

## (4) Sample Pretreatment for HPLC

Pretreatment before HPLC analysis is often required for samples of low concentration or samples containing analytical contaminants. It improves reproducibility and sensitivity in analysis, and protects HPLC columns. The pretreatment methods are different for each sample. The following are examples of different methods.

### 1) Filtration

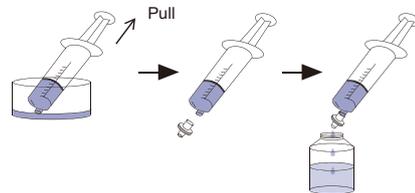
Filtration is a common method used for separating solids from liquids. It extends a column's life by minimizing damage from solid contaminants such as particles, sediments, and colloids. It also improves reproducibility of analytical data. We offer both syringe-type and spin-type filters for sample filtration.

	Syringe Filter	Centrifugal Filter
Product	Cosmonice Filter	Cosmospin Filter
Configuration		
Usage	Easy to use Just attach a filter on top of a syringe	Easy to use by centrifugation
Type	W (for aqueous solutions) S (for organic solvents)	Pore diameter: 0.2 $\mu\text{m}$ Pore diameter: 0.45 $\mu\text{m}$
Required Equipment	Syringe, Sample Bottle	Centrifuge

#### Cosmonice Filter

How to use :

1. Fill a syringe with the sample you want to filter.
2. Attach a Cosmonice filter to the syringe.
3. Push the syringe plunger to filter the sample.
4. Analyze the filtered sample by HPLC.

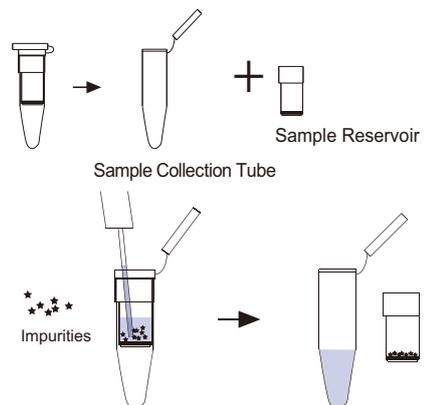


#### Cosmospin Filter

Components : Sample Reservoir  
Sample Collection Tube

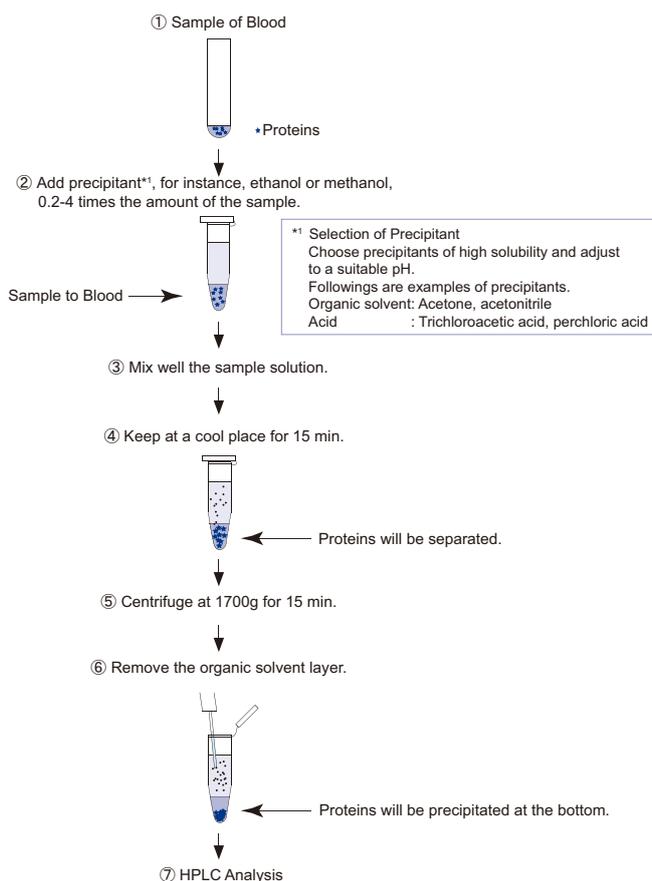
How to use :

1. Insert the sample reservoir into the sample collection tube.
2. Add a sample to the sample reservoir.
3. Close the sample collection tube cap and centrifuge.
4. Remove the sample reservoir, and collect the filtered sample in the sample collection tube.
5. Analyze the filtered sample by HPLC.



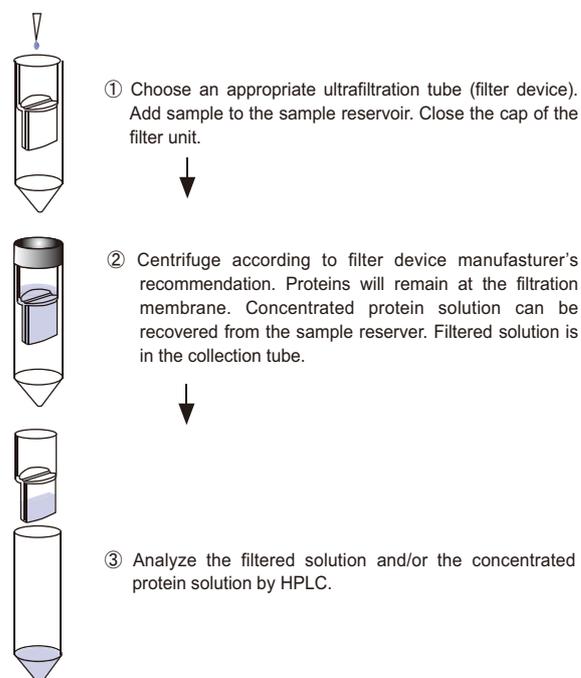
## 2) Protein Precipitation

Protein precipitation is commonly used to remove proteins in samples for downstream analysis. For example, when analyzing drug concentration in blood samples, proteins have to be removed first. Otherwise, proteins may be adsorbed in columns and interfere with the analysis. Common methods of protein precipitation include salting out, isoelectric precipitation, and precipitation with organic solvents. A general procedure for protein precipitation with organic solvents is shown on the right.



## 3) Ultrafiltration

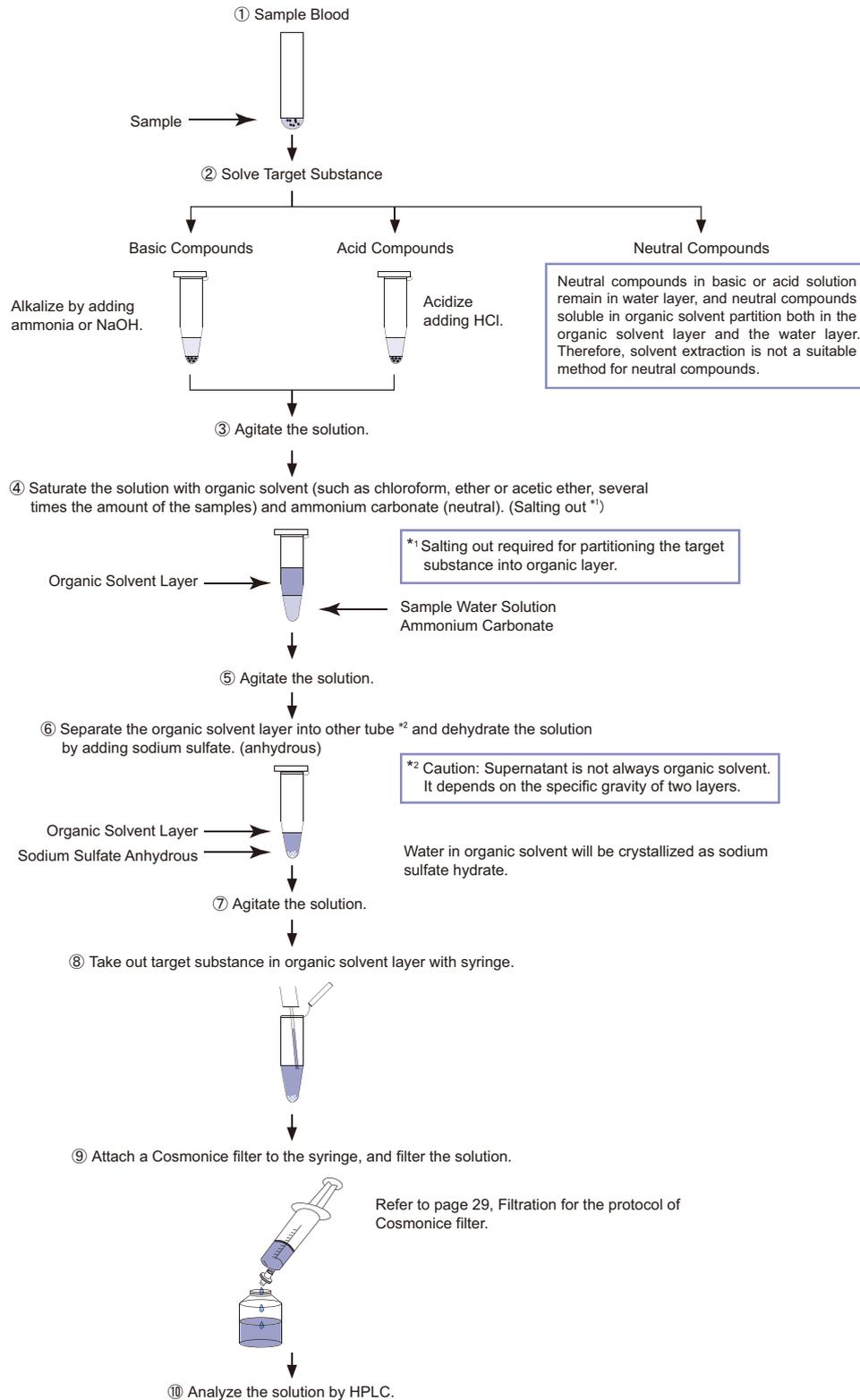
Ultrafiltration is a method to concentrate proteins or other macromolecules through a semipermeable membrane with defined pores. Ultrafiltration is used for sample desalting, concentrating proteins from a dilute solution (such as urine samples), or removing proteins from samples with high protein concentration (such as blood serum or plasma). A general procedure for ultrafiltration is shown at the right.



## 4) Solvent Extraction Method

Solvent extraction is a method to separate compounds using their unequal solubility in two immiscible liquid phases, usually water and an organic solvent. This method is used to concentrate highly hydrophobic compounds, and thus increase analytical sensitivity. A buffer solution is added to the sample to optimize the pH, and the target substance is then extracted by an organic solvent such as ether or chloroform. However, when target substance is bound to protein, solvent extraction may not be effective.

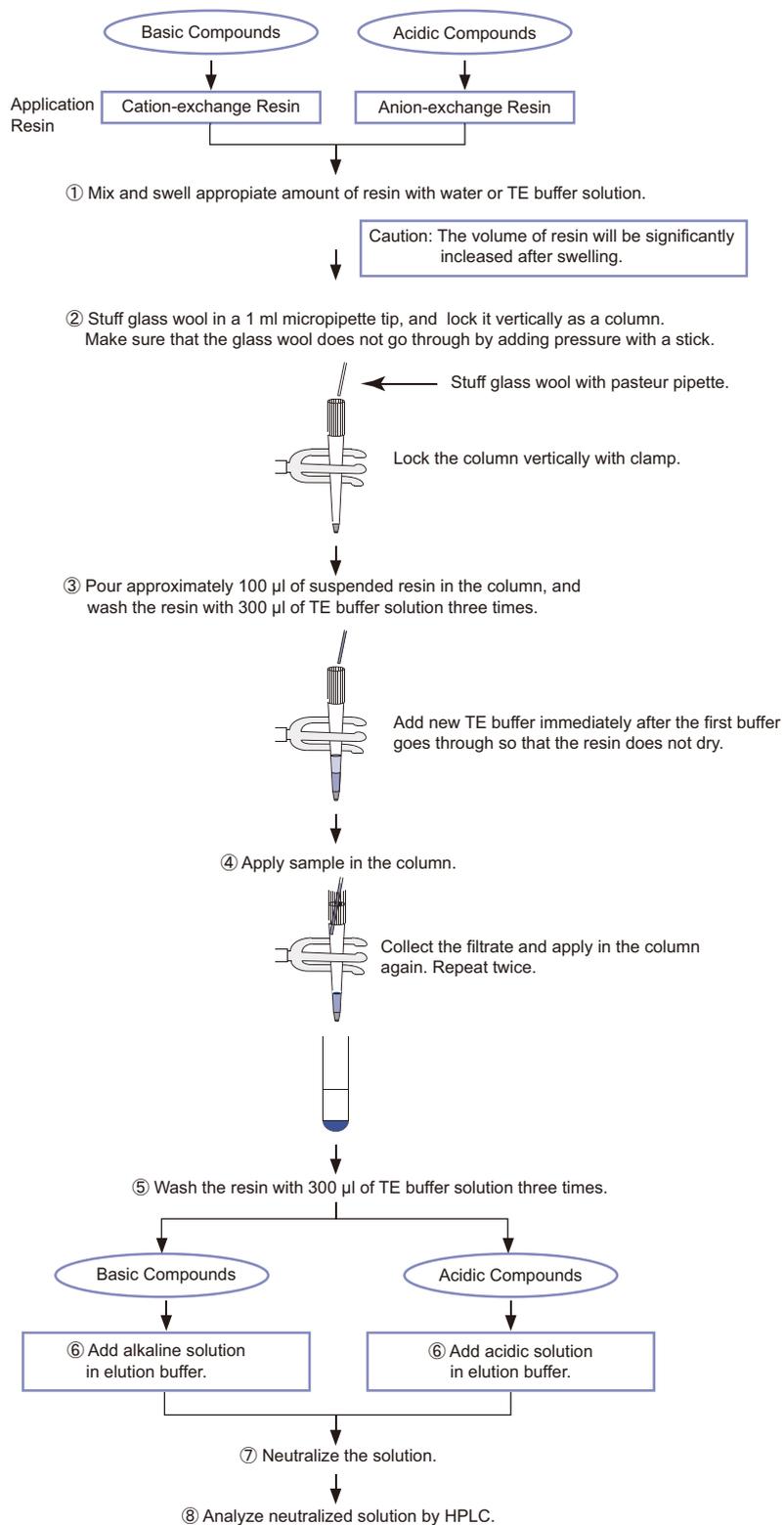
General Procedure for Solvent Extraction:



## 5) Ion Exchange

Pretreatment by ion-exchange resin can be effective for samples that the solvent extraction method cannot separate. A preliminary experiment may be required for the selection of resin and experimental conditions. For example, a negatively charged compound is strongly adsorbed on an anion-exchange resin, such as DEAE cellulose resin. The target compound is collected by increasing salt concentration of buffer solution or adjusting pH of elution buffer after washing off other weakly adsorbed, undesired substances.

General Procedure for Ion Exchange:



## 6) Solid Phase Extraction

