Application News

Preparative Purification Liquid Chromatograph – Nexera[TM] Prep

High Throughput Preparative LC with Auto Switching Dilution Injection Function

Kosuke Nakajima[1,2], Devadiga Navin[3]
[1]Shimadzu Corporation, [2]Shimadzu (Asia Pacific) Pte Ltd., [3]Shimadzu Analytical (India) Pvt Ltd.

User Benefits

◆ Large volume injection enables to improve the throughput of preparative LC.
◆ Dilution injection shows a better peak shape with strong elution dissolution solvents.
◆ The auto switching setup makes the system which is suitable for typical injection as well as for large volume injection with strong elution dissolution solvents.

■ Introduction

Preparative LC is a widely used purification technique. High throughput purification is required in various industries. However, large volume injection which is required to achieve high throughput purification, causes peak broadening. This article introduces a large amount of injection and a high throughput preparative LC using dilution injection.

■ Importance of large volume injection to improve the throughput of purification

Loading amount is one of the critical parameters to determine the throughput of preparative LC. In most cases, the amount of crude sample to purify is larger than the loading capacity of conventional preparative LC. Therefore, multiple preparative operations are necessary to purify target quantity of sample. Increasing the loading amount leads to a less number of repetitions of the process and improves the purification throughput. The combination between highly concentrated sample and injection volume determines the loading amount of each preparative process. (Table 1) Especially, large volume injection is required because making high concentrate sample causes precipitation in the preparative LC.
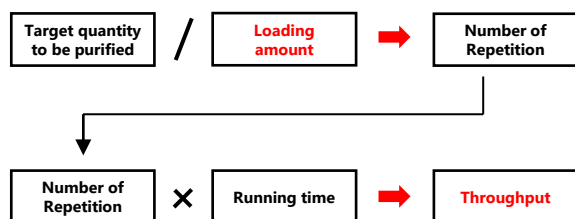

Fig. 1 The relationship between loading amount and throughput

Table 1 Loading amount in the sample

Sample Concentration	Loading amount	
	500 µL injection	2000 µL injection
1 mg/mL	0.5 mg	2 mg
5 mg/mL	2.5 mg	10 mg
20 mg/mL	10 mg	40 mg

■ Limitations of large volume injection

Large volume injection enables to improve the purification throughput. However, large volume injection affects peak shapes due to the lack of mixing between dissolution solvent and mobile phase. Especially, large volume injection causes peak broadening when the dissolution solvent has a higher eluotropic strength than the starting mobile phase of preparative LC (Fig. 2). Table 2 describes the analysis condition for typical injection. The effect of large volume injection with high elution strength solvent on the peak shape can be seen in Fig. 3. The peak shape has become worse in 2000 µL injection due to dissolution solvent.
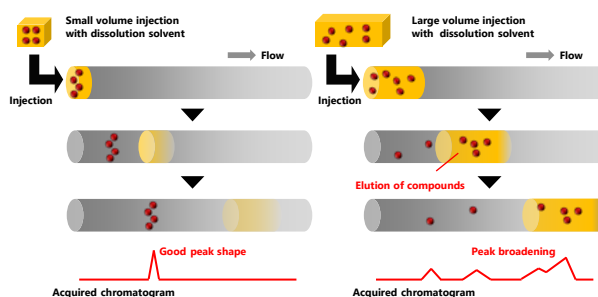

Fig. 2 Effect of injection volume on the peak shape

Table 2 Analysis conditions for typical injection

Column	: Shim-pack[TM] GISS C-18 [*1] (100 mm × 20 mm I.D., 5 µm)
Mobile phase	: A: Water B: Acetonitrile
Flow rate	: 20 mL/min
Time program	: B conc. 5 % (0 min)→ 90 % (4.5-6.5 min) →5 % (6.51-8 min)
Column temp.	: Ambient
Sample Solvent	: Dimethyl sulfoxide (DMSO)
Analytes	: 5 mg/mL caffeine, 50 mg/mL sulfamethazine
Vial	: 10 mL screw vial [*2]
Detection	: PDA 250 nm

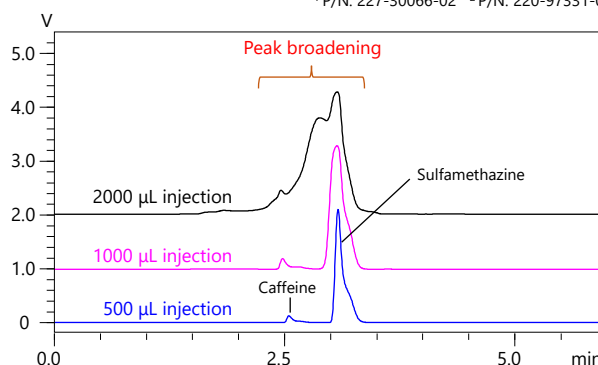[*1] P/N: 227-30066-02 [*2] P/N: 220-97331-09

Fig. 3 Chromatograms of two compounds with different injection volume

■ Dilution injection for large volume injection

Dilution injection is a technique to improve peak shape for large volume injection. Dissolution solvent is diluted by diluent with a lower eluotropic strength than starting mobile phase, and the target compounds are concentrated at the tip of the columns. Fig. 4 shows the improvement of peak shape by dilution injection. Fig. 5 shows the flow diagrams of the switching system between typical injection and dilution injection. Fig. 5a shows the flow path for typical injection whereas Fig. 5b shows the flow path for dilution injection.
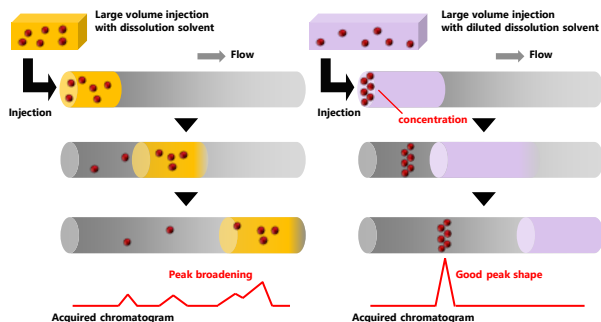
Large volume injection with dissolution solvent

Flow

Injection

Large volume injection with diluted dissolution solvent

Flow

Injection

concentration

Peak broadening

Acquired chromatogram

Good peak shape

Acquired chromatogram

Fig. 4 Improvement of peak shape by dilution injection

Mixer (for dilution)

Pump A (Mobile Phase A)

Valve (position A)

Column

Pump B (Mobile Phase B)

Mixer (for gradient)

Auto sampler

Mobile Phase A

Mobile Phase B

Dissolution Solvent
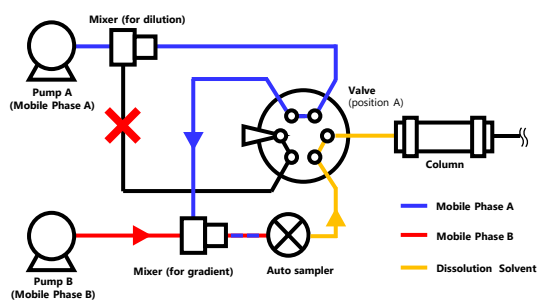
Fig. 5a Flow path diagram for typical injection

Mixer (for dilution)

Pump A (Mobile Phase A)

Valve (position B)

Column

Pump B (Mobile Phase B)

Mixer (for gradient)

Auto sampler

Mobile Phase A

Mobile Phase B
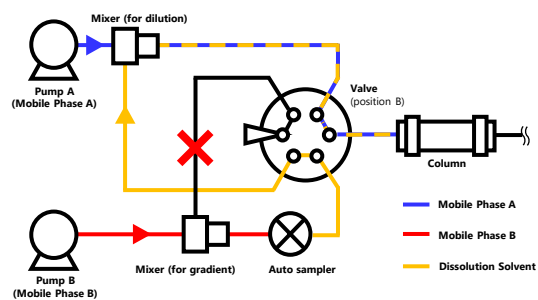
Dissolution Solvent

Fig. 5b Flow path diagram for dilution injection

■ Large volume injection of caffeine and sulfamethazine with dilution injection

In this study, we used caffeine and sulfamethazine as target compounds with dilution injection. Table 3 shows the analysis condition for dilution injection. The acquired chromatograms are as shown in Fig. 6. Typical injection showed peak broadening, however, dilution injection leads good peak shapes and separation between the two analytes.

Table 3 Analysis conditions for dilution injection

Column	: Shim-pack GISS C-18 (100 mm × 20 mmI.D., 5 μm)
Mobile phase	: A: Water, B: Acetonitrile
Flow rate	: 20 mL/min
Dilution	: B conc. 5 % (0-2.2 min, diluent: water)
Time program	: B conc. 5 % (0 min)→ 50 % (4.5 min) →5 % (4.51-6 min)
Column temp.	: Ambient
Sample Solvent	: Dimethyl sulfoxide (DMSO)
Analytes	: 5 mg/mL caffeine, 50 mg/mL sulfamethazine
Vial	: 10 mL screw vial
Detection	: PDA 250 nm

Typical injection (2000 μL)

Sulfamethazine
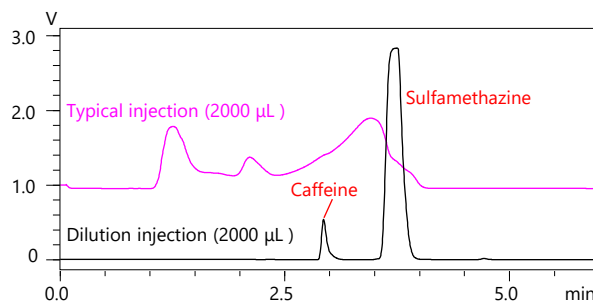
Caffeine

Dilution injection (2000 μL)

Fig. 6 Comparison between the typical injection and dilution injection

■ Consecutive dilution injection

In the dilution process, another capability of the system is to achieve large loading amount by consecutive injections as described in Fig. 7. Consecutive injections for mixture of caffeine and sulfamethazine was performed with the same analysis condition as shown in Table 3. In this process, more than 500 mg of target compounds were introduced into the separation column by five times consecutive injections. The acquired results showed achievement of higher loading amount with good separation and peak shape, as shown in Fig. 8.
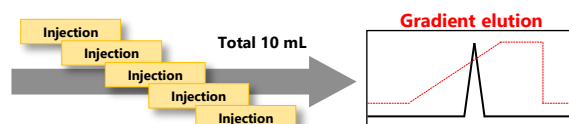
Injection

Injection

Injection

Injection

Injection

Total 10 mL

Gradient elution

Fig. 7. Schematic representation of consecutive dilution injection

Consecutive injection (2000 μL, 5 times)
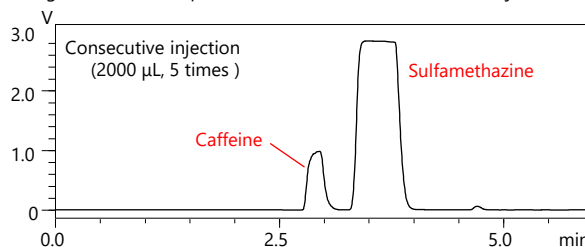
Sulfamethazine

Caffeine

Fig. 8 Chromatogram of consecutive dilution injection

■ Conclusion

This article is an example of the large volume injection. In most cases, a large volume injection affects the peak shape and separation. Shimadzu Nexera prep system with switching system for both typical injection and dilution injection enables better chromatographic performance for samples with large loading amount.

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