

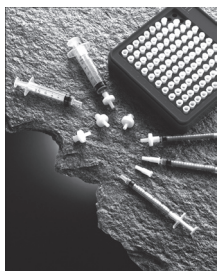

TECHNICAL NOTE

3. 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 preparation methods are different according to the each sample. The followings are examples of different pretreatments.

1) Filtration

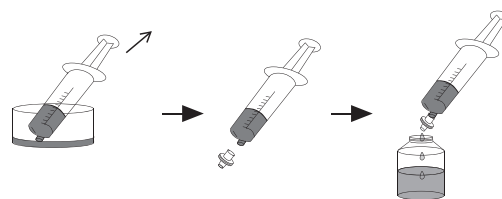
Filtration is a common method used for separating solids from liquids. It extends a column's life by minimizing column damages from solid contaminants such as particles, sediments and colloid substances. It also improves reproducibility of analytical data. We offer both syringe-type and spin-type filters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

■ Syringe filter	■ Centrifugal filter
Cosmonice filter	Cosmospin filter
	
Easy to use. Just attach a filter on top of a syringe.	Easy to use by centrifugation.
<ul style="list-style-type: none"> • W (aqueous system) • S (solvent system) 	<ul style="list-style-type: none"> • pore diameter : 0.2 μm • pore diameter : 0.45 μm
■ Required equipment	
Syringe · Sample bottle	Centrifuge
■ Product information	
Please see page 73	Please see page 73

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.

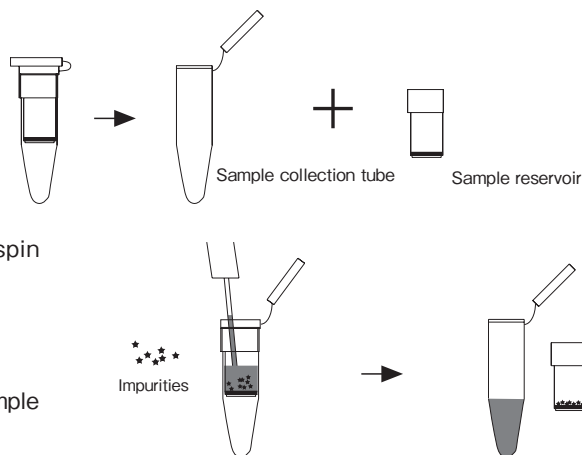


Cosmospin filter

Components : · Sample reservoir
· Sample collection tube

How to use :

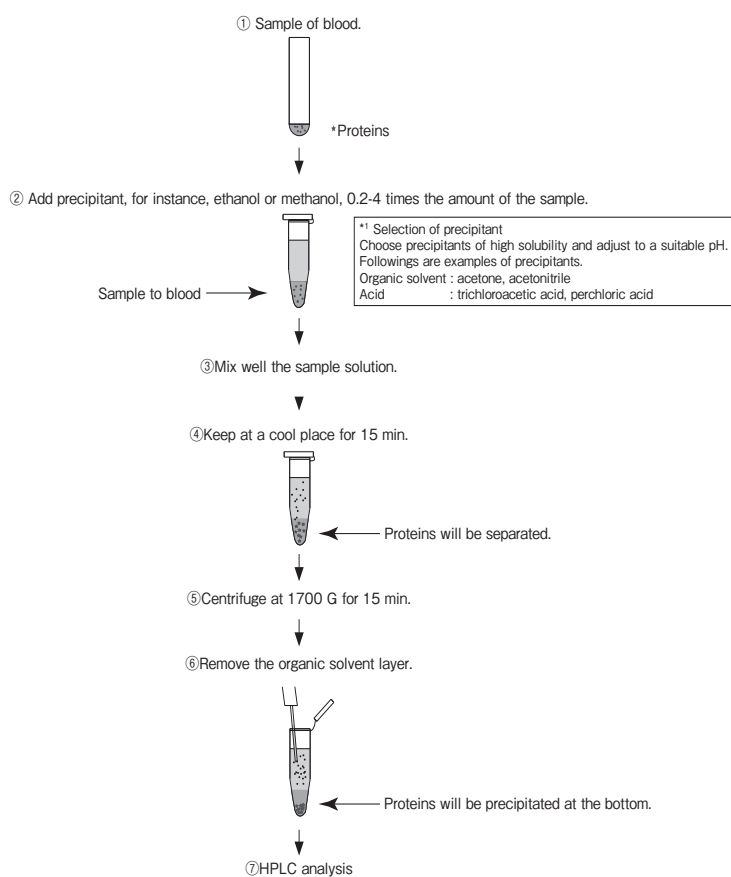
- ① Insert a Cosmospin sample reservoir into a Cosmospin sample collection tube.
- ② Add a sample into the Cosmospin sample reservoir.
- ③ Close the sample collection tube cap and centrifuge.
- ④ Remove the sample reservoir and collect the filtered sample in the sample collection tube.
- ⑤ Analyze the 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 for protein precipitation include salting out, isoelectric point precipitation and precipitation with organic solvents. The following shows a general procedure for protein precipitation with organic solvents.

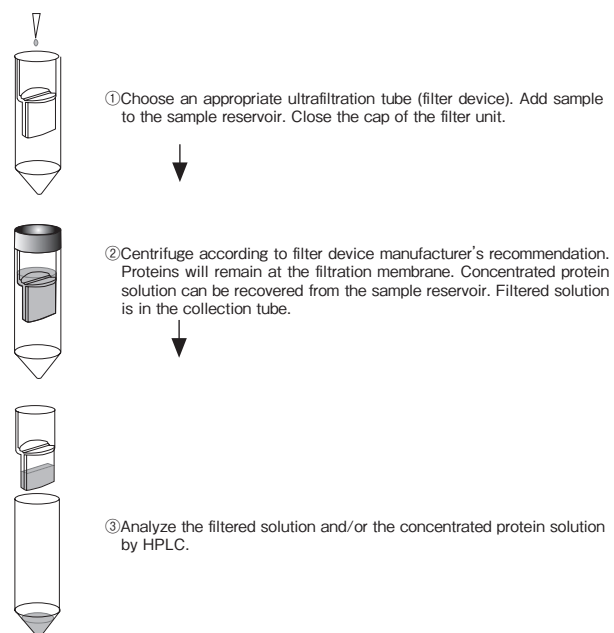
Procedure for protein precipitation :



3) Ultrafiltration

Ultrafiltration is a method to concentrate proteins or other macromolecules through a semi-permeable membrane with defined pores. Ultrafiltration is applicable for sample desalting, concentrating proteins from dilute solution such as urine samples, or deproteinizing samples with high protein concentration (i.e. blood serum or plasma). Following is a general procedure for ultrafiltration.

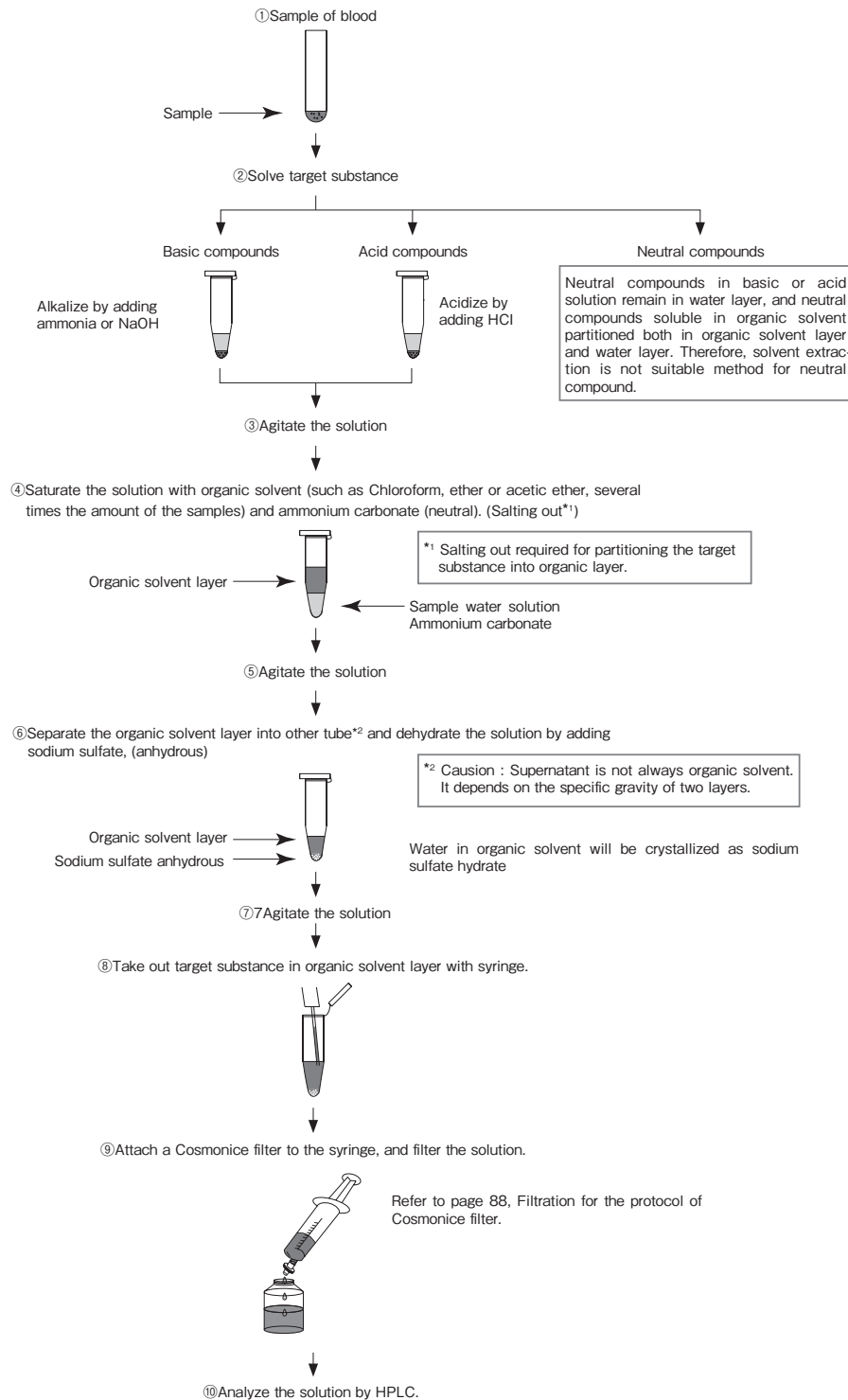
Procedure for ultrafiltration :



4) Solvent extraction method

Solvent extraction is a method to separate compounds due to their unequal solubility in two immiscible liquid phases, usually water and an organic solvent. The method is used to concentrate highly hydrophobic compounds, and consequently increase analytical sensitivity. A buffer solution is added to sample to optimize the pH and target substance is then extracted by an organic solvent such as ether and chloroform. However, when target substance is combined with proteins, solvent extraction may not work well.

Procedure for solvent extraction method :



5) Ion exchange

Pretreatment by ion-exchange resin may be effective for samples that the solvent extraction method cannot be adapted due to its emulsification. 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. Therefore, 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.

Procedure for ion exchange :

