methanol for more than half an hour before the injection. If I only wash it for half an hour and start to use, is that ok? Will it affect the peak?

A: First of all, if it is only the activation of a new column, it is recommended to strictly follow the the manufacturer's instructions; Secondly, not all determinations require sample aging for new columns. However, it is a good habit to inject several times according to the method and start the formal determination after ensuring that the retention time of peak area no longer changes obviously.

9

Q: What columns are generally used for the determination of peptides? If the mobile phase includes acetonitrile, water and little TFA, or short peptide of tripeptide, what column should I choose?

A: Polypeptides with low molecular weight can generally be determined by conventional C18 or C8 columns, as well as ion exchange columns and HILIC hydrophilic columns. If the mobile phase contains acid additives such as trifluoroacetic acid, it is recommended to use columns that resists low pH, such as Ultisil® LP series columns.

10

Q: When the amino column enters the acidic sample, it will damage the column. If the peak shape changes after using column for a period of time, what should I do to maintain the column?

A: If you use amino column to determine acidic sample, HILIC mode of amino column may be used. Acids may protonate slightly negatively charged amino functional groups, resulting in changes in the retention properties of some analytes or decreased column efficiency after using the column for a period of time. Suggestion: flush the column with 5~10 times column volume containing 0.5~1.0% NH3 acetonitrile / water (50/50) solution (after that, wash off excessive ammonia with alkali-free mobile phase). A little ammonia, such as 0.05%, is recommended to be added in the mobile phase when analyzing this kind of acidic analytes.

If you have any problem or require further information, please contact info@welchmat.com.

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