

1. Preparation of Mobile Phase for HPLC

1) Organic Solvent / Aqueous Mixed Mobile Phase

(e.g.) 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.

Attention; The better approach is to prepare the mobile phase gravimetrically rather than volumetrically. Following is example of preparation.

Composition table for mobile phase 1L (Methanol : water)

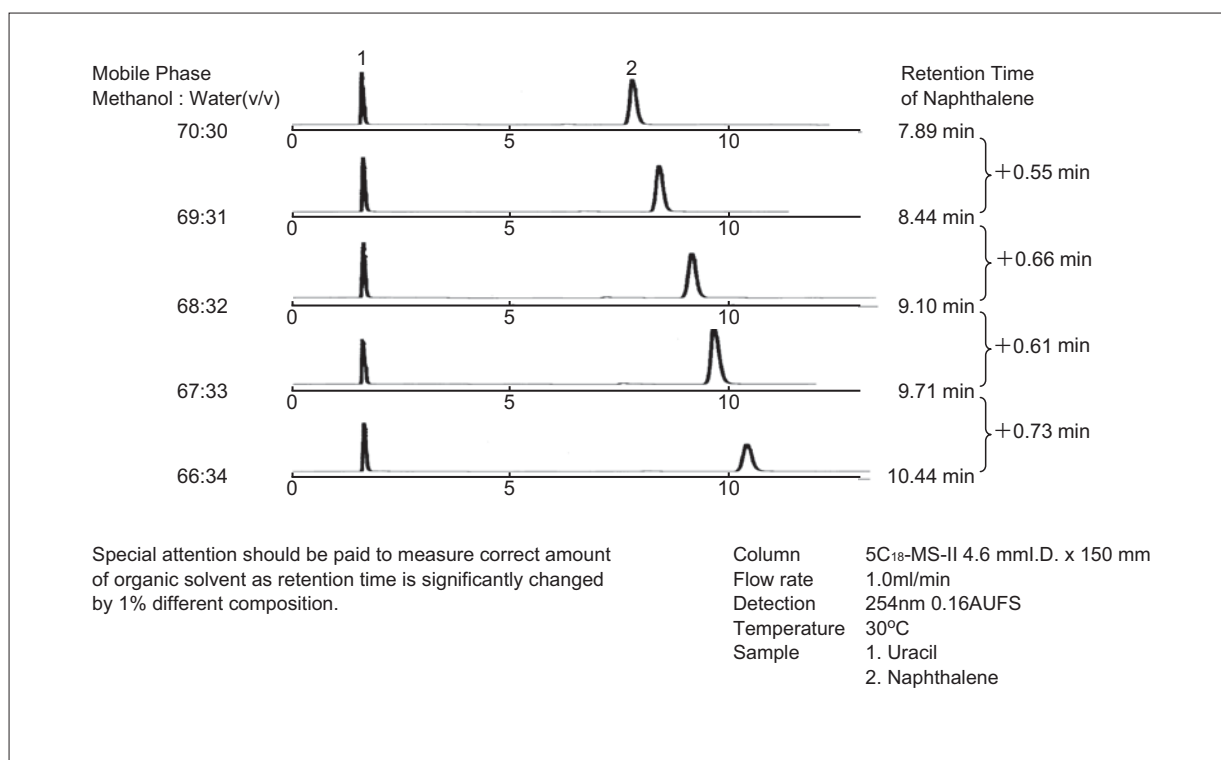
Methanol / Water	Methanol (g)	Distilled Water (g)
90 : 10 (v/v)	711.9	99.8
80 : 20 (v/v)	632.8	199.6
70 : 30 (v/v)	553.7	299.5
60 : 40 (v/v)	474.6	399.3
50 : 50 (v/v)	395.5	499.1
40 : 60 (v/v)	316.4	598.9
30 : 70 (v/v)	237.3	698.7
20 : 80 (v/v)	158.2	798.6
10 : 90 (v/v)	79.1	898.4

Composition table for mobile phase 1L (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, do not use for medical purpose. Always process in a laboratory hood and wear an eye protection and a mask.

(Reference) Influence of organic solvent composition in mobile phase on the retention time.



2) Organic Solvent / Buffer Mixed Mobile Phase

(e.g.1) Preparation of 20 mmol/l phosphate buffer (pH2.5)

1. Preparation of 20 mmol/l sodium dihydrogenphosphate aqueous solution (Dissolve 2.40g of sodium dihydrogenphosphate, Anhydrous (Product No. 31720-65) in distilled water to make 1L solution.)
2. Prepare 20 mmol/l phosphate aqueous solution (Dissolve 2.31g of Phosphoric acid (Purity: 85%), (Product No. 08964-92) in distilled water to make 1L solution.)
3. Adjust the pH to 2.5 by mixing 1 with 2.
4. Filter under reduced pressure to remove insoluble substance (0.45 µm or smaller pore size is recommended.). Attention; Filter solids from the solution to prevent clogging to pump and columns.
5. When mix with organic solvent, mix by volume ratio. Attention; The solid may precipitate after mixing.

For more information on adjusted solution, Phosphate Buffer Solution (pH 2.5) (5x) (Product No, 08969-71), Please refer to page 77.

(e.g.2) Preparation of 20 mmol/l phosphate buffer (pH7.0)

1. Preparation of 20 mmol/l sodium dihydrogenphosphate aqueous solution (Dissolve 2.40 g of sodium dihydrogenphosphate, Anhydrous (Product No. 31720-65) in distilled water to make 1L solution.)
2. Prepare 20 mmol/l di-sodium hydrogenphosphate aqueous solution (Dissolve 2.84 g of di-Sodium Hydrogenphosphate, (Product No. 31801-05) in distilled water to make 1L solution.)
3. Adjust the pH to 7 by mixing 1 with 2.
4. Filter under reduced pressure to remove insoluble substance (0.45 µm or smaller pore size is recommended.). Attention; Filter solids from the solution to prevent clogging to pump and columns.
5. When mix with organic solvent, mix by volume ratio. Attention; The solid may precipitate after mixing.

For more information on adjusted solution, Phosphate Buffer Solution (pH 7.0) (5x) (Product No, 08968-81), Please refer to page 77.

(e.g.3) Preparation of 5 mmol/l Sodium 1-hexanesulfonate, 20 mmol/l phosphate buffer (pH2.5)

1. Prepare 5 mmol/l Sodium 1-hexanesulfonate, 20 mmol/l phosphate buffer (pH2.5) aqueous solution (Dissolve 10 ml of Sodium 1-hexanesulfonate (0.5 M solution) (Product No. 31532-06) and 2.40 g of sodium dihydrogenphosphate, Anhydrous (Product No. 31720-65) in distilled water to make 1L solution.)
2. Prepare 5 mmol/l Sodium 1-hexanesulfonate, 20 mmol/l phosphate aqueous solution (Dissolve 10 ml of sodium 1-hexanesulfonate (0.5 M solution) (Product No. 31532-06) 2.31g of phosphoric acid (Purity: 85%), (Product No. 08964-92) in distilled water to make 1L solution.)
3. Adjust the pH to 2.5 by mixing 1 with 2.
4. Filter under reduced pressure to remove insoluble substance (0.45 µm or smaller pore size is recommended.). Attention; Filter solids from the solution to prevent clogging to pump and columns.
5. When mix with organic solvent, mix by volume ratio. Attention; The solid may precipitate after mixing.