

# How to Use Preparative HPLC -Part 3

## About column temperature control in preparative

Since preparative HPLC uses a large amount of solvent, it is important to keep the temperature of the mobile phase (eluent) uniform. Analytical columns are generally temperature controlled, but preparative columns are sometimes kept at room temperature without temperature control.

Even with a preparative column, a constant retention time leads to accurate preparative separation, so it can be said that temperature control of the preparative column is particularly beneficial when performing repeated preparative separations.

This time, following technical notes No. 133 and No. 134, we will introduce the following points for column temperature control as "How to use preparative separation well, Part 3".

(K. Kanno)

**Point 1 Keep the temperature of the mobile phase and column as uniform as possible**

**Point 2 Recommendation for using a preheat mixer**

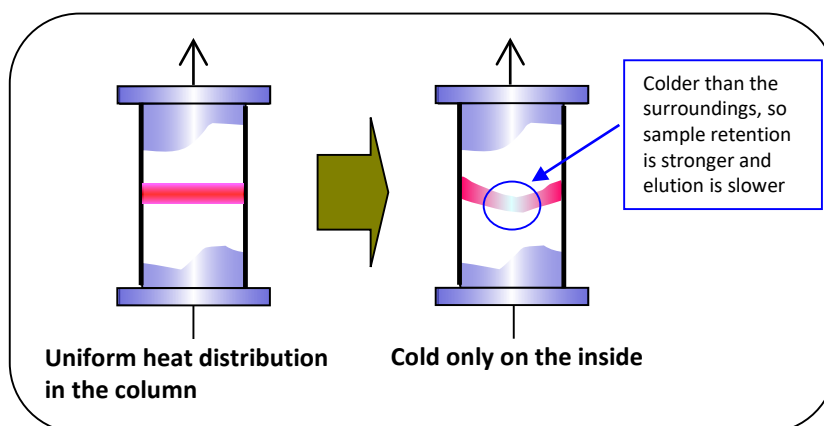
**Point 3 Reducing the impact of environmental temperature fluctuations**

**Point 4 Effect of increasing the processing amount of heating**

**Point 5 Isomer separation effect of cooling**

### Point 1. Keep the temperature of the mobile phase and column as uniform as possible

By controlling the temperature of the column during preparative separation, fluctuations in retention time due to changes in room temperature can be suppressed, leading to stable preparative purification. When using an analytical column with an inner diameter of 4.6 mm, it may be less important to pay attention to the temperature of the mobile phase. On the other hand, in the case of preparative columns, since the inner diameter of the column is large, it is not possible to heat the center of the column. This results in shoulder peaks, or worse, splitting into multiple peaks. Therefore, in preparative HPLC, it is important to keep the mobile phase temperature as uniform as possible at the column temperature.

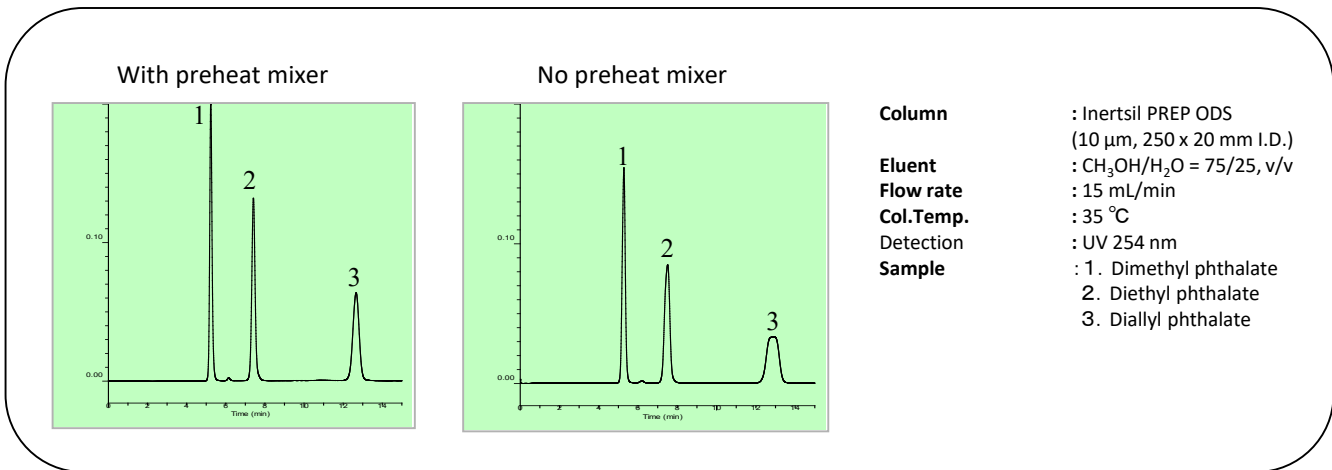
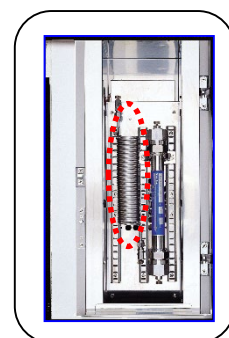


Difference in temperature distribution in the column

**Point 2. Recommendation for using a preheat mixer**

It is effective to use a preheat mixer to bring the mobile phase temperature to the same level as the column temperature. The preheat mixer has a mixing effect during the gradient and a temperature control effect that makes the mobile phase temperature uniform with the column temperature.

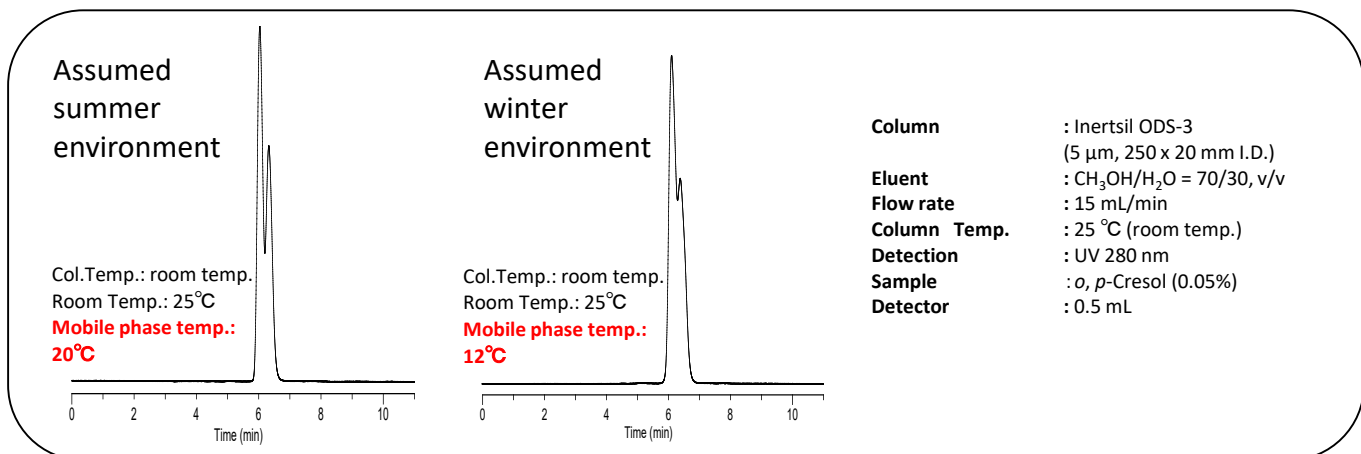
The figure below shows a comparison of chromatograms with and without a preheat mixer. You can see that the peaks with the preheat mixer are sharper than without the preheat mixer.



Effect of difference in temperature distribution in column

**Point 3. Reducing the impact of environmental temperature fluctuations**

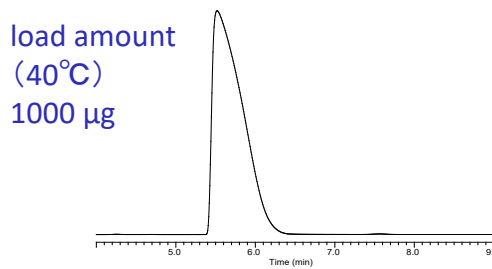
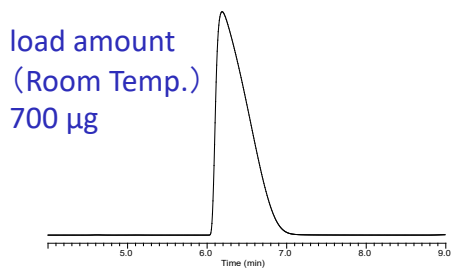
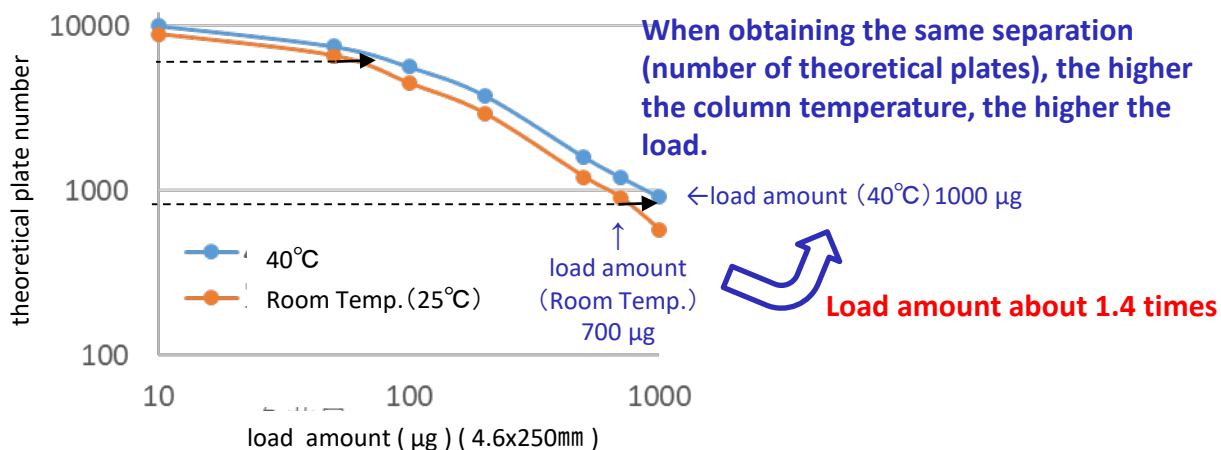
As the difference between column temperature and mobile phase temperature increases, the peak shape tends to deteriorate. Temperatures in the lab are controlled and may not vary much throughout the year. However, the temperature of the solvent varies greatly depending on the storage location. If the solvent is stored outdoors, the difference between the room temperature and the solvent temperature tends to be large, especially in winter. Therefore, it is recommended to control the temperature by preheating even when fractionating at room temperature.



**Point 4. Effect of increasing the processing amount of heating**

As the load on the column increases during preparative separation, the number of theoretical plates decreases and the separation gradually deteriorates. Below are the results of comparing the number of theoretical plates at room temperature (25° C) and heated (40° C) conditions using an analytical size column (4.6 mm inner diameter x 250 mm length) when sample is loaded.

When obtaining separation (theoretical plate number) equivalent to room temperature conditions under heating conditions, the load under heating conditions is higher, and heating the column has the effect of increasing the maximum throughput.



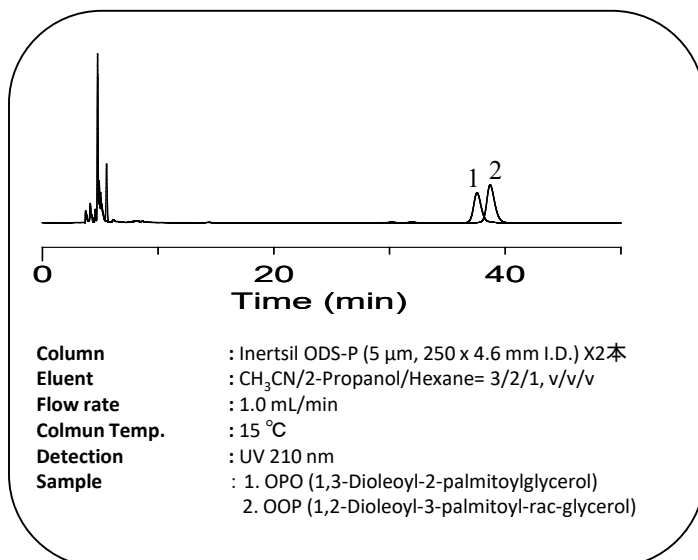
**Column** : Inertsil ODS-HL (5 µm, 250 x 4.6 mm I.D.)  
**Eluent** : CH<sub>3</sub>OH/H<sub>2</sub>O = 40/60, v/v  
**Flow rate** : 1.0 mL/min  
**Column Temp.** : 40 °C  
**Detection** : UV 300 nm  
**Sample** : Caffeine (10 mg/mL) Dilute with water, load onto the column at the injection volume

**Point 5. Isomer separation effect of cooling**

In the column separation of isomers by HPLC, the separation is better at low temperature, so the temperature may be controlled by a column oven (cool function). In this case as well, it is ideal to control the temperature as shown in Point 2.

The following points should be noted when using at low temperatures.

- Lower load (see point 4)
- Column pressure rise
- Increased retention time



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