

# 3. Technical Information

## (1) Preparation of Mobile Phase for HPLC

### 1) Organic Solvent / Aqueous Mixed Mobile Phase

#### (Example) Methanol : Water = 70 : 30 1L

Prepare mobile phase by volume ratio.

1. Measure 700 ml of methanol in a measuring cylinder.
2. Measure 300 ml of distilled water in a measuring cylinder.
3. Mix 1 and 2 thoroughly and degas.

Note: The better approach is to measure by mass rather than volumetrically. This is more precise in general and reduces the effect of temperature on measurement.

Composition table for 1 L of mobile phase (methanol / water)

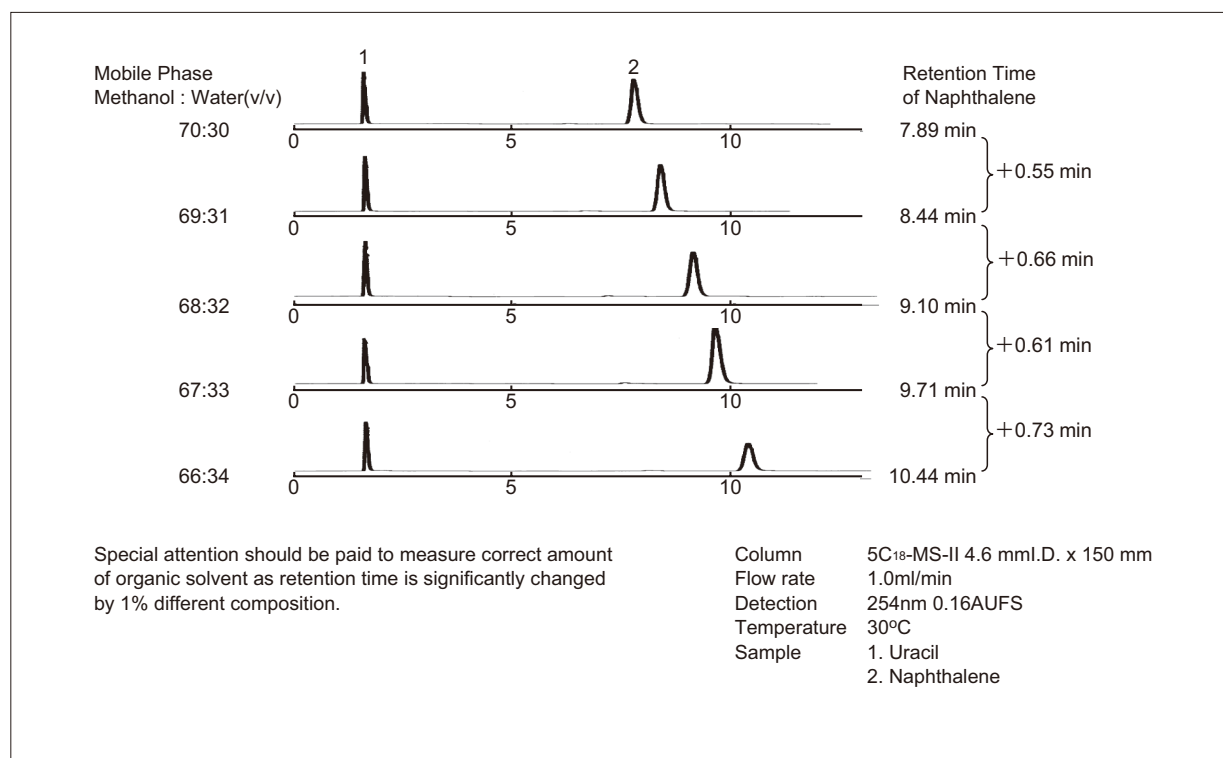
Methanol / Water	Methanol (g)	Distilled Water (g)
90 : 10 (v/v)	711.9	99.8
80 : 20 (v/v)	632.8	199.6
70 : 30 (v/v)	553.7	299.5
60 : 40 (v/v)	474.6	399.3
50 : 50 (v/v)	395.5	499.1
40 : 60 (v/v)	316.4	598.9
30 : 70 (v/v)	237.3	698.7
20 : 80 (v/v)	158.2	798.6
10 : 90 (v/v)	79.1	898.4

Composition table for 1 L of mobile phase (acetonitrile / water)

Acetonitrile / Water	Acetonitrile (g)	Distilled Water (g)
90 : 10 (v/v)	707.4	99.8
80 : 20 (v/v)	628.8	199.6
70 : 30 (v/v)	550.2	299.5
60 : 40 (v/v)	471.6	399.3
50 : 50 (v/v)	393.0	499.1
40 : 60 (v/v)	314.4	598.9
30 : 70 (v/v)	235.8	698.7
20 : 80 (v/v)	157.2	798.6
10 : 90 (v/v)	78.6	898.4

Caution: Methanol and acetonitrile are hazardous substances. Always process in a laboratory hood, and wear eye protection and a mask.

Influence of mobile phase composition on retention time.



## 2) Organic Solvent / Buffer Mixed Mobile Phase

### (Example 1) Preparation of 20 mmol/l phosphate buffer (pH 2.5)

1. Prepare 20 mmol/l sodium dihydrogenphosphate solution: Dissolve 2.40 g of anhydrous sodium dihydrogenphosphate (Product No. 31720-65) in distilled water to make a 1 L solution.
2. Prepare 20 mmol/l phosphoric acid solution: Dissolve 2.31 g of 85% phosphoric acid (Product No. 08964-92) in distilled water to make a 1 L solution.
3. Adjust the pH to 2.5 by mixing the two solutions.
4. Filter to remove insoluble substances (0.45  $\mu\text{m}$  or smaller pore size is recommended). Unfiltered solutions may clog pump and columns.
5. When mixing with organic solvent, mix by volume ratio.  
Solids may precipitate after mixing. Please ensure that the buffer is soluble in the final mobile phase.

### (Example 2) Preparation of 20 mmol/l phosphate buffer (pH 7.0)

1. Prepare 20 mmol/l sodium dihydrogenphosphate solution: Dissolve 2.40 g of anhydrous sodium dihydrogenphosphate (Product No. 31720-65) in distilled water to make 1L solution.)
2. Prepare 20 mmol/l disodium hydrogenphosphate solution: Dissolve 2.84 g of disodium hydrogenphosphate, (Product No. 31801-05) in distilled water to make a 1 L solution.
3. Adjust the pH to 7 by mixing the two solutions.
4. Filter to remove insoluble substances (0.45  $\mu\text{m}$  or smaller pore size is recommended). Unfiltered solutions may clog pump and columns.
5. When mixing with organic solvent, mix by volume ratio.  
Solids may precipitate after mixing. Please ensure that the buffer is soluble in the final mobile phase.

### (Example 3) Preparation of 5 mmol/l Sodium 1-hexanesulfonate, 20 mmol/l phosphate buffer (pH 2.5)

1. Prepare 5 mmol/l sodium 1-hexanesulfonate, 20 mmol/l sodium dihydrogenphosphate solution: Dissolve 10 ml of 0.5 M sodium 1-hexanesulfonate (Product No. 31532-06) and 2.40 g of anhydrous sodium dihydrogenphosphate (Product No. 31720-65) in distilled water to make a 1 L solution.
2. Prepare 5 mmol/l sodium 1-hexanesulfonate, 20 mmol/l phosphoric acid solution: Dissolve 10 ml of 0.5 M sodium 1-hexanesulfonate (Product No. 31532-06) and 2.31g of 85% phosphoric acid (Product No. 08964-92) in distilled water to make a 1 L solution.
3. Adjust the pH to 2.5 by mixing the two solutions.
4. Filter to remove insoluble substances (0.45  $\mu\text{m}$  or smaller pore size is recommended).  
Unfiltered solutions may clog pump and columns.
5. When mixing with organic solvent, mix by volume ratio.  
Solids may precipitate after mixing. Please ensure that the buffer is soluble in the final mobile phase.