Injection of high-concentration samples with the Agilent 1100 Series purification system

Application

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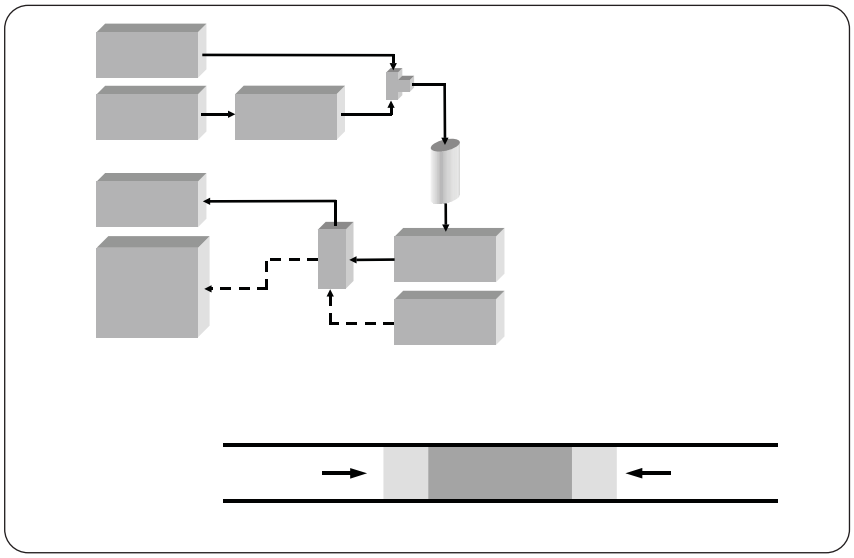

Abstract

To increase throughput in preparative HPLC it is necessary to load high amounts of sample onto the column. Since the compounds have to be applied onto the column in much higher concentration than in analytical HPLC it is necessary to use a modified injection principle to avoid precipitation and clogging of the flow path. In this Application Note we describe two common techniques – sandwich injection and organic-phase injection, and how they can be performed easily on an Agilent 1100 Series purification system.[1,2]

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Introduction

Preparative HPLC is nowadays widely used for the purification of compounds in drug discovery because it is the only technique fast enough to catch up with high-throughput and robotic synthesis. Since the purified compounds go to activity testing an amount of about 20–50 mg crude sample is typically loaded onto the column to get at least 5–10 mg pure material. Such sample amounts can usually be loaded onto one-inch columns operated at flow rates between 20 and 35 mL/min under reversed phase conditions with water/acetonitrile or water/methanol as mobile phases and a generic gradient starting at 5 or 10 % organic mobile phase. For optimal chromatography the sample should be dissolved in the starting composition of the gradient, which is very often not possible. Many compounds are not very soluble in highly aqueous mobile phase, which results either in low column loading or high injection volumes. In this Application Note we describe two approaches to increase the column loading – the sandwich injection and the organic-phase injection. Both techniques can be carried out on the Agilent 1100 Series purification system using software features of the autosampler or with minor hardware modifications.

Equipment

The experiments were performed on an Agilent 1100 Series purification system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS

The system was controlled using the Agilent ChemStation (rev. A.09.03) and the Purification/HighThroughput software (rev. A.01.02).

Results and Discussion

Since the compounds to be purified are very often not soluble in the mobile phase of the starting conditions of the gradient run they have to be dissolved in strong solvents such as DMSO or DMF. The problem is that as soon as the sample starts to mix with the mobile phase, precipitation occurs, which leads to blockage of the flow path. Very often the precipitation happens in the switching valve of the autosampler, which not only means the purification run has to be stopped but also that the valve has to be taken apart for cleaning. To avoid precipitation in the injector valve two techniques can be applied – the sandwich injection or the organic-phase injection.

Sandwich injection

For the sandwich injection the sample is injected between two plugs of the sample solvent. If, for example, the compound is dissolved in DMSO a plug of DMSO is placed before and after the sample (figure 1).

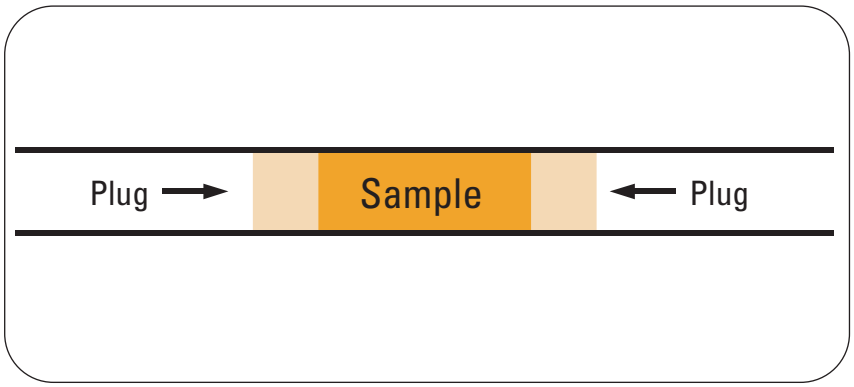

Figure 1
Sandwich injection

When the sandwich starts to mix with the mobile phase the mixing begins from the end of the plugs where the sample concentration is zero and therefore no precipitation can occur. With the Agilent ChemStation the sandwich injection can be done easily using an injector program (figure 2).
In the example shown in figure 2 a vial with pure solvent (DMSO) was placed into position 111 of the autosampler. From this vial 50 µL were drawn before and after the defined sample volume. The chromatographic results of sandwich injections with different plug sizes compared to a standard injection are shown in figure 3. The size of the plugs before and after the sample is critical. If the plugs are too small the mobile phase could come in contact with the sample, which lead to precipitation. If the plugs are too large the chromatographic performance can suffer.

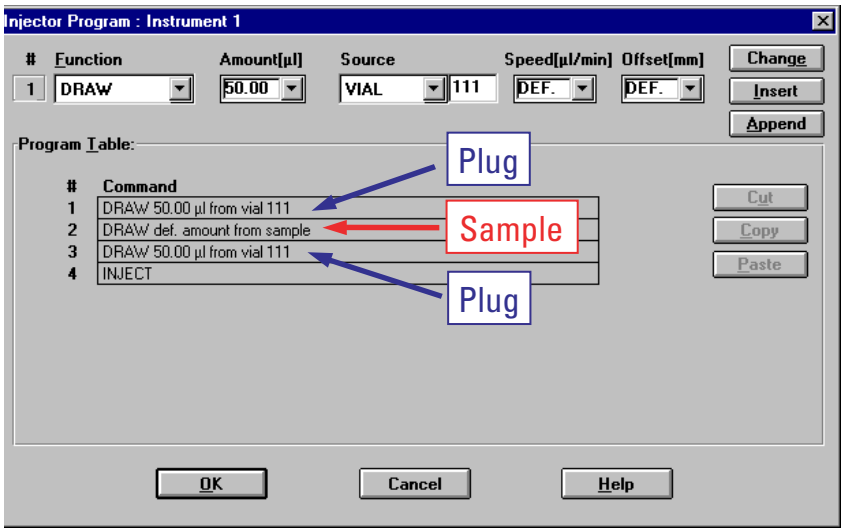

Figure 2
Injector program for sandwich injection

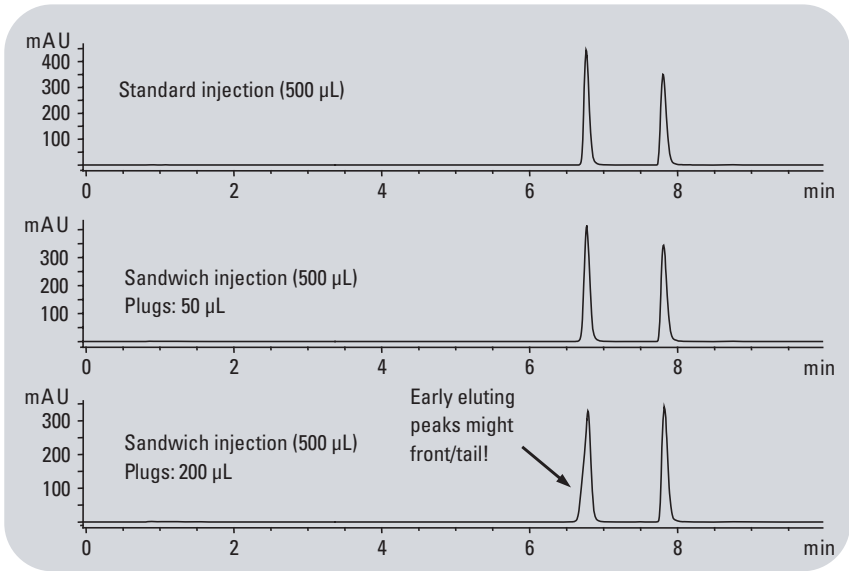

Figure 3
Sandwich injection with different plug sizes.

Advantages of sandwich injection

- The sample does not come in contact with the mobile phase until it reaches the column. Therefore no precipitation in the critical part of the flow path can occur.
- No hardware re-configuration required. Sandwich injection can be done on a standard system using an injector program

Disadvantages of sandwich injection

- The sample must be retained on the column in a rather narrow band. The strong sample and plug solvent leads to a significant band broadening of the sample on the column head.
- If the ratio of plug and sample volume compared to the column volume is too large some of the compound can elute with the solvent front as column breakthrough.
- The plug and sample solvent might disturb the equilibrium of the stationary phase.

Organic-phase injection

Organic-phase injection[3] requires re-plumbing of the preparative pumps and the injector. The idea is that the injector is connected into the flow path right after the pump delivering the organic solvent prior to the mixing point of the aqueous and organic mobile phase (figure 4). This means the sample has only contact to the organic phase until it reaches the mixing tee and the pre-column. Therefore precipitation can not occur in the critical part of the flow path, which is the switching valve of the autosampler. The mixing tee and the pre-column must be close together so that the sample has moved already to the head of the pre-column before precipitation occurs. Due to the low percentage of organic mobile phase at the beginning of the gradient only a low flow goes from the pump delivering the

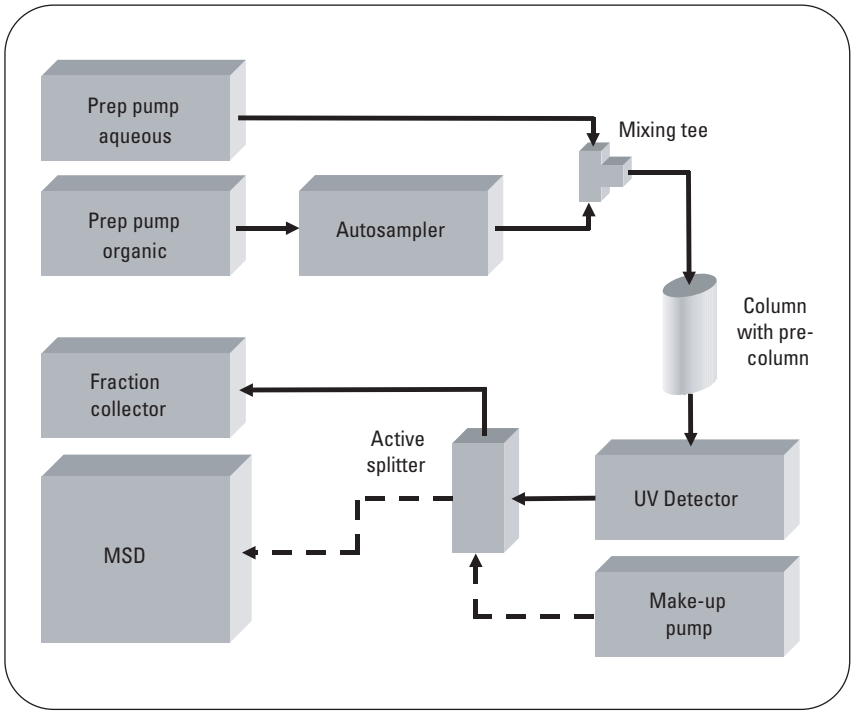

Figure 4
Organic phase injection

organic phase through the autosampler to the mixing tee. If, for example, the overall flow rate is 25 mL/min
and the starting condition of the gradient is 10 % organic mobile phase the flow through the autosampler is only 2.5 mL/min. That means the starting conditions of the gradient have to be kept long enough to make sure the sample reaches the head of the pre-column. Then the gradient can be started. The chromatographic result of a organic-phase injection compared to a standard injection is shown in figure 5. The comparison of the peak shapes for standard and organic-phase injection shows a peak broadening. This is due to the lower mobile phase mixing performance of the mixing tee in organic-phase injection compared to the static mixer in the standard configuration. The selection of the mixing tee regarding inner diameter etc. is critical to get a good mixing performance on one hand but no precipitation on the other hand.

Advantages of organic-phase injection

- No strong sample solvent is required, which could lead to additional peak broadening or column breakthrough.
- Since the Agilent 1100 Series preparative pump in the gradient version consists of two physical pumps the gradient programming is very easy. It can be set up in the same way as for the standard configuration, no flow gradient programming is necessary.

Disadvantages of organic-phase injection

- Although the autosampler valve is the critical part for sample precipitation in the flow path clogging can still occur in the mixing tee or on the head of the pre-column. While precipitation on the column head usually leads only to an increased pressure clogging in the mixing tee is critical.
- Setting up the purification system for organic-phase injection requires re-plumbing of the system. This means it is not possible to perform organic-phase and standard injection on the same system without hardware changes.

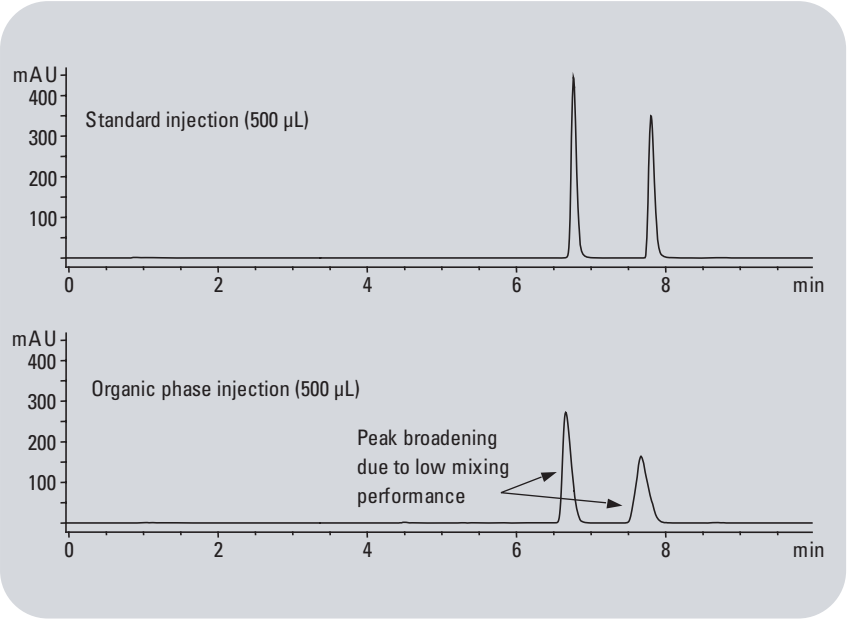

Figure 5
Organic phase injection

Conclusion

In this Application Note we showed two different ways to inject high-concentration samples onto an Agilent 1100 Series purification system. Using the sandwich injection the sample is placed between two plugs of pure solvent to avoid mixing of the sample with the mobile phase of the gradient starting conditions. This can be achieved easily by using an injector program in the Agilent ChemStation. When doing organic-phase injection the autosampler is connected into the flow path of the organic mobile phase prior to the mixing point with the aqueous mobile phase. This means mixing and precipitation does not occur in the autosampler valve but very close to the pre-column head where it does not cause problems. Which injection method is suitable depends completely on the purification application, both can be performed easily on the Agilent 1100 Series purification system.

References

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