

Adjustable range of chromatography conditions has been specified in [2.00 General theory of chromatography] which has been stipulated based on the contents harmonized and agreed upon by Japanese/U.S./European Trilateral Pharmacopoeia at the first supplemental public comment of the Japanese Pharmacopoeia, 18th Edition, which was disclosed in September 2021. Based on this, the acceptable changeable range of conditions is widened, enabling easier requirement study.

In this report, an analysis was implemented by changing the column size within the range specified in [2.00 General theory of chromatography] for JP18 Purity Test (2) of Macroglol 400. The change of analytical conditions is for the content to be applied from the first supplemental revision of JP18 and is not for the content announced in the past. Accordingly, this Technical Note is simply just for reference.

## Changeable Items in JP

In [2.00 General theory of chromatography], the change of LC column and instrumental conditions is allowed by satisfying the requirements of system suitability.

Items in Pharmacopoeia		Japanese Pharmacopoeia <Public comment proposal in September 2021> International harmonization by Trilateral Pharmacopoeia
Stationary phase	Particle size	Can be reduced down to a maximum of 50%, but cannot be increased (packed column)
	Film thickness	-50% ~ +100%(Capillary column)
Column size	<b>Length</b>	<b>-70% ~ +100%</b>
	Inner diameter	±50%
Instrument	Column temperature	±10%
	Temperature program	Adjustment of temperature is acceptable as described above. Adjustment of temperature increase rate and retention time for each temperature is tolerable within ±20%.
Carrier gas	Flow rate	±50%
	Injection volume and Split ratio	If the requirement of system suitability is within the established tolerable range, injection volume and split ratio can be changed. For reducing the injection volume or increasing the split ratio, it is necessary to pay particular attention to detection (detection limit) of peak response and reproducibility. Increase of injection volume or decrease of split ratio is allowed only if linearity and resolution of measurable peak satisfy the requirement of system suitability.
	Injection port temperature and conditions for transfer line temperature at static head space	±10°C unless decomposition or concentration occurs

## JP18 Purity Test (2) of Macrolog 400

Referring to JP18 Purity Test (2) of Macrolog 400 as an analysis example, analysis was implemented by changing the column size within the changeable range specified in JP [2.00 General theory of chromatography]

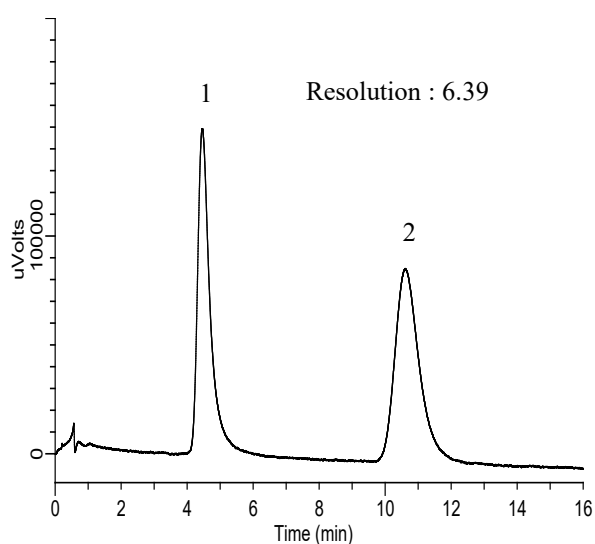
## 【Purity Test (2)】

## &lt;Operation conditions&gt;

- Detector: Hydrogen flame ionization detector  
 Column: Diatomaceous earth for gas chromatography of 150 – 180  $\mu\text{m}$  coated with D-sorbitol for gas chromatography at a ratio of 12% is packed in the tube of approximately 3 mm in inner diameter, and approximately 1.5 m in length.  
 Column temperature: Constant temperature in the vicinity of 165°C  
 Carrier gas: Nitrogen or helium  
 Flow rate: Adjust so that the retention time of Diethylene glycol may become approximately 8 min.  
 Column selection: Use the column by which ethylene glycol and diethylene glycol are eluted in order and also each peak can be completely separated when operated in the above conditions for each 2  $\mu\text{L}$  of standard solution.

Column size described in test method is approximately 1.5 m in length and changeable range is -70% - +100%, corresponding to  $0.45 \text{ m} \leq 1.5 \text{ m} \leq 3.0 \text{ m}$ . In this analysis, the test is carried out by changing the column length to 0.5 m.

## &lt;Before Condition Changed&gt;



1. Ethylene glycol
  2. Diethylene glycol
- (Standard solution)

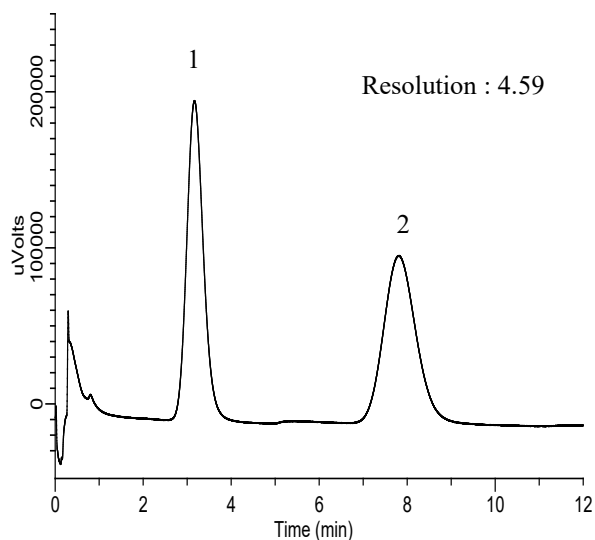
Conditions

- Column : Sorbitol 12% Uniport HP 80/100  
 Glass  $\frac{1}{4}$  " O.D. X 1.5 m X 3.0 mm I.D.  
 Col.Temp. : 165 °C  
 Carrier Gas :  $\text{N}_2$   
 Detector : FID 200°C  
 Injection : 170°C  
 Sample Size : 2  $\mu\text{L}$

**Column pressure : 200 kPa**

**Retention time of diethylene glycol : 10.6 min**

Before changing the conditions, even under column pressure of 200 kPa, the retention time of diethylene glycol was 10 min, exceeding the stipulation of test method, but resolution was within the specifications.

**<After Condition Changed>**

1. Ethylene glycol
  2. Diethylene glycol
- (Standard solution)

Conditions

Column : Sorbitol 12% Uniport HP 80/100  
Glass 1/4" O.D. X **0.5 m** X 3.0 mm I.D.

Col.Temp. : 165 °C  
Carrier Gas : N<sub>2</sub>  
Detector : FID 200°C  
Injection : 170°C  
Sample Size : 2 µL

**Column pressure: 40 kPa**

**Retention time of diethylene glycol: 7.81 min**

After changing the conditions, under column pressure of 40 kPa, the resulting retention time of diethylene glycol, was approximately 8 min and the resolution achieved the stipulation. It can be said that an analysis is capable without applying pressure load to the column.

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