SHISEIDO HPLC HOME

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PC HILIC

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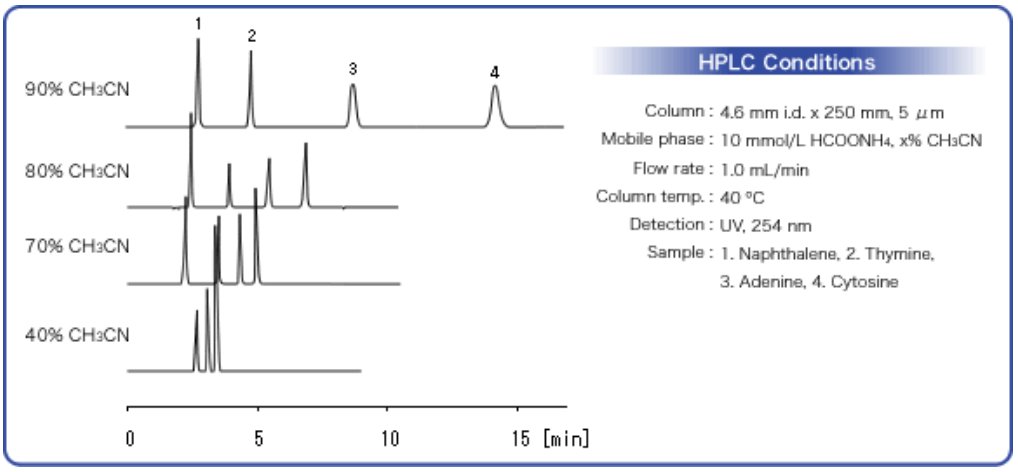
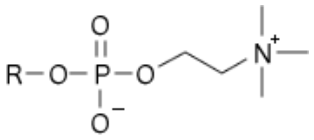
What is HILIC?

Hydrophilic interaction liquid chromatography (HILIC) is a relatively new LC technique that uses a hydrophilic stationary phase, in most cases, with organic-dominant mobile phase. The elution order of substances in HILIC mode is roughly the reverse of that in reversed-phase mode.



What is PC?

Phosphorylcholine (PC) is a partial structure of phosphatidylcholine (lecitin), one of the phospholipids forming cell membranes. PC has a betaine structure and shows high hydrophilicity, biocompatibility, and inhibitory effect of protein adhesion. Its superhydrophilic character is suitable to the application as a HILIC phase.



【Chemical structure of PC】

Features

·A silica-based HILIC column with phosphorylcholine (PC) group

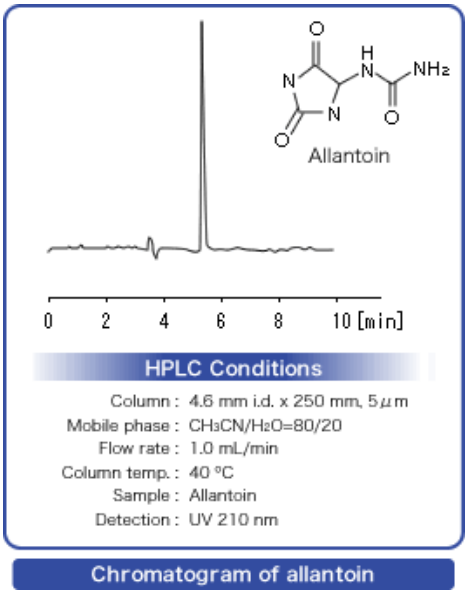
·Excellent retention and separation of very polar and hydrophilic compounds

·Large number of theoretical plates and outstanding peak profiles

·Also available in worldwide

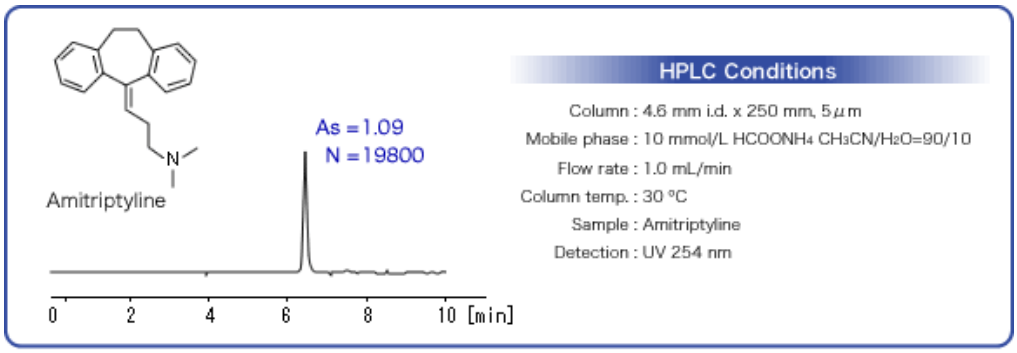
Strong retention of polar compounds

HILIC mode provides another alternative to handle extremely polar and hydrophilic compounds, which are unretainable in reversed-phase (e.g. a chromatogram of allantoin, shown below)



Chromatogram of allantoin

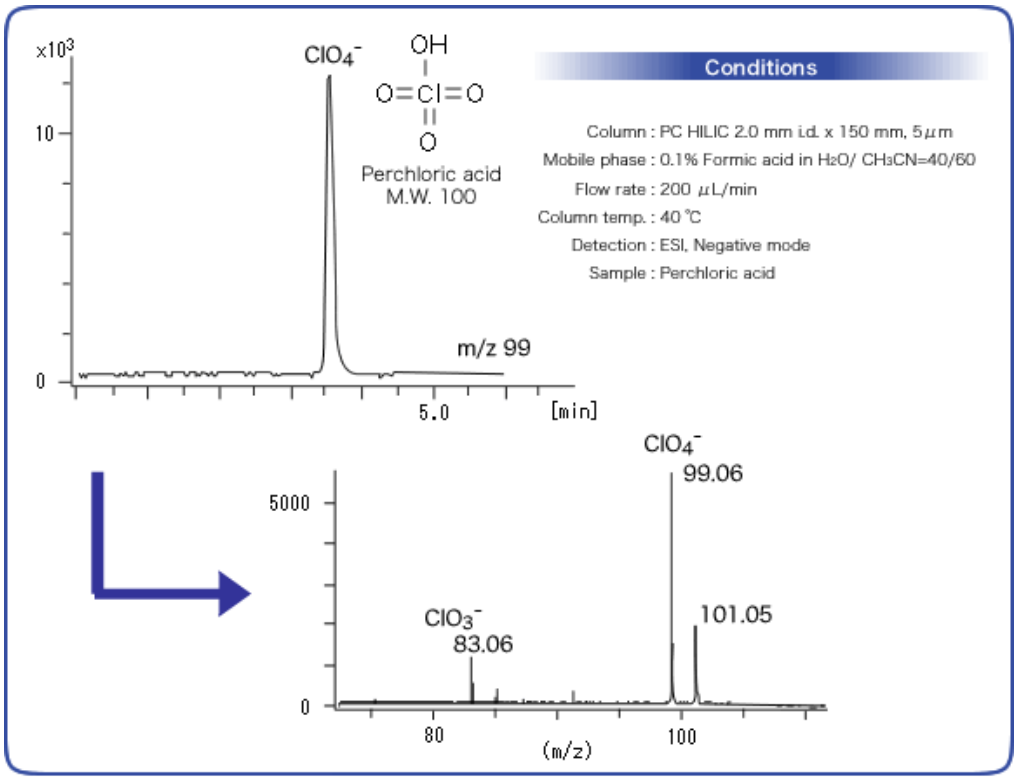
Amitriptyline, a compound with a strong basicity, is often used for discussing the quality of columns. PC HILIC provides excellent peak shapes for basic compounds, too.



Chromatogram of amitriptyline

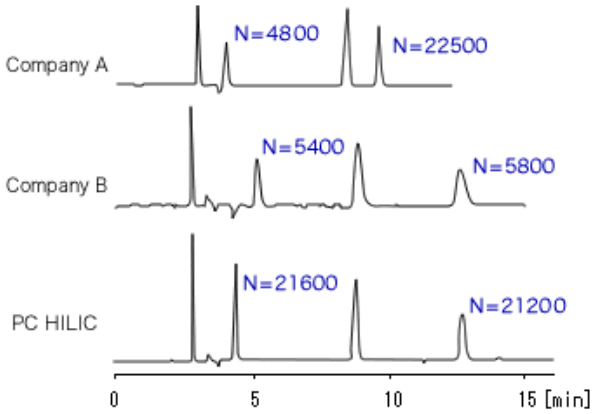
◆ High sensitivity in LC/MS

In LC-MS of very hydrophilic or ionic compounds, HILIC columns are often chosen to avoid extremely water-rich, or ion-pair containing mobile phases used under reversed-phase mode. The preferable nature common to PC HILIC seems to be the use of mobile phases of higher-organic contents, which are advantageous in providing larger diffusion constants of analytes during their migration through the columns, and also better ionization efficiency in electrospray ionization (ESI)



Separation of perchloric acid

PC HILIC shows large numbers of theoretical plates, compared to conventional HILIC columns.



HPLC Conditions

Column : 4.6 mm i.d. x 250 mm, 5μm
Mobile phase : 10mmol/L $HCOONH_4$ (pH3.5) / CH_3CN = 10/90
Flow rate : 1.0 mL/min
Column temp. : 40 °C
Detection : UV 254 nm
Sample : 1. Naphthalene, 2. Thymine
3. Adenine, 4. Cytosine

Chromatorgam of nucleobase

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PC HILIC User's Manual

PC HILIC is made of totally porous spherical silica bonded with phosphorylcholine (PC) groups.

1. Handling the Column

1. Handle the column with great care. A strong shock may cause damage.
2. Attach or detach the column when the pressure gage indicates zero.
3. The maximum column operating pressure is 20 MPa.

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2. Attaching the Column

1. The column joint is of the male nut type for tubing of 1/16 inch OD. Check that the tubing joints of the system fit correctly and that the ferrule tips are deeply inserted into the joints. (See Fig. 1.)
2. Before attaching the column, replace the liquid in the system with the mobile phase to be used. Note the replacement procedure to avoid salting out. The shipment solvent is described in the column report enclosed with the column.
3. Attach the column according to the direction of the arrow.

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3. Analysis

3-1. Mobile Phase

1. A mobile phase should be a mixture of acetonitrile and water (or aqueous buffer). Mobile phase has to contain more than 70 % of acetonitrile, and minimum 3 % of water (or aqueous buffer) for the HILIC mode to function. Upon preparing a buffer solution, ammonium formate or ammonium acetate are recommended to avoid precipitation and accidental pressure rise.
2. The acceptable pH range for PC HILIC is 3 to 7.5.
3. After complete degassing, filtrate the mobile phase using a membrane filter 0.45 µm or smaller to remove dust. A 2-µm filter is used at the column inlet. To prevent foreign matter from clogging the column inlet filter, the use of a line filter is recommended.
4. A brand new column contains the mobile phase used in the test for shipment. When using a mobile phase containing inorganic salts, cares should be taken in arranging a liquid replacement procedure to avoid a sudden pressure increase by salt precipitation.
5. To prevent column deterioration, avoid the following:
   •Frequent mobile phase change, or changing directly to a mobile phase of low compatibility
   •Rapid pressure change in column inlet
   •Large column pressure under the use of a high-viscosity mobile phase.
   •Leaving 100% water flowing through the column for more than one hour.

3-2. Preparing a Sample Solution

1. IF possible, prepare the sample with the mobile phase, or something closer to it.
2. Using a solvent with high content of water may lower the separation efficiency. Use a solvent with more than 50% content of organic solvent.
3. Sample solutions should be aqueous. Remove insoluble matters if any by using a filter of 0.45 µm or smaller.
4. The pH of the sample solution should be set in acceptable pH range for packing material.

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4. Storing the Column

1. Seal the column with the accessory plug and store it in a cold place where there is little temperature fluctuation.
2. For storage, replace the mobile phase with a solution of organic solvent and water having the same composition as the mobile phase and then fill it with the solvent used at the time of shipment. (Refer to the column report.)

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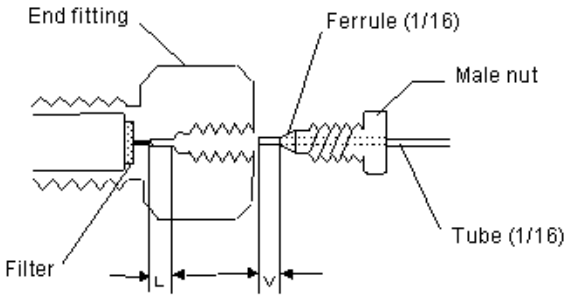
5. End Fittings

1. An analytical column of up to 6-mm ID uses a filter-embedded end fitting as shown in Fig. 1. The filter cannot be changed alone. If the filter is clogged or the column pressure is high, replace the end fitting. See Table 3 for the replacement parts and repair items.
2. See Fig. 1 for the column connection. If the tubing is inappropriate, especially if a tube for a different type of column is used, the length after the ferrule tip (V in Fig. 1) is often different from the end fitting length L, and a problem may occur.
   If L is greater than V, dead volume may be generated and cause peak broadening or tailing or deterioration of separation performance.
   If L is smaller than V, liquid may leak because of inadequate ferrule adhesion.
   Therefore, we recommend replacing the ferrule together with the column.
   *If the column is replaced frequently, the male nut may crush the ferrule and liquid may leak. Since tightening the nut too much may cause its head to come off, replace the ferrule at an early stage.



【Fig. 1】 Column connection

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6. Replacement Parts and Repair Items

Table 3 Replacement parts and repair items

Part No.	Part Name	Description
21105	End fitting (4.6 mm)	2 pieces
21107	End fitting (6 mm)	2 pieces
21110	Ferrule (1/16)	Ferrules (1/16) 10pieces

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7. Troubleshooting

Problems in high performance liquid chromatography are attributable to various causes that cannot all be listed up. The table below describes some comparatively common problems related to the column.

Symptom	Cause	Measures
1. Column pressure rise.	Blocking with foreign matter 1. Dust or insoluble matter in the mobile phase or sample solution. 2. Dirt in the tubing. 3. Plunger seal fragment. 4. Precipitation of sample components.	• Sonicate the filter or replace it. • Filtrate the mobile phase and sample solution in advance using a membrane filter. • Attach a line filter. Clean the tubing and replace the plunger seal.

		• Prepare a sample solution with the mobile phase.
2. Peak splitting, tailing, and broadening.	1. Void in the column head. 2. Dead volume due to inappropriate connections. 3. Inappropriate mobile phase conditions. • Ion suppression method: Inadequate suppression (Too much sample). • Ion-pair method: Inadequate concentration of the ion-pair agent (Too much sample). 4. Column deterioration. * Not repairable in the case of column deterioration or damage to the packing condition.	• Reconnect the tubing. • Review the pH, salt concentration, sample amount, and other conditions. • Check the column performance using standard inspection solution.
3. Retention time too long or unstable.	1. Liquid leak (Indicated on the pressure gage of the pump). 2. Inappropriate mobile phase conditions. 3. Inadequate column equilibration time.	• Check the pump and tubing for any leaks. • Secure adequate equilibration time.
4. Retention time too short.	1. Hydrolysis (deterioration) of a bonded groups by strong acid or base. 2. Inappropriate mobile phase conditions. 3. Inadequate column equilibration time.	- • Secure adequate equilibration time.

PC HILIC is shipped after a strict performance check. However, if you should find any defect, please contact your dealer or Shiseido for replacement.
Note that Shiseido does not warrant the product against column life or deterioration caused by the failure to follow the above handling instructions.
Ten or more days after reception by the customer, Shiseido will assume that the product was delivered in good condition, and will not accept a later replacement request.

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