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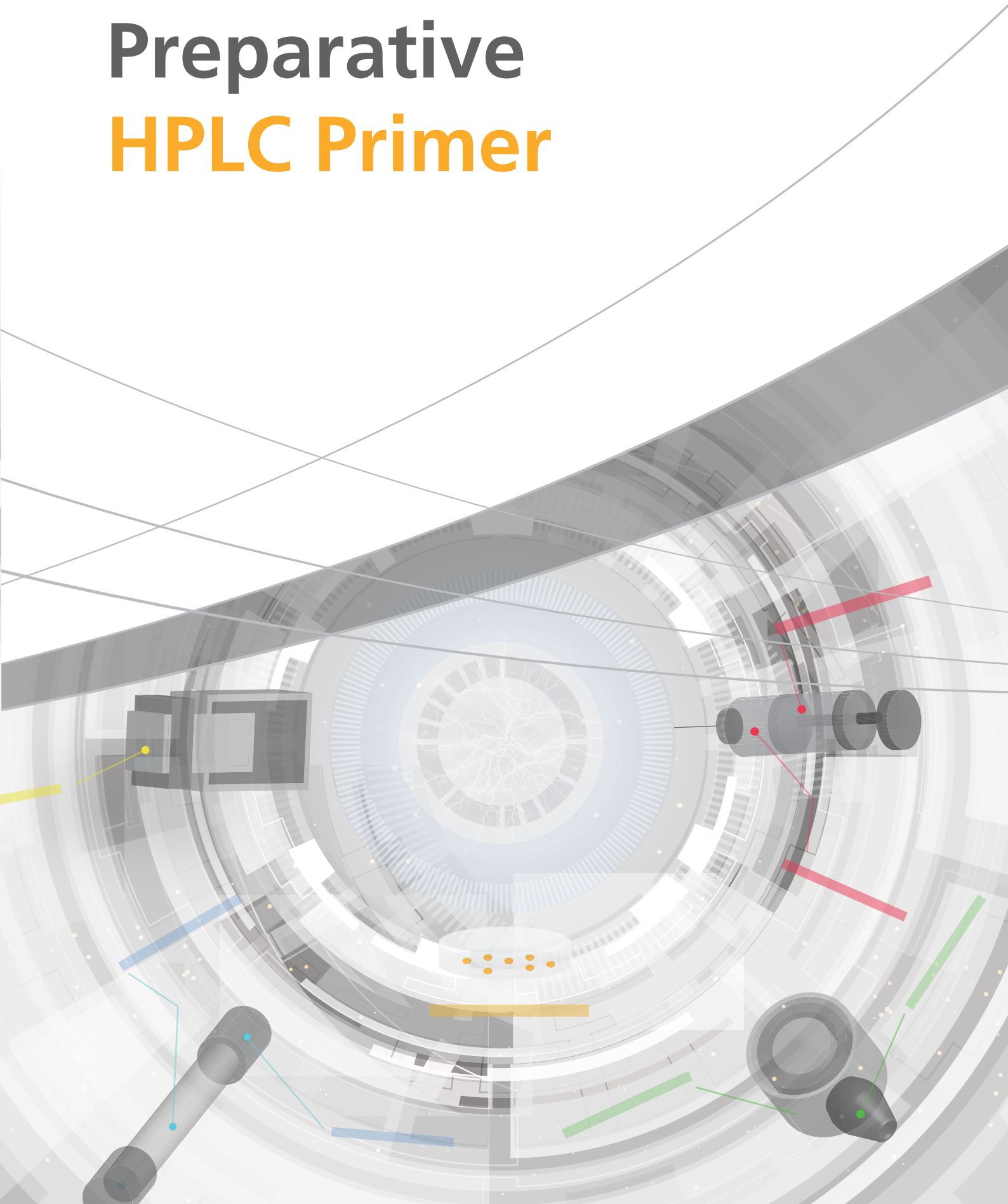
C190-E271

Preparative HPLC Primer



SHIMADZU
Excellence in Science

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Prep LC Primer

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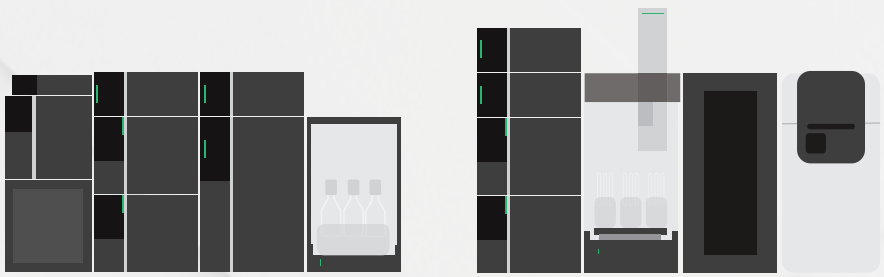
Prep LC Primer

Introduction

Liquid chromatography is an analytical instrument widely used across wide range of industries, commonly used in pharmaceuticals, chemistry, food and environment. In recent years, commercially available HPLC equipment has advanced automation and software control, and can be easily used in routine work following established SOP. On the other hand, HPLC is also widely used as a “tool” for the purpose of fractionation / purification of compounds, and in such a case, there are caveats for this specific application. Since the choice of columns and equipment depends on the purpose of application needs, that is, what purified compound is used for in the next step of analysis, we need a strategy that fully considers the subsequent analysis. In such as case, when HPLC is used for Purification purpose, the technic is termed as Preparative HPLC. Here, we will summarize the procedures and precautions for using Preparative HPLC.



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Prep LC Primer

Preparative HPLC

Preparative HPLC is a liquid chromatograph (LC) for separating and purifying the target compound from the mixed solution after the synthesis reaction and/or extract from the natural product and recovering it with high purity. By obtaining the target compound with high purity, it is possible to conduct structural elucidation analysis, various functional evaluation / analysis or the next step analysis more reliably.

The technique is also sometimes used for purification of the final product on an industrial scale basis. Appropriate scaling up from the required amount and degree of purity of the target compound, it is possible to select a system configuration that is superior in efficiency and cost performance. The preparative LC can be categorized as the below table based on the scale.

Table 1 : Category of preparative LC by scale

Scale	Column I.D.	Purpose
Semi-micro	2mm	Bio-chemical preparation
Conventional	4 - 6mm	Structural analysis and biochemical preparation
Semi-preparative	10 - 20mm	Structural analysis, pretreatment and purification
Large Scale preparative	30 - 50mm	Purification and pretreatment
Industrial Scale	100mm -	Purification

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Prep LC Primer

Preparative HPLC

In the preparative HPLC system, the key objective is to separate desired compound from the coexisting components in the crude material. The same Liquid Chromatograph principles apply in the Preparative workflow. Therefore, as with ordinary HPLC systems, its basic instrument setup includes a liquid feed pump, a sample injection unit, a separation column, and a detector. Additionally, as a characteristic feature of the preparative system, a mechanism for fractionating the target substance has been added after elution. In the case of a very simple preparative HPLC system, manual injection / manual fractionation is adopted by using a manual injector for the sample injection part and a manual flow path switching valve for the fractionating part. It is also possible to configure a fully automated system by setting an autosampler or fraction collector.

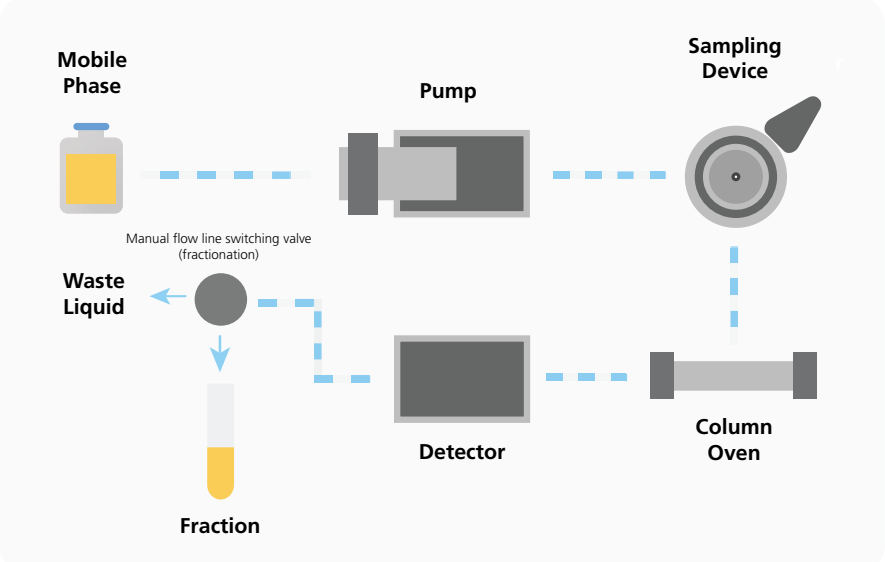

Figure 1 : An example of simple preparative system

This article provides the knowledge required to select an appropriate preparative HPLC and compatible columns for the intended purpose and introduces various advanced combinations of Preparative HPLC systems.

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Prep LC Primer

Preparative HPLC

Below is a process outline of system selection and condition setting of preparative HPLC as explained in Chapter 2 and later.

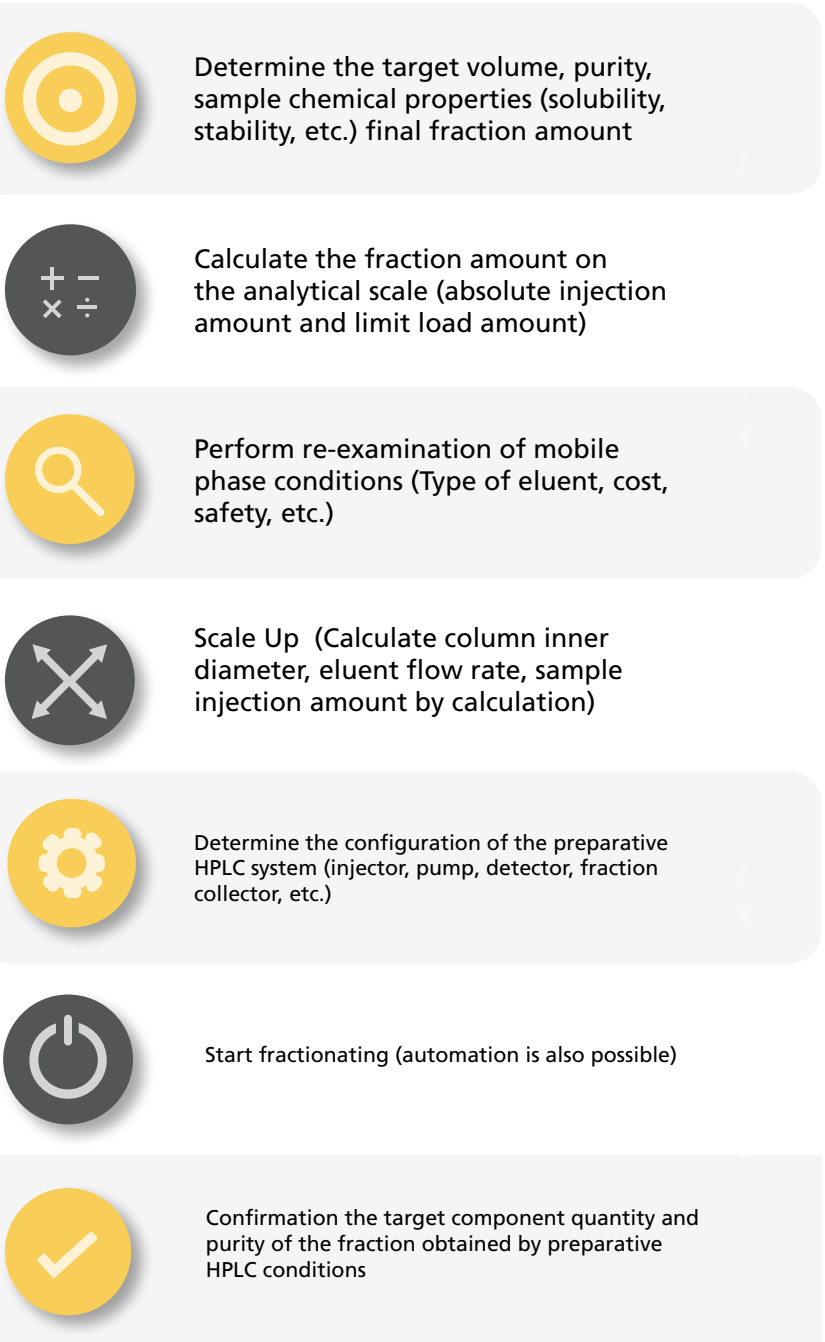
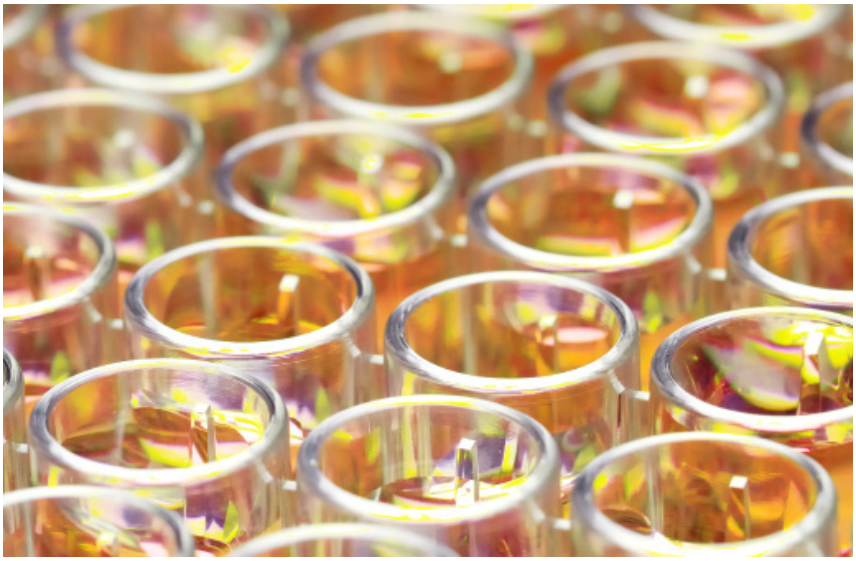


Figure 2 : Process outline of system selection and condition setting of preparative HPLC

Setting the Fraction Volume Target



2.1 To contemplate purpose of preparative HPLC

Since the strategy changes according to the purpose of preparative separation, first clarify the purpose of using preparative HPLC and advance target setting assuming actions and effects that can occur by preparative work. For example, if it is said that the target component that has been fractionated and purified is used for structural analysis by NMR, the required amount will vary depending on the performance of NMR to be used, and it is necessary to reduce other components, e.g. residual solvent, buffer and moisture as much as possible.

1 H-NMR is more sensitive than 13 C-NMR, but sometimes it may interfere if too much eluent components such as moisture remain.

When 13 C-NMR or 2D NMR is used, it is necessary to set the target value for obtaining the sufficient sensitivity. In drug development, etc., when conducting bioassay after sample purification, substances that may inhibit the bioassay (such as a highly toxic solvent) should not be included in the eluent. Even acetonitrile, which is often used under HPLC analysis conditions, may be avoided in many cases. Hence, effective and complete drying process is very critical.

Setting the Fraction Volume Target

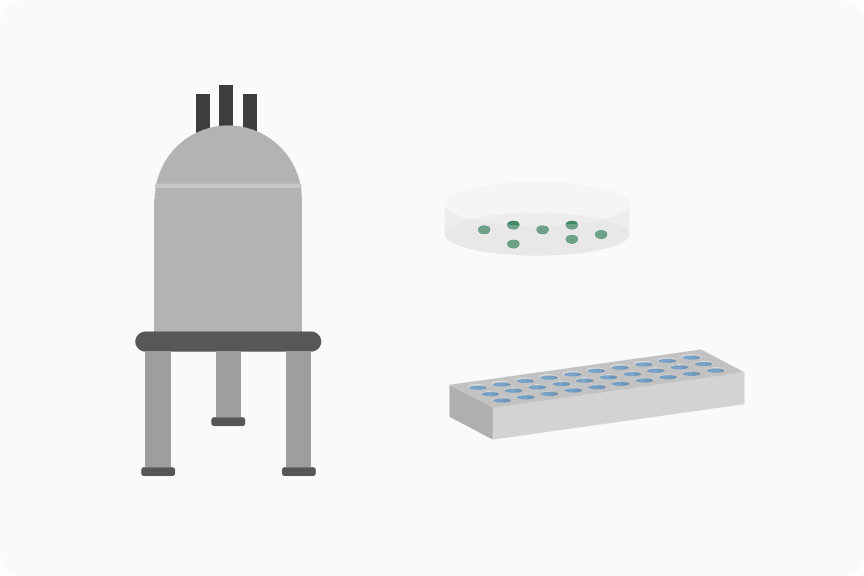


Figure 1: Preparative HPLC strategy needs to be developed for next process or purpose to use the fraction.

For example, NMR analysis or assay should define target purity and volume

As other example, when preparative purification is carried out to evaluate the functionality of the target component in the fine chemical and specialty chemical fields, it may be necessary to pay specific attention so that the preparative work does not affect the functionality of the desired compound itself. The chemical structure may be possibly change in an isolated state when separating the stabilizer and additives originally present in the sample. Also, light sensitive compound structure may alter when passing through the UV detector or heat-sensitive compound may degrade during evaporation process, where heat may be utilized to speed up the drying process.

In other words, instead of scaling up following the same condition (column type and solvent) on the analytical scale, in some cases change the type of salt in mobile phase, change the type of organic solvent, avoid the use the gradient condition, or changing the reverse phase system to the normal phase system, such bold strategies may be considered. To fully understand the chemical profile of the sample, the use and effect of overall flow of preparative HPLC and subsequent work before starting up the scale up is really important.

2.2 Setting the fraction volume and purity target

In preparative HPLC, it is important to clarify target amount and purity to be obtained together with the purpose of preparative. This is because the target value determines the column and device configuration to be used. This is to avoid in time wastage, labor and cost reduction.

Setting the Fraction Volume Target

2.3 Understand the current situation on the analysis scale

(Calculation of absolute injection amount and limit load amount)

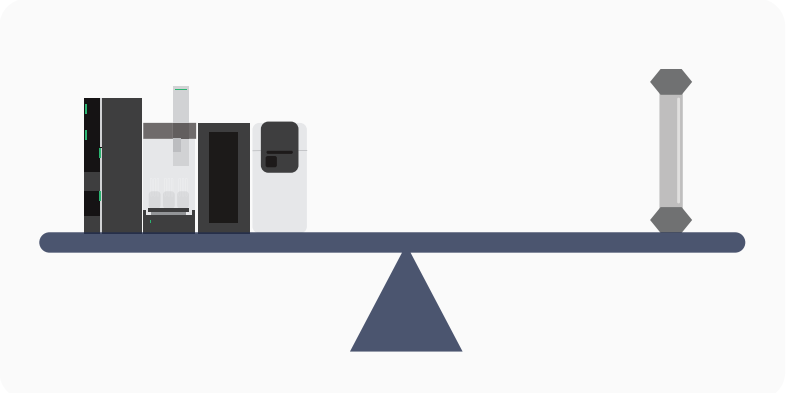
The correct instrument and column selection is significantly affected by the fractionation volume. First rule is to investigate preparative HPLCs by determining the minimum fraction volume required.

When fractioning target components by HPLC, for the sake of subsequent processing, it is important to be able to specify the fraction volume or at least the minimum meaningful fraction volume. For example, a specialized fraction collector is not required if a fraction volume in the order of micrograms is sufficient. This fractioning may be possible using a conventional size of instrument and column.

Conversely, if a fraction volume in the order of grams is required, a preparative scale of instrument and column is probably needed. Therefore, the instrument and column selection is significantly affected by the fraction volume required.

2.4 Understanding the current status at conventional scale

First, we calculate the volume that can be fractioned on a conventional scale (using a normal HPLC instrument and column). An understanding of the fraction volume at a conventional scale permits selection of the appropriate preparative HPLC to obtain the target fraction volume. (This assumption is based on HPLC analysis conditions that have been established to measure the concentration of the analysis target components in the actual sample, and that the eluent used does not cause any problems during post-fractioning processing.)



Setting the Fraction Volume Target

2.4.1 Calculating the absolute injection volume

If the concentration of the target component in the sample solution and the volume injected into the HPLC are both known, they can be multiplied together to determine the absolute injection volume. (For example, if the concentration of the target component in the sample solution is 1 mg/L and the injected volume is 10 µL, the absolute injection volume is 0.01 µg.) The most commonly measured concentration range by HPLC with a UV detector extends from sub-mg/L to several hundred mg/L. It is easy to see that fraction volumes of just a few micrograms can be obtained by fractioning with such an instrument. Obtaining a 1 mg (1000 µg) fraction requires the injection of 100 µL of a 1 % (10,000 mg/L) sample solution.

▼ **Table 2:** Sample concentration and loading amount

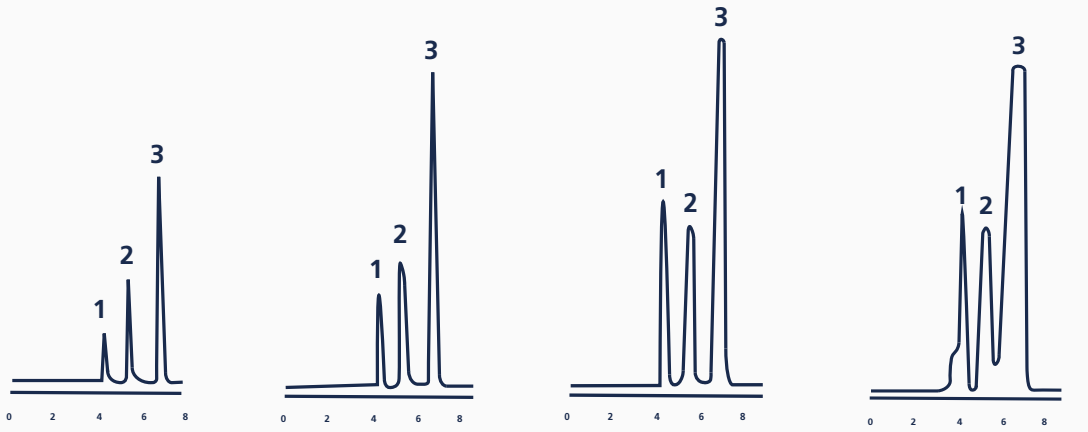
	Loading Amount (µg)	
Sample Concentration (mg/L)	10 µL injection	100 µL injection
0.01	0.0001	0.001
1	0.01	0.1
100	1	10
10000	100	1000

Setting the Fraction Volume Target

2.4.2 Determining the load limit

Next, it is recommended to determine how high the analysis target concentration can be increased in the sample solution and how much the injection volume can be increased using a conventional-scale instrument. In preparative HPLC, even if the peak shape and separation ability are impaired or the peak exceeds the detection range of the detector, it is still possible to perform fractionating if the target component can be separated from coexisting components. Before scaling up, check to what extent the sample concentration and injection volume can be increased on the analysis scale. Figure 3 shows the chromatogram when changing sample concentration and injection volume.

▼ **Figure 3:** Example of test results to increase the injection volume



10 µL injection (Benzene5 mg) | **20 µL injection (Benzene10 mg)** | **50 µL injection (Benzene25 mg)** | **100 µL injection (Benzene50 mg)**

1 - Benzoic acid
2 - Benzene
3 - Naphthalene

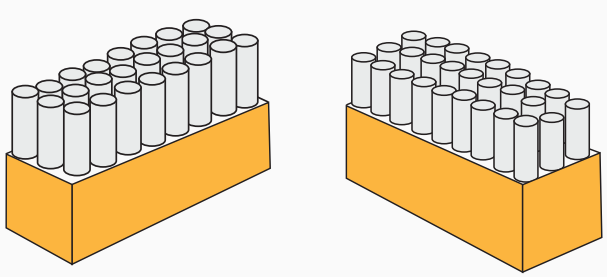
* To prevent the peaks for high-concentration benzene going off-scale, detection was performed at 270 nm to avoid the maximum absorbance.

Setting the Fraction Volume Target

This example shows chromatograms of a mixed solution of 500 mg/mL benzene with trace levels of benzoic acid and naphthalene analyzed using a conventional-sized ODS column (250 mm × 4.6 mm, 5 µm). The results of these tests show that the peak shape can be maintained up to 50 µL injection volume, which corresponds to 25 mg when converted to a benzene load.

The tests above were performed while increasing the injected volume. However, it is also possible to gradually increase the sample concentration. As a detailed investigation is not required, the injection volume or sample concentration can be increased in steps of 5 or 10 times to determine the limit to which the target component peak can be separated from the adjacent impurity peaks. It is important to determine only whether preparative LC can separate the target peak from coexisting peaks. It does not matter if the peak shape is somewhat deformed or the detector goes off-scale. In order to ascertain whether the target component has been isolated, the fraction can be confirmed in the chromatogram by re-injecting it into Analytical HPLC. In advanced system configuration case, a combined Preparative and Analytical system can be setup, to run both Preparative separation and purity check.

If the load limit is known at the conventional scale, it is then possible to calculate how much the fraction volume can be increased by scaling up the column size. However, when scaling up bigger size column, a more specific configured Preparative system may be required.



Setting the Fraction Volume Target

2.5 Determination of Preparative HPLC by scale-up prediction

Once the fraction volume has been established at the conventional scale, it can be compared with the fraction volume target value.

1) Target approximately equals the fraction volume at the conventional scale.

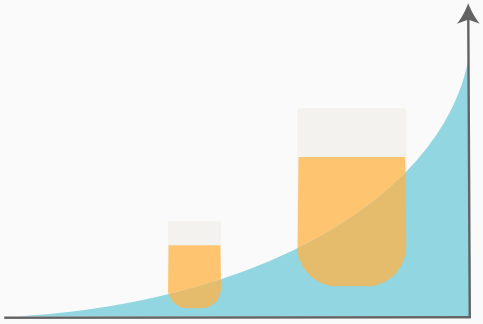
→ Specialized fraction collector or larger column is not required. If the fraction volume is slightly below the target value, repeat fractioning several times.

2) Target is 10 to 100 times the fraction volume that can be obtained at the conventional scale.

→ Introduce laboratory preparative HPLC to achieve the target. Select the appropriate instrument and column to run laboratory preparative HPLC.

3) Target is more than several hundred times the fraction volume that can be obtained at the conventional scale.

→ Target may be difficult to achieve with a laboratory HPLC instrument. Consider an industrial preparative LC or use a separation method using a different principle from HPLC.

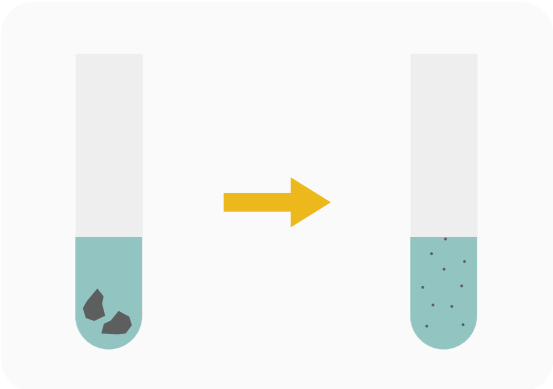


Setting the Fraction Volume Target

2.6 Judgment of application of various analytical conditions to preparative scale

When an analysis scale with established conditions that offers problem-free analysis is scaled up for fractioning, complications may occur. Therefore, it is necessary to re-check the overall analysis conditions.

- Depending on the target fraction volume, in some cases it may be necessary to inject large quantities of high-concentration sample to achieve efficient fractioning. Some compounds may dissolve adequately in the eluent at the analysis scale but not at the preparative scale. In such cases, it is important to review the eluent composition and separation mode. In certain situation, minimal amount of THF is added to improve solubility; filtration is also required to remove any undissolved particle to prevent column clogging.



- If you inject a large amount of sample, the compound may not be retained on the column if the dissolution power of the solvent in which the compound is dissolved is higher than that of the eluent. Therefore, when injecting a large amount of sample, it is necessary to weaken the elution force by mixing with the eluent at the stage of dissolution or dilution. One example is when dissolving sample in big amount of DMSO, the compound may not retain in the column, and will be eluted out without any separation.

- Since the target component obtained by fractionating dissolved in the eluent, it must remain stable without decomposition, in the eluent. Also, although it is rare, it may be altered by UV irradiation in the UV detector, and it may have a different structure in the fraction.

Setting the Fraction Volume Target

- It is also important to suppress the content of non-volatile salt, as salt presence may interfere the evaporation of the reversed phase eluent to dryness. Phosphoric acid is often used for analytical HPLC. However, it is often replaced by formic acid, acetic acid, or trifluoroacetic acid for preparative HPLC. In addition, it is important to avoid using ion-pair reagents in the reverse-phase mode and to use organic solvent-rich rather than water-rich compositions. If a bioassay is to be performed after fractioning, it is also important that the eluent does not contain substances that may inhibit the bioassay (such as highly toxic solvents). Besides, the use of additive in the mobile, may also result in salt formation with the desired compound. In such a case, an additional free-basing step should be included to remove free-base the desired compound for next step analysis.

- If the analytical condition is a gradient elution method, if applied directly to the preparative scale, the eluent corresponding to the initialization time will be discarded. In the case of mild gradient conditions, optimization is carried out under isocratic conditions drastically or unnecessary components after elution of the target component are eluted in a short time with a solvent having a high elution power even under a gradient condition, preferably as much as possible early initialization, etc. can also be considered.
- Also, as the fractions obtained are dissolved in the eluent, it is necessary to confirm in advance that no complication will surface during post-processing (concentration, purification, analysis by other instruments, etc.). Since water is contained in the eluent in the reversed phase system, if the condition is changed to a normal phase system, and it is easy to perform post treatment such as evaporation to dryness, in separation condition with highly volatile organic solvent system, can be one of the strategies. However, change in adopting which method for separation has to be fully assessed, as different separation mode gives different degree of separation. Additionally, separation pattern will change depending on which mode of separation.

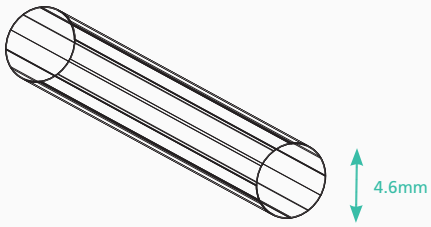
Scale up

3.1 Basic Principles of Scaling Up

Basically, fractioning is performed by simply increasing the size in the analysis conditions, as described below. However, this may lead to problems with inadequate solubility in the eluent with some samples. As described in Determining the Load Limit, injections of 10 to 100 mg/cm2 are possible by increasing the sample load before scaling up.

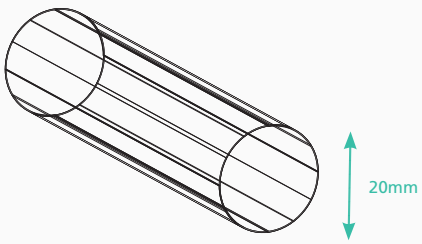
When the analytical scale is scaled up to semi-preparative or preparative size, if the packing is kept the same, it is thought that the eluent flowrate and sample load can be increased in proportion to the column cross-sectional area to obtain approximately the same separation.

Figure 4 : Column size example of scale-up to get similar separation



Column ID: 4.6mm

Column Length: 150mm
Particle Size: 5 µm
Flow Rate: 1mL/min



Column ID: 20mm

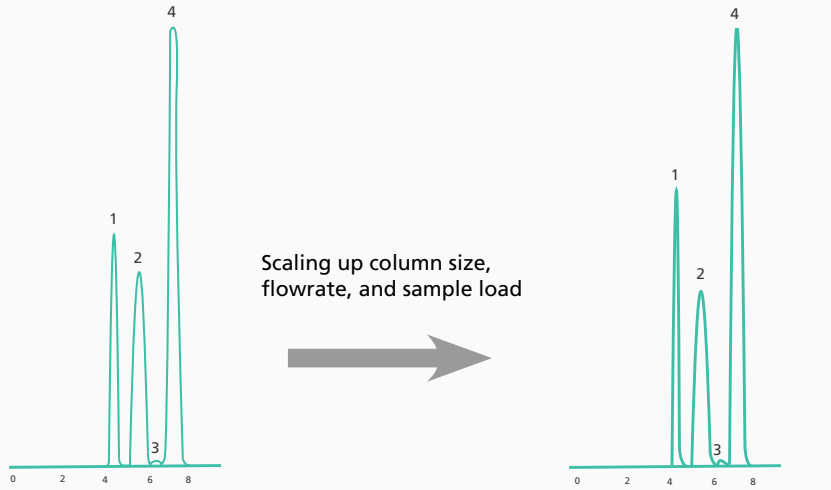
Column Length: 150mm
Particle Size: 5 µm
Flow Rate: 19mL/min

Scale up

The diagrams below show an example of scaling up using columns with the same packing. A 4.6 mm I.D. column was used at the analysis scale and a 20 mm I.D. column was used at the preparative scale. As the 20 mm I.D. column has approximately 19 times the cross-sectional area of the 4.6 mm I.D. column, the flowrate was scaled up from 0.8 mL/min to 15 mL/min and the sample injection volume can also be scaled up from 50 µL to 1 mL. This resulted in approximately identical chromatogram patterns, and same degree of separation, but in larger injection volume.

Therefore, scaling up is easy if a set of columns of different inner diameters but similar packing properties is available.

▼ **Figure 5 : Example of Scaling Up from Analysis Scale to Preparative Scale**



Despite the injection volume being increased 20 times from the analysis scale, approximately equal sensitivity and area values are obtained at both the analysis scale and preparative scale (excluding naphthalene). This is thought to occur because the eluent flowrate setting is increased by almost the same ratio, so that the dilution in the eluent results in approximately the same component band concentrations being retained in the detection cell for the same amount of time.

3.2 Selecting Column

Select a column with the appropriate inner diameter by comparing the load limit for the conventional size with the target fraction volume. Determine the eluent flowrate according to the column cross-sectional area by following the Basic Principles of Scaling Up described above.

The table below shows examples of the cross-sectional area ratio and set flowrates scaled up for a variety of columns, based on 4.6 mm I.D. and 1.0 mL/min eluent flowrate.

It may not be possible to use some flowrates shown above with preparative columns in practice, due to issues with the eluent viscosity and column pressure resistance. In this case, first determine the flowrate for the analytical column and then convert it back to the flowrate for the preparative column.

▼ **Table 3 : Relationship Between Column Cross-Sectional Area and Flowrate**

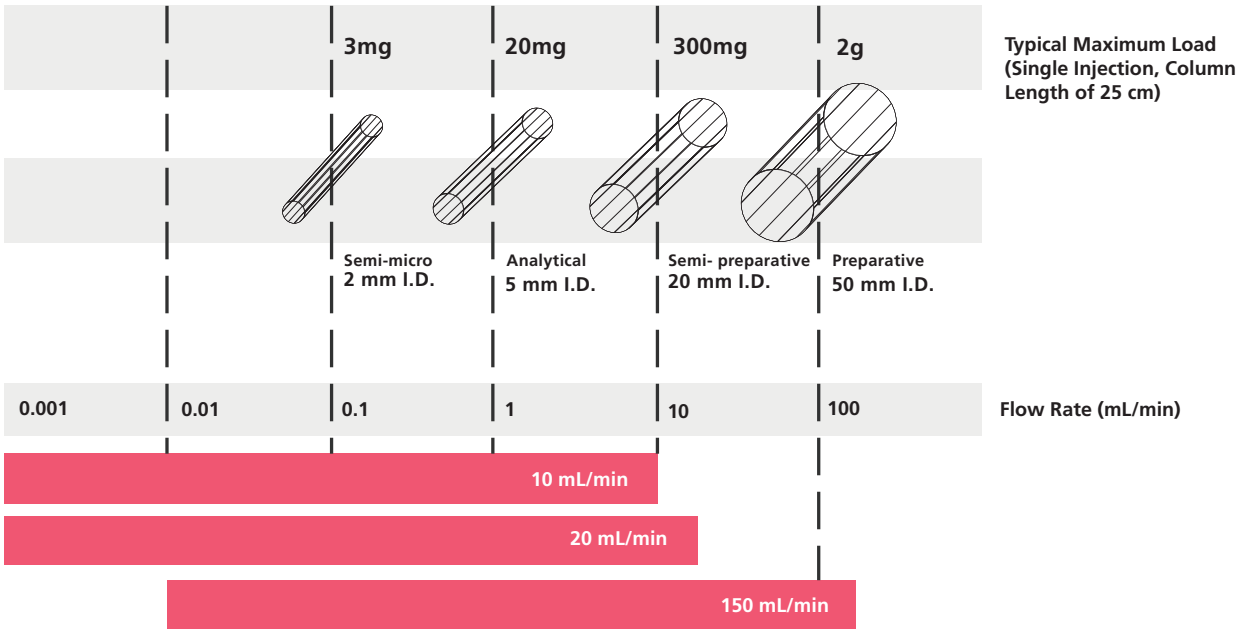
Column I.D. (mm)	Section Area (cm^2)	Columns Cross-Sectional Area Ratio	Flow Rate (mL/min)	Max. Amount (mg)
2	0.03	0.17	0.15	3
4.6	0.17	1	0.8	17
20	3.1	18.9	15	300
50	20	115	90	2000

04 Configuration of Preparative HPLC

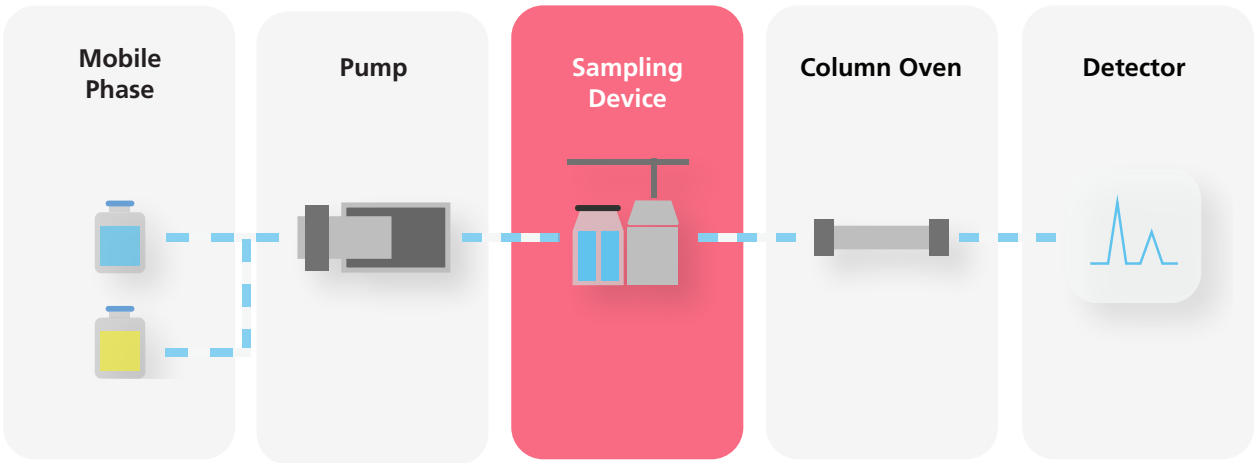
4.1 Solvent Delivery Units

The table below shows the standard flowrates at analytical and preparative scales and the corresponding solvent delivery units to handle them. Select the solvent delivery pump according to the determined eluent flowrate. A maximum flowrate setting is prescribed for each solvent delivery unit for HPLC. To use 1/2 to 2/3 of the maximum flowrate setting as the upper limit for practical applications is recommended. Continuous operation at a high flowrate may lead to rapid deterioration of the plunger seal, which result in higher cost of maintenance. Depending on the specification, it is configured in combination with degassing unit, gradient unit, solvent switching valve, etc.

▼ **Table 4 : Flow Rates and Corresponding Solvent Delivery Units**



Configuration of Preparative HPLC

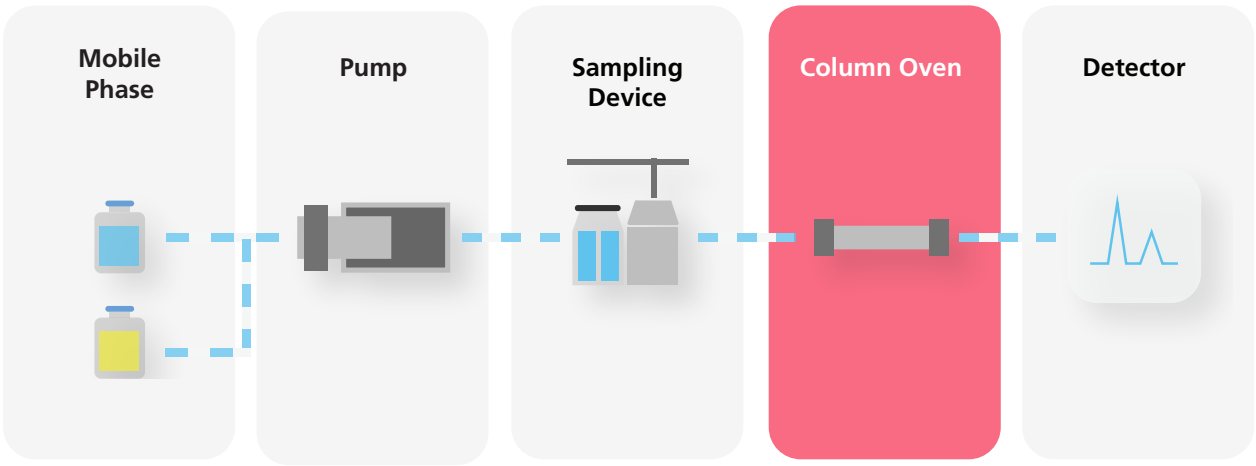


4.2 Sampling Device

In a series of preparative HPLC work, if only one or two injections of sample are done, the manual injector is sufficient for use. When using a manual injector, both analysis scale and preparative scale can be performed depending on model and injection volume. If you want to inject multiple samples continuously or if you want to repeatedly inject the same sample, you can automate by introducing an autosampler. By scaling up the sample injection volume according to the cross sectional area ratio, it is possible to select injectors suitable for injection volume. When using the autosampler, the maximum injection volume and setting range vary depending on the model. Sometimes it is possible to increase the injection volume of an existing autosampler by adding an optional sample loop. When introduced in conjunction with the fraction collector it is more automated as a whole and more effective.

In preparative HPLC, a high concentration sample is injected in a large volume, so samples may remain in the sample injection area such as needle and seal (carryover). When using the same system on the analysis scale or when checking the purity of the fraction, this carryover is an obstacle, so measures such as thorough washing are necessary.

Configuration of Preparative HPLC

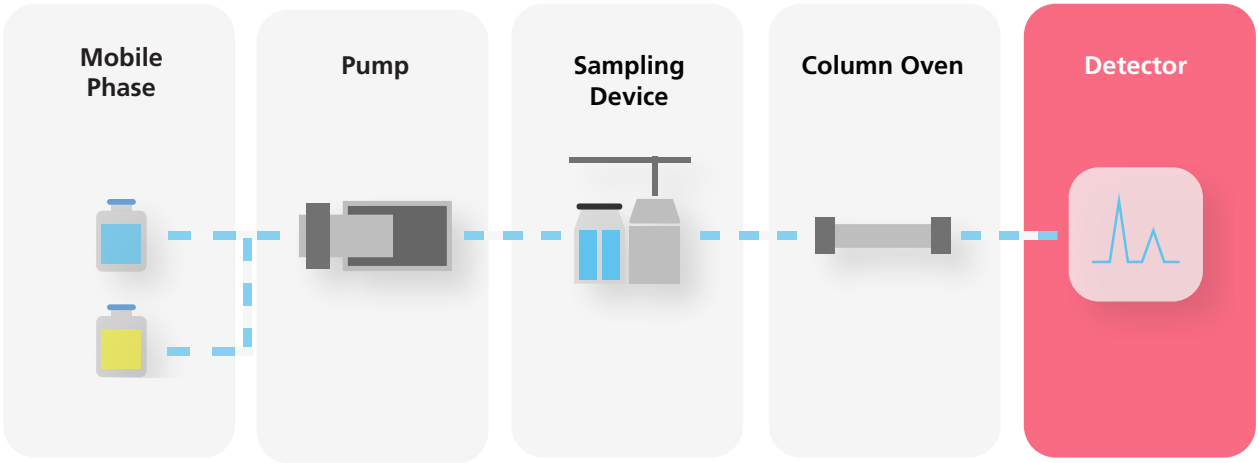


4.3 Column Ovens

The column oven is selected according to the temperature range to use, the column size, the storage of valves, etc. For the connection of the column, it is necessary to apply the inner diameter and length of piping so that pressure is not applied too much in accordance with the inner diameter, and the separated peaks do not diffuse more than necessary. Also, since the preparative column tends to be heavier than the analytical column, use a holder that can securely fix the column. For analytical / preparative switching systems, analytical and preparative columns can be connected to a single device via flow path switching valves.

Also, if the difference between the mobile phase and the column oven temperature is large, the internal temperature of the preparative column does not reach the set temperature, which may affect the peak shape as a result. As a countermeasure, it is conceivable to provide a preheating coil before the entrance of the column. Owing to this issue, normally column oven is not necessary when performing preparative LC.

Configuration of Preparative HPLC



4.4 Detectors

UV / VIS detectors and PDA detectors are most commonly used. In preparative HPLC, a high concentration sample is injected in a large volume, so select a wavelength with low sensitivity or use a detector cell with a short optical path length so that the peak on the chromatogram is not saturated.

When the target compound or its impurities in the sample solution do not have sensitivity for those detectors, such as ELSD (Evaporative Light Scattering) or MS detector (mass spectrometer) can be used. However, since these detectors exhaust and consume the sample passing through, it is necessary to divide the flow path after leaving the column into the detection section and the fraction section. ELSD measures the scattered light by atomizing the target compound by evaporating the eluent eluted from the column. It has the feature that most substances can be detected if it is a nonvolatile substance, the baseline is stable and gradient analysis is also possible. Fractionation using an MS detector can achieve fractionation of high purity target components from a sample containing many impurity components by utilizing the high selectivity of LCMS.

RI (Refractive Index) is also used to detect compounds that are not sensitive to UV / VIS detectors, but for reasons such as requiring time to stabilize, relatively low sensitivity, weak against back pressure, isocratic method, etc. Another key factor for RI Detector is, only isocratic method can be utilized. It is not very suitable for preparative HPLC.

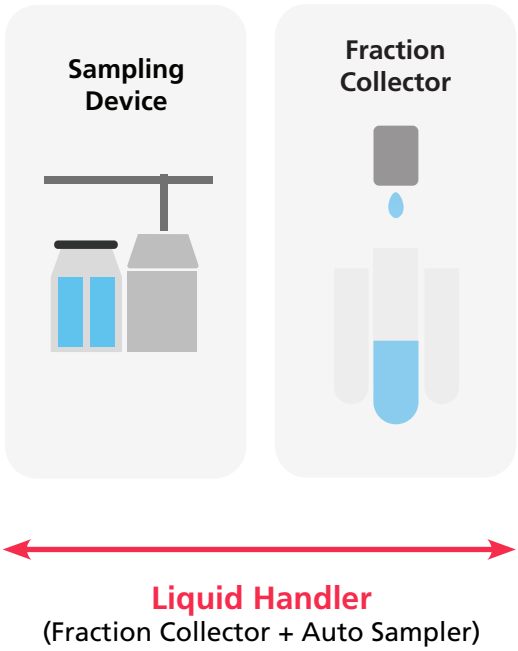
4.5 Fraction Collectors

Manual fractioning can also be performed when target amount of target component can be separated by one to several sample injections. But when multiple fractioning is repeated, or when multiple components are fractionated by one sample injection, it is convenient to use a fraction collector. It is possible to start and terminate fractionation with retention time, detector signal height and molecular weight of target component as MS triggers. Multiple signals can also be triggered. In a preparative HPLC with a relatively small scale, it is necessary to confirm and set the difference between the time at which the sample signal is divided by the detector signal and the time at which the target component actually reaches the fraction collector. Depending on the amount of fraction and the capacity of the fraction collector fractionation vessel, you may collect multiple fractions in one container, or separate one fraction into several smaller volume containers.

If the target component is an unstable compound, it may be necessary to consider the temperature of the environment in which the fractionation solution is stored, shading, etc.

• Liquid Handler

Liquid handler is a unit with auto injector and fraction collector function. It is very convenient because you can perform suction / injection of sample and recovery of fractionation solution with one unit, and re-inject fraction to check the purity.



• Measures to handle solvent leaks

A semi- or large-scale preparative system uses a lot of solvent, so that measures must be taken to cope with liquid leaks. For example, position a tray below the plunger head to cope with liquid leaks from the pump plunger seal. Take special care if using a flammable solvent. Recommended measures include not positioning equipment that is a potential ignition source nearby and

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Prep LC Primer

Application Systems

Shimadzu strives to improve the purity, speed, and cost efficiency of preparative HPLC. Shimadzu has a worldwide sales record of over 2000 preparative HPLC units, which are being used across a range of scales and applications. This page introduces the features of each system, to allow the customer to select the appropriate preparative system for the target fraction volume and sample type.

Large-Scale Preparative System

Semi-Preparative System

LCMS / ELSD Preparative System

Analytical / Preprative Automatic Switching System

High-Performance Preparative Recycle System

High-Performance Semi-Preparative Recycle System

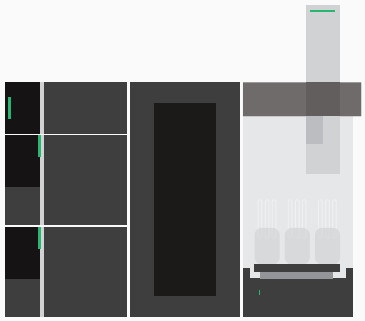
Open Solution - Supports Compound Check and Purification

Ultra Fast Purification Liquid Chromatograph System

Semi-Preparative SFC System

Application Systems

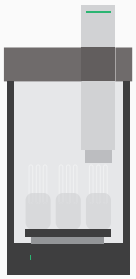
5.1 Large-Scale Preparative System



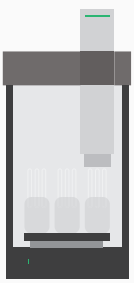
▶ Large-Scale Preparative System (20AP type)

The large-scale preparative system employs an LC-20AP solvent delivery unit. This is a powerful unit offering a maximum flow rate of 150 mL/min that is suitable for automated continuous fractioning with a 20 to 50 mm I.D. column. It can also be used to investigate separation conditions and load conditions and to test the purity of liquid fractions using an analytical column (1 mL/min).

The system can be configured using a variety of options, including five sample injectors, three recycle valves, and multiple fraction collectors.



▶ Liquid Handler, LH-40



▶ Fraction Collector, FRC-40

The new Liquid Handler LH-40 and Fraction Collector FRC-40 are the latest additions to the Shimadzu Preparative LC product family. Specifically for LH-40, the liquid handler is capable of performing large volume injection, standard injection volume is 2 mL and up to 20 mL injection volume optional is available, and variable volume sizes fraction collection at the same time.

Application Systems

The flexible configuration of LH-40 can also be further extended to perform re-injection to carry out purity check on each fraction. To further increase the efficiency, the system configuration can be extended to have combinations of multiple LH-40 and FRC-40. The optional liquid level detection makes sure all remaining sample is fully aspired, without having to manually input the accurate injection volume. In addition, this functionality also avoids the injection of air bubble into separation column, which will disrupt the separation performance.

Simple software operation for both LH-40 and FRC-40 makes the overall preparative parameters setting hassle-free. The new patented technology promises not only user-friendly software, but the algorithm calculation ensures excellent judgment on the desired peak collection, to avoid fractionation of unwanted noise peak.

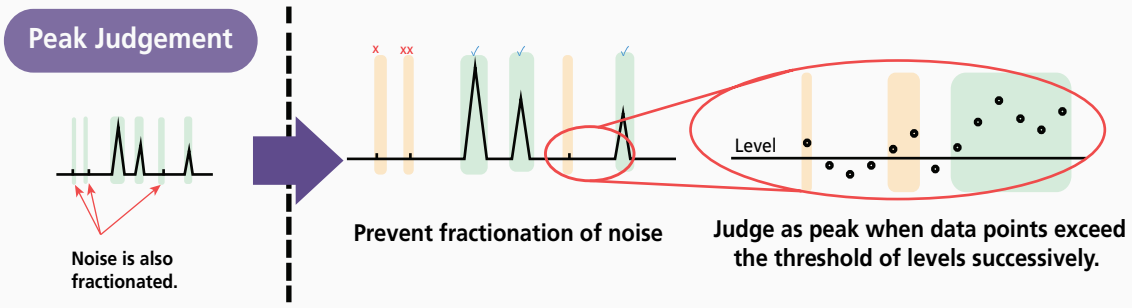
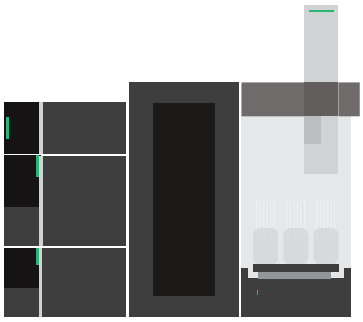


Figure 6: Accurate sample peak judgment for sample fractionation

The sample rescue function also assists in recovering loaded sample, when issue surfaces, e.g. column clogging, and etc. This is especially useful when purifying precious sample, to avoid any sample loss.

Application Systems

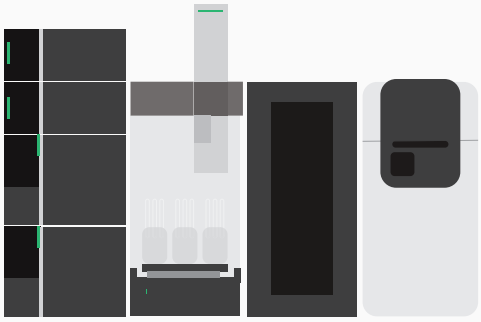
5.2 Semi-Preparative System



▶ Semi-Preparative System (20AR type)

A system using LC-20AR supports columns from analytical columns to 20 mm I.D. semi-preparative columns and a system using LC-20AT supports columns from semi-micro analytical columns to 10 mm I.D. semi-preparative columns. Systems can be configured to suit their purpose, from simple systems with manual injection and isocratic separation to automated systems with Autosampler injection and gradient elution.

5.3 LCMS/ELSD Preparative System



▶ LCMS / ELSD Preparative System

If the target compound does not absorb light or if the impurities in the sample solution do not absorb light, the preparative purification efficiency can be enhanced by also using a detector not based on the principle of light absorption, such as an ELSD (Evaporative Light Scattering Detector) or MS (Mass Spectrometer). In particular, fractioning using an MS trigger can exploit the high selectivity of LCMS to achieve high purity of the target component from samples that contain many impurity components. In some cases, user requirement to have multi-detection system; the system can be easily configured to have UV/PDA, with ELSD and/or MS.

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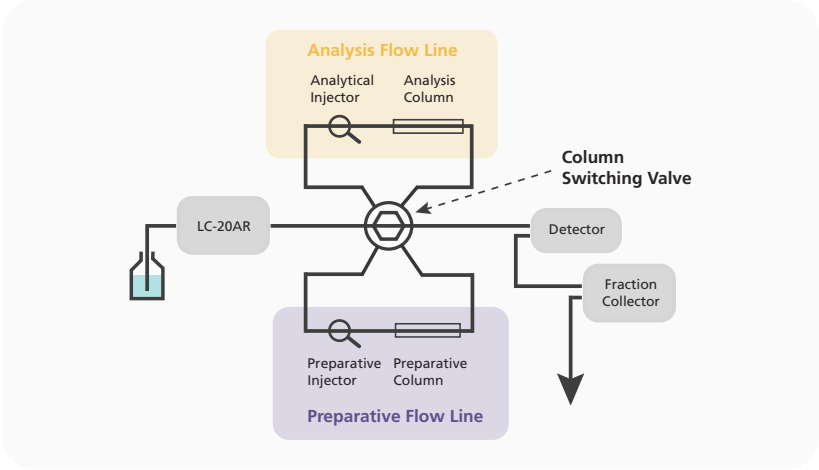
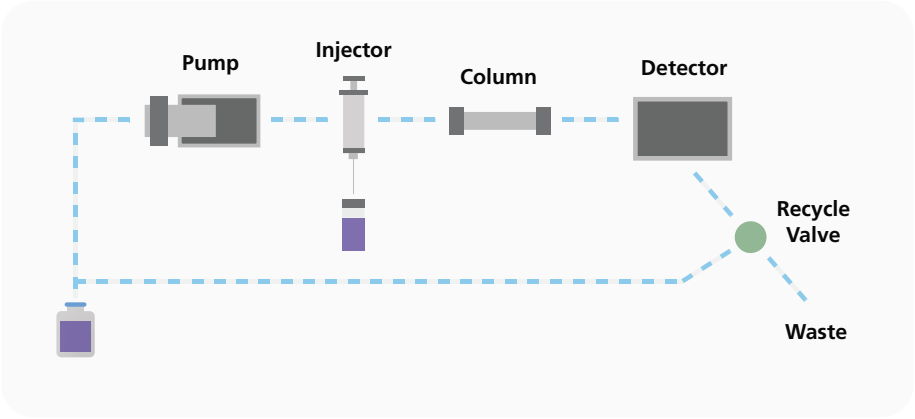
5.4 Analytical/Preparative Automatic Switching System



▶ Analytical / Preparative Automatic Switching System

The Analytical/Preparative Automatic Switching System permits smooth investigation of the fractioning conditions at both the analysis and preparative scales. Simply operating a flow-line switching valve makes it easy to switch between the analysis and preparative scales. Using a single system to handle all processes from investigating separation conditions and sample loads with a conventional column to scaling-up reduces the consumption of mobile phase solvent. After fractioning, this system allows smooth and efficient switching to the analytical mode.

5.5 High-Performance Preparative Recycle System



▶ Closed-Loop Recycle Flow Diagram

The recycle separation method achieves an effect equivalent to increasing the column length by repeatedly introducing into the same column the eluate band containing the target component that eluted from the separation column.

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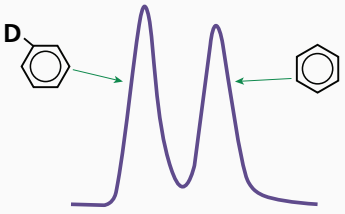
5.6 High-Performance Semi-Preparative Recycle System (20AR type)

This preparative recycle system using a 20AR solvent delivery unit (max. flowrate: 20 mL/min with 20AR, 150 mL/min with 8A) incorporates a kit to minimize the internal volume and permit highly effective recycle separation (closed-loop recycle separation). Combining this system with the appropriate column can achieve a theoretical plate number greater than 1 million. This permits separation of benzene and mono-deuteriated benzene substitute, which have only extremely small differences in properties (see diagram below).

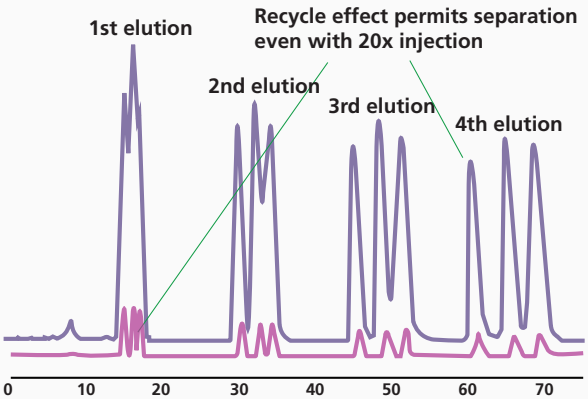
Also, if the elution volume until the peak appears is 35 mL or more, recycle separation can be performed with an analysis size column (see diagram below).

Recycle separation is perceived as a time-consuming technique. However, in separation of proximate components, the fractioning rate per unit time can be increased in some cases by using recycle separation with the injection of a large amount of sample (see diagram below).

▶ **High-Performance Semi-Preparative Recycle System (20AR type)**



▶ **Separation of Benzene and Mono-Deuteriated Benzene Substitute Column: STR ODS-II (250 x 20 mm I.D.) x 2**



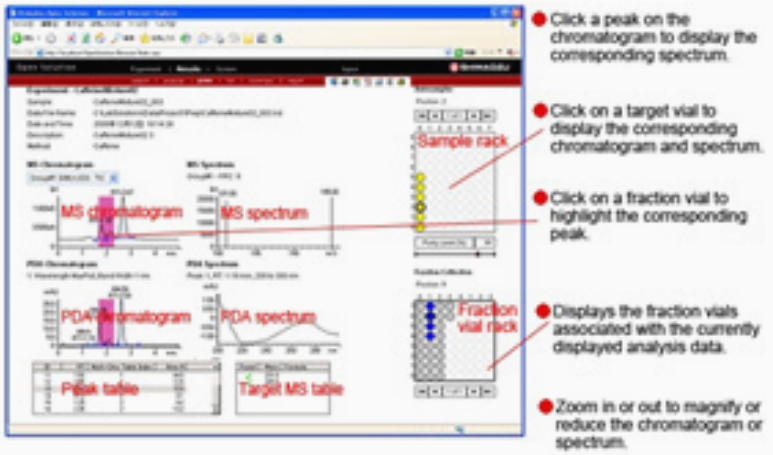
▶ **Increased Fractioning Rate Using Recycle Separation with Analytical ODS**

Column Column: STR ODS-II (150 x 6 mm I.D.)

Flowrate: 2 mL/min, sec-/iso-/n-butylbenzene

Application Systems

5.7 Open Solution — Supports Compound Check and Purification

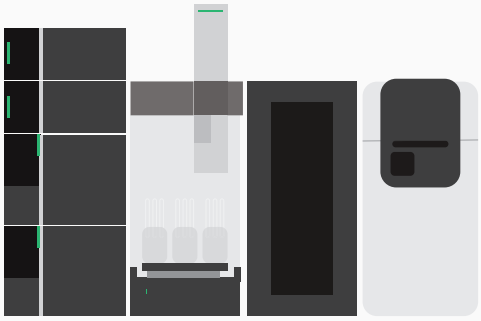


▶ **Open Solution Fractioning Results Check Window**

Preparative systems are often used for the synthesis checks and purification of compounds. However, the large number of samples handled results in fractioning to a huge number of vials, which makes it difficult to determine which peak was fractioned to which vial. Therefore, error-free sample tracking is extremely important for the samples supplied for fractionation, their respective chromatograms and peaks and the associated vials (target component fractions).
Open Solution displays the mutual relationship between the vial rack diagram, the fractionation results, and the fraction vials. Click on a vial to immediately check the relationship between the chromatogram, fraction vial, and spectra.

Application Systems

5.8 Ultra Fast Purification Liquid Chromatograph system

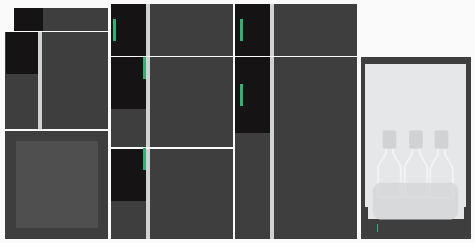


UFPLC System

The UFPLC system drastically saves the preparative purification process by bringing the preparative steps from preparative to concentration, purification and recovery on-line. The system can be conveniently extended to the preparative system to give complete online purification/ recovery system. The UFPLC system utilizes the solid phase extraction technology, by trapping the fractioned compound in the Shimadzu proprietary trapping column technology.

After successful trapping of desired compound, the flow is reversed and trapped compound is eluted out with organic solvent. Since highly volatile organic solvent is used for recovery of the target component, the time required for evaporation to dryness can be shortened to less than 1/10 of the conventional time. In addition, washing off the counter ion with a rinsing solution step can be added before eluting the desired compound; in such scenario, the compound can be free-based and recovered as a pure free base (free base form) compound. This step significantly reduces additional manual free-base step, to avoid cross-contamination. The purified compound can be obtained in shorter time, excellent purity and high recovery. The quality of the discovery research of new drugs, such as drug efficacy screening and pharmacokinetic testing of drug candidates will be greatly improved.

5.9 Semi-Preparative SFC System



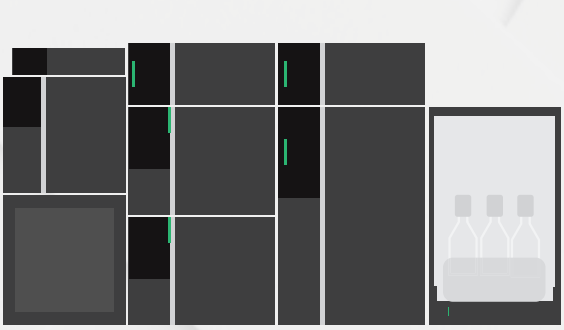
Semi-Preparative SFC System

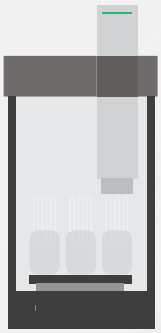
The Nexera UC Prep is a new preparative supercritical fluid chromatography system that offers both the high basic performance developed for the previous Nexera UC model and original state-of-the-art preparative SFC technologies.

Due to the low viscosity of CO2, higher flowrate can be achieved, and faster analysis can be performed especially for chiral or isomer separation. Additionally, the complete drying process can be significantly shortened because usage of CO2 as mobile phase. The most unique Nexera UC Prep is the introduction of LotusStream gas-liquid separator, which promises superior sample recovery, and excellent low carryover at the same time. Nexera UC Prep resolves a number of issues in preparative tasks, reducing labor and improving efficiency while fitting into pre-existing workflows. Not only does the Nexera UC Prep achieve superior recovery rates for purification, it provides flexible system configurations in a compact design, requiring low installation space and allowing you to maximize lab resources.

Summary

- We will start with clarifying the purpose of sorting, grasping the overall flow from sorting to post-treatment, and establishing a strategy
- Before starting preparative HPLC, we will examine separation conditions on analysis scale
- Fractionation conditions evaluate loading, particle size of the column and other influencing factors
- By appropriate fractionation conditions, you can obtain a chromatogram similar to the chromatogram on the analytical scale
- Proper recycling sorting can improve insufficient separation
- By using an autosampler or fraction collector, it is possible to efficiently and reliably perform sorting





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