

HPLC manual (for chiral HPLC analysis)

A) Column

- (1) choose the appropriate column
(We recommend AD-H and OD-H on your first try.)
- (2) change the column and CLAMP it
- (3) cap the previous column and return it to the box

-notice-

DO NOT dry or let the air goes in the column.

Columns are very expensive: 180,000 yen for each.

-TIPS-

It's very difficult to predict which column can separate your samples.

You need to try columns one after another in order to find appropriate column.

You can connect two columns in series if any single column can't separate your samples.

B) Solvent

- (1) decide the appropriate solvent
(We recommend hexane/IPA = 10/1 on your first try.)
- (2) **DEGAS the solvent by sonication** (ca. 5 min)
- (3) change the solvent

-how to prepare solvent-

Use HPLC grade IPA and EtOH.

Use hexane from 18-liter square can directly, not from solvent tank.

-notice-

You can ONLY use hexane, IPA and EtOH.

When you want to use other solvents, please ask manager.

-TIPS-

Generally, injection shock appears at ~5 min.

You should choose solvent to detect your sample's peak after 10 min.

C) Setting Conditions and Purge

- (1) set your analytical conditions
(We recommend 1.0 mL/min, 254 nm on your first try.)
- (2) open purging valve
- (3) turn on the pump (ca. 5 min)
- (4) close purging valve
- (5) solvent displacement (ca. 20-30 min)

-TIPS-

You can use shorter wavelength (i.e. 210 nm) when your sample is difficult to detect at 254 nm. (When you use 210 nm conditions, you can't use solvents that have absorbance at 210 nm such as EtOAc and acetone.)

However, we strongly recommend that you convert your samples to aromatic compounds that have absorbance at 254 nm.

D) Preparation of Sample

- (1) **dissolve your sample COMPLETELY** in solvent
(concentration: 1 mg sample in 1 mL solvent)

-notice-

Be careful to dissolve your sample completely especially when your sample is prone to crystallize.

When you isolate your sample with column chromatography or PTLC, you should correct all fractions containing desired compounds.

-TIPS-

Solvent for dissolving your sample should be LESS POLAR than mobile phase solvent if the sample can be dissolved.

Polar solvent sometimes cause a lowering of separation ability.

E) Measurement

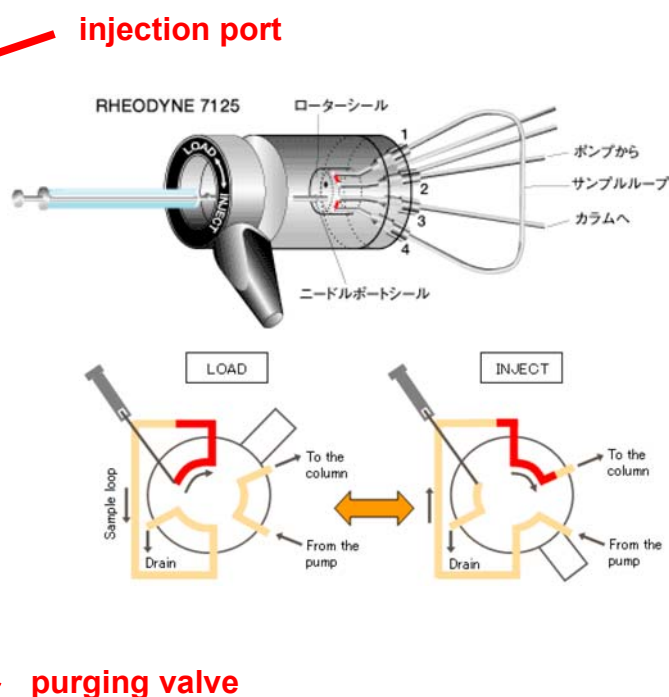
- (1) inject your sample (5 μ L)
(insert syringe \rightarrow turn to "LOAD" \rightarrow inject sample \rightarrow turn to "INJECT" \rightarrow extract syringe)
- (2) WRITE HPLC NOTE
(date, your name, machine number, column, solvent, flow rate, pressure)
- (3) turn OFF the pump after the measurement

-notice-

Wash the syringe by MeOH or EtOH after you use.

Keep plunger and syringe separately.

For data collection, we recommend that you analyze racemic samples and chiral samples successively to maintain the retention times constantly.



F) Print

- (1) 「レポートスタイルを指定して印刷」
(DO NOT select 「印刷」 It's a waste of color ink)

G) Clean Up

- (1) KEEP CLEAN!!
(don't leave your sample, pipettes etc.)
- (2) Don't forget to SHUT DOWN the HPLC and PC.

If you want to know more details about chiral column, please see the online handbook from DAICEL.

<https://www.daicelchiral.com/img/mainpage/column/pdf/handbook.pdf>