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)

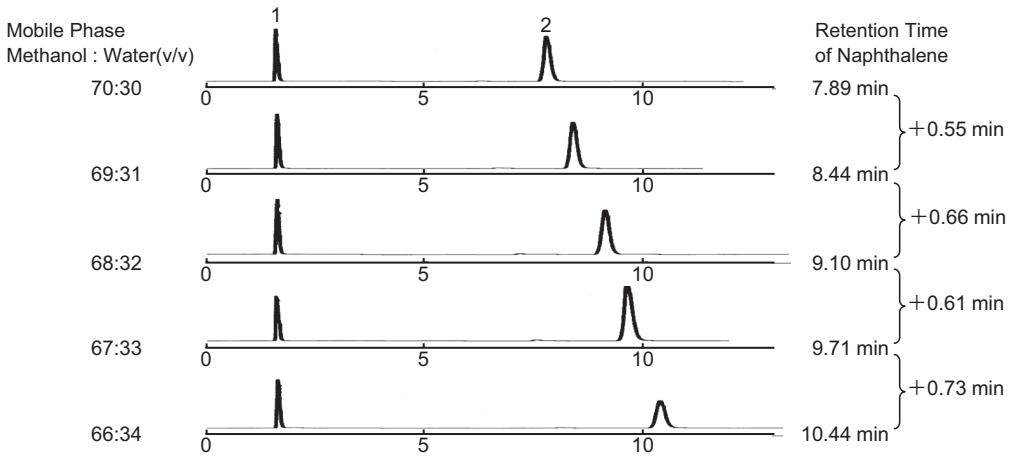
Methanol / Water	Methanol (g)	Distilled Water (g)
90 : 10 (v/v)	711.9	99.8
80 : 20 (v/v)	632.8	199.6
70 : 30 (v/v)	553.7	299.5
60 : 40 (v/v)	474.6	399.3
50 : 50 (v/v)	395.5	499.1
40 : 60 (v/v)	316.4	598.9
30 : 70 (v/v)	237.3	698.7
20 : 80 (v/v)	158.2	798.6
10 : 90 (v/v)	79.1	898.4

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.



Special attention should be paid to measure correct amount of organic solvent as retention time is significantly changed by 1% different composition.

Column	5C$_{18}$-MS-II 4.6 mmI.D. x 150 mm
Flow rate	1.0ml/min
Detection	254nm 0.16AUFS
Temperature	30°C
Sample	1. Uracil
	2. Naphthalene

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.