See also: **II/Chromatography:** Countercurrent Chromatography and High-Speed Countercurrent Chromatography: Instrumentation. **Chromatography: Liquid:** Countercurrent Liquid Chromatography. **III/Alkaloids:** Gas Chromatography; High-Speed Countercurrent Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography.

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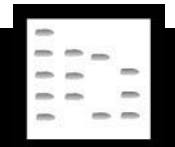
MEDIUM-PRESSURE LIQUID CHROMATOGRAPHY



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Introduction

Medium-pressure liquid chromatography (MPLC) is one of the various preparative column chromatography techniques. Separation under pressure renders the use of smaller particle size supports possible and increases the diversity of usable stationary phases. MPLC was introduced in the 1970s as an efficient technique for preparative separation of organic compounds. MPLC overcame one major drawback of low pressure liquid chromatography (LPLC), i.e. the limited sample loading. This separation method is now routinely used beside or in combination with the other common preparative tools: open-column chromatography, flash chromatography, LPLC or preparative high performance liquid chromatography (HPLC). The distinction between low pressure, medium pressure and high pressure LC is based on the pressure ranges applied in these techniques and the overlap is often considerable. MPLC allows purification of large compound quantities and, unlike open-column chromatography and flash chromatography, faster and improved separations are obtained. Packing of material with lower particle size under pressure enhances separation quality and moreover the solid phase can be reused. **Table 1** provides a comparative description of these different methods. Simplicity and availability of the instrumentation, together with recycling of packing materials and low maintenance costs, contribute to the attractiveness of this technique. More details about experimental conditions are given below.

Instrumentation

A schematic representation of a simple MPLC setup is shown in **Figure 1**. The instrumentation is made up of

Table 1 Comparative description of various preparative column chromatographic techniques

Technique	*Stationary phase particle size (μm)*	*Pressure (bar)*	*Flow rate (mL min^{-1})*	*Sample amount (g)*	*Solvents*	*General*
Open-column chromatography	63–200	Atmospheric	1–5	0.01–100	General solvent used	Frequent packing; RP not possible
Flash chromatography	40–63	1–2	2–10	0.01–100	General solvent used	Frequent packing
Low pressure LC	40–63	1–5	1–4	1–5	More solvent required	Prefilled Lobar columns
Medium pressure LC	15–40	5–20	3–16	0.05–100	More solvent required	Infrequent packing
Preparative HPLC	5–30	> 20	2–20	0.01–1	High purity solvent required	Higher resolution

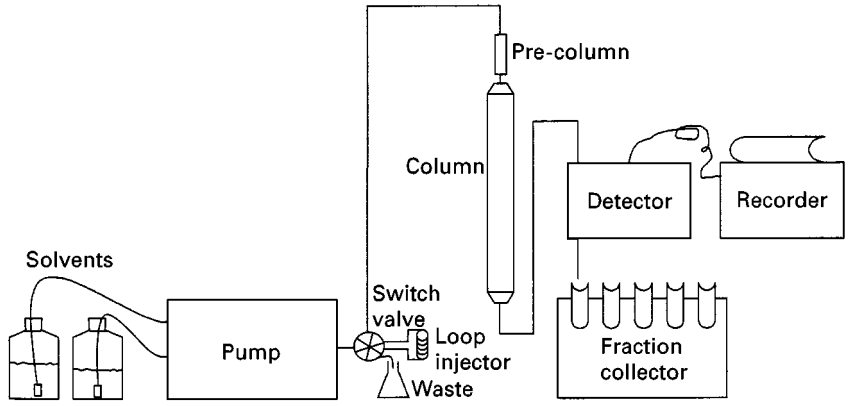

Figure 1 Typical MPLC instrumental setup.

a pump for solvent delivery, a sample injection system, and a self-packed column. Product separation can be followed either manually by monitoring with thin-layer chromatography (TLC) or automatically with a detector and a recorder connected to the column outlet. Separated compounds are collected by means of a fraction collector.

Pump

Chromatographic separation of 0.1–100 g sample within a few hours requires flow rates ranging from about 5 to 200 mL min^{-1}, with a maximal pressure of 40 bar. Several companies provide pumps suitable for MPLC. Criteria for selecting an MPLC pump include: flow rate range; presence or absence of a pulse-damper, which provides regular flow rates and pressures during separation and increases the reproducibility of the separation; presence of a pressure cut-off device; and presence of a gradient former. Some manufacturers provide pumps with exchangeable piston heads, thus allowing flow rates from 0.5 to 160 mL min^{-1} with pressures up to 40 bar.

Column

The column is the central point when optimizing a preparative chromatographic separation and criteria such as amount of sample to be purified, amount of packing material and column length versus column diameter, have to be carefully considered. MPLC columns are generally made of thick glass coated with protective plastic and can withstand pressures up to 50 bar; however, some columns from some manufacturers cannot withstand pressures exceeding 20 bar. The columns vary in length as well as in internal diameter (i.d.) and sizes are expressed as filling volumes. Filling volumes range from 63 mL (9 mm i.d. × 100 mm) to 15 000 mL (105 mm i.d. × 1760 mm) for the larger columns available. Selection of the column dimensions depends on the sample amount to be separated, ranging from 0.1 g with the smallest columns up to 100 g with large columns. Selectivity (α) and retention factor (k) are the prominent factors influencing resolution. Sample loading can greatly affect resolution. Therefore, when separations are 'easy' ($\alpha > 1.2$; high resolution between the eluted compounds), larger sample amounts can be loaded. Increasing the column diameter allows injection of a larger sample mass (higher throughput), but also makes use of smaller particle size material possible. On the other hand, increasing the column length results in higher resolution but has little or no effect on sample throughput. The back pressure increase with longer columns often implies the use of larger particle size material. The influence of column dimensions on resolution has been studied through the separation of standard mixtures. The correlation between resolution and amount of packing material was shown to be linear either when testing columns with identical internal diameter and different lengths or when varying the internal diameter in a set of columns of the same length. However, a lesser increase in resolution was observed with the use of larger internal diameter and a constant length. Consequently, longer columns are preferred in order to improve the separation of a given sample. The column system supplied by Büchi (Flawil, Switzerland) gives the possibility to couple columns together vary simply by means of a Teflon sealing joint, resulting in an increased resolving power.

Detector and Recorder

Monitoring of a MPLC separation can be performed by TLC of the collected fractions. Online detection is also routinely used with single-wavelength UV/Vis detectors. Most available UV detectors are designed for analytical purposes and are of little use for

preparative separations. Accommodation of high flow rates is a prerequisite for a preparative chromatographic detector. This results in a loss of sensitivity, which is compensated by the usual high concentration of the eluate. In fact, these concentrations are often so large when coming through the detection cell that the detector is overloaded. This problem can be solved by the use of detectors with a splitting system before the UV cell: one part of the sample goes directly from the column outlet to the fraction collector, while another part is diverted through the detector. Detectors with a pathlength < 0.1 mm are also very useful. Selection of a detection wavelength where absorption of the products is low can also be an alternative to avoid detection overload. The Gow-Mac 80-800 LC-UV detector is a specially designed detector for preparative separations: flow rates up to 500 mL min^{-1} are possible and the eluate arrives through a needle and passes as a thin film on a 6.5 cm wide quartz cell. Connection to a recorder allows visualization of the chromatographic separation.

Fraction Collector

Automatic collection of fractions can be performed by connecting a fraction collector to the column or detector outlet. The volume of the collected fractions is of course strongly dependent on the internal diameter of the column and the flow rate; it is in most cases time-monitored. Presence of a built-in peak detector or connection to an external one allows peak-monitored fraction collection. In its standard MPLC setup, the Büchi system provides a fraction collector with a total capacity of 240×20 mL tubes, 120×50 mL tubes or 48×250 mL tubes. This type of fraction collector has proved to be particularly suitable for MPLC.

Column Packing

Packing Material

Selection of the stationary phase is probably the most crucial parameter affecting separation quality. Several types of packing material are commonly used in MPLC and various factors have to be considered when choosing the packing material:

- particle size
- column length
- operating pressure
- type of sample
- cost.

With regard to cost-effectiveness, the most frequently utilized stationary phase is silica gel. Beside its economic advantage, silica gel possesses other advantages such as a wide range of possible solvents as eluents, easy evaporation of the fractions and elution with high flow rates. The risk of irreversible adsorption is a possible major drawback of this support.

A wide range of particle sizes is commercially available. The smallest average particle size (5–10 μm) is used for analytical HPLC, while for preparative LC, stationary phases with sizes starting at 15 μm are the most convenient. Optimal separations are generally obtained with sizes around 20 μm. The influence of particle size on resolution has been investigated. A large decrease in resolution was observed when the average particle size changed from 15 μm to 30 μm. Using particle sizes of 52 μm or 130 μm resulted in a slower resolution decrease but the retention times increased significantly: 3 h more with an average particle size of 130 μm than with one of 15 μm.

The use of modified silica gel phase (bonded phases) has become more common. These inherent advantages, such as a lower risk of sample decomposition and less irreversible adsorption, allowing an easier recycling of the column sorbent. Reversed-phase (such as RP-18, RP-8 or RP-4) or dihydroxypropylene-bonded (Diol, Merck, Germany) silica gels are frequently used for MPLC separations. Moreover, it is possible to use other commercially available bonded phases for MPLC separations.

Column Packing Methods

Different filling methods are described for packing MPLC columns. Filled columns should possess an optimal homogeneity and a good density. Two methods are most frequently used: dry filling and the slurry method.

Dry filling Dry filling is generally applied for silica gel. This method usually gives a 20% better packing density than the slurry method. The 'tap-and full' technique can be used with particle sizes larger than 20–30 μm; however, when applying eluent pressure up to 40 bar, the packing density obtained may not be sufficient. Packing under nitrogen pressure allows use of 15 μm particle size silica gel and provides a high packing density. Filling is carried out manually by connecting a reservoir to the top of the column, which is then filled with dry stationary phase until it contains approximately enough phase to fill another 10% of the column (**Figure 2**). The system is then connected to a nitrogen cylinder and a 10 bar pressure is applied (with the column outlet open) until the level of packing material remains constant. The nitrogen valve can be closed and the pressure slowly goes down to atmospheric. Vacuum at the column exit can

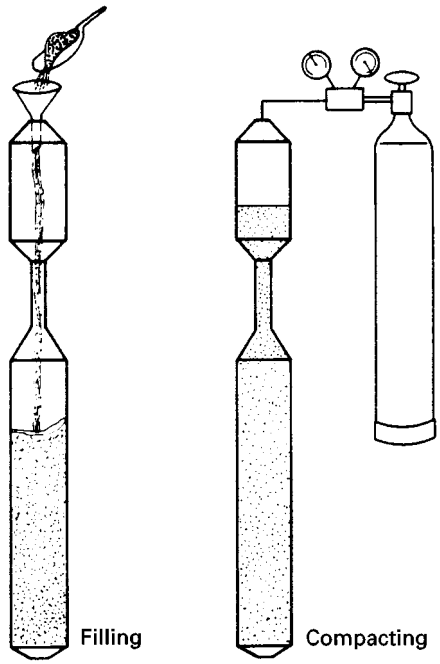

Figure 2 Dry filling of Büchi MPLC glass columns. (Reproduced with permission from Hostettmann *et al.*, 1997.)

be used as an alternative to nitrogen pressure. An automatic mechanism has been suggested to slowly and homogeneously fill the column (3–4 g min^{-1}). Passing mobile phase through the column induces compression of the stationary phase, which is compensated by the further addition of dry silica gel.

Slurry method The slurry method is an alternative method to pack silica gel, with the inconvenience of a lower packing density. However, slurry filling is the preferred method for packing bonded phases. The slurry is prepared by suspending homogeneously an appropriate amount of stationary phase in the eluent. The mixture is then poured into the column and the eluent is passed through the column until the stationary phase level is constant.

Column preparation and regeneration Before sample introduction, it is recommended that a separation test is performed with a standard mixture of compounds. A mixture of phthalic acid dimethyl-, diethyl- and dibutyl esters is convenient for testing silica gel columns, whereas the separation of benzene and naphthalene can be used for reversed-phase columns.

Usually, packing material is regenerated after each chromatographic separation. For silica gel supports, this can be performed by washing successively with methanol, ethyl acetate and *n*-hexane. However, after a certain time, the stationary phase should be changed and the column repacked. Regular elution of a test mixture is a good method to determine column quality. Bonded-phase columns are generally easier to clean (for example with a mixture of methanol/tetrahydrofuran (1 : 1)) and thus have a longer working life. In order to prolong column lifetime, a pre-column can be used. Contaminating material remaining at the top of the column is thus eliminated after each MPLC separation.

Solvent Selection

Selection of the eluent system is also a crucial point in development and optimization of a MPLC separation. The ideal case would be successive direct testing of various solvent mixtures on the MPLC column. However, in routine practice such an approach is obviously impossible because of the waste of time due to column equilibration, together with loss of sample, etc. Two methods are mainly used for solvent selection: optimization by TLC or transposition of analytical HPLC conditions on MPLC.

Preliminary TLC allows rapid screening of numerous possible solvents and it is now well established how TLC results on silica gel plates can be transposed to silica gel columns. Solvent testing on silylated TLC plates can be used for reversed-phase columns. One important factor that has to be considered is that the surface areas of silica gel used in TLC is about twice that of the column packing material. Therefore, it is recommended that sample constituents display a retention factor (R_F) lower than 0.3 on the TLC plate. The major drawback of this method is the lower separation and resolution observed when reducing the solvent strength to obtain an $R_F \leqslant 0.3$. An alternative has been suggested to circumvent this problem: the use of overpressured-layer chromatography (OPLC) as a pilot method for MPLC. In a first step, a suitable multicomponent eluent with a good selectivity is searched for by means of TLC. Adjustment of the solvent strength and fine tuning are performed with OPLC. Unlike TLC, OPLC is a closed and equilibrated system and can be viewed as a 'planar column'. Because of these properties, direct transposition from OPLC to MPLC is an accurate and efficient method. Such an approach is also applicable to the other preparative pressure chromatography techniques using normal silica gel as stationary phase.

Because of the similarities of the phases used in analytical HPLC and preparative packing materials, separation optimization on an analytical HPLC column very often provides excellent results and transposition to MPLC is straightforward and direct. This is particularly evident for separations on

reversed-phase sorbents, where studies with TLC are more difficult. Due to the wider range of solvents available for normal-phase chromatography, preliminary tests on TLC are of major use prior to analytical HPLC optimization. Examples of transposition of analytical HPLC conditions to MPLC are given below.

Once the ideal conditions have been selected, a compromise has to be found between speed of separation and sample loading: decreasing the solvent strength (for example, by adding water to the solvent system in reversed-phase separations) will increase the separation between the different components and afford higher sample loading, but will require a considerable longer separation time. The influence of solvent strength on the resolution of a standard mixture has been studied and a linear decrease of resolution was observed when increasing the solvent strength. Running a gradient is also possible with MPLC, provided a suitable solvent delivery system is used. Peak sharpening can be obtained by a simple stepwise change of mobile phase composition.

Evaporation of large quantities of solvent takes place after fraction collection in order to concentrate the purified compounds. This procedure can cause the accumulation of considerable amounts of nonvolatile impurities from the solvent. As high purity solvents are very expensive, preliminary distillation of ordinary grade solvents to prepare the eluent can be a good compromise between solvent purity and quantity employed. Use of such lower grade solvents often implies an additional purification step by gel filtration, for example.

Sample Introduction

Several criteria have to be considered before sample injection on a MPLC column:

- sample preparation
- sample mass and volume
- solubility characteristics of the sample
- type of injection used.

If solubility is not a problem, the eluent should be chosen to dissolve the sample. However, even in such a case, care has to be taken to adjust the sample volume: a sample that is too dilute (injection of a large volume) results in decreased separation efficiency, while precipitation at the top of the column may be observed by injection of samples that are too concentrated. High concentrations of the sample may alter the viscosity of the solution, which is then very different from that of the mobile phase. High viscosity leads to severe tailing, while fronting may result from lower sample viscosity compared to the mobile phase. Despite these inconveniences, injection of a small volume of a concentrated solution is usually preferred. Sample solubilization in a solvent different from the mobile phase is also possible, but special care has to be taken with such an approach: solubility after mixing the sample solution with mobile phase has to be checked in order to avoid sample precipitation on the top of the column. Samples can be injected either directly on the column through a septum, or by means of a sample loop. In both cases, injection success depends on the quality of the column packing to ensure a homogeneous distribution of the sample at the top of the column.

The various problems mentioned above are more frequently encountered with separations on reversed-phase columns. The mobile phase usually contains a large proportion of water and organic compounds are often encountered that are insoluble in water. Solid injection or solid introduction is an alternative to circumvent low sample solubility. The introduction mixture is prepared by mixing dry powdered sample with a suitable amount of column packing material. The sample can also be preadsorbed on stationary phase by removing the volatile solvent (e.g. dichloromethane, ethyl acetate, acetone, etc.) in which it was solubilized from the suspension containing the stationary phase. Homogeneity of the injection powder is a prerequisite for efficient separations. The proportions of the introduction mixture are generally one part sample mixed with two to five parts stationary phase. The prepared sample is then placed directly onto the column inside a small precolumn and the eluent is passed through the system for separation.

Applications: MPLC in Natural Product Isolation

MPLC has recently become widely used in the pharmaceutical, chemical and food industries, and many applications are found in natural product isolation. Both applications given below have been selected as examples of the transposition of analytical HPLC conditions to MPLC.

The methanol extract of *Halenia corniculata*, a Gentianaceae plant from Mongolia, was first passed through a Sephadex LH-20 gel column and the glycoside-rich fraction (300 mg) was then purified by MPLC on a reversed-phase RP-18 column, yielding six xanthone glycosides (1–6). The search for optimal conditions was performed by analytical HPLC (**Figure 3A**) and was followed by direct transposition to MPLC separation (**Figure 3B**).

The dichloromethane extract from the roots of *Tinospora crispa* (Menispermaceae) was first

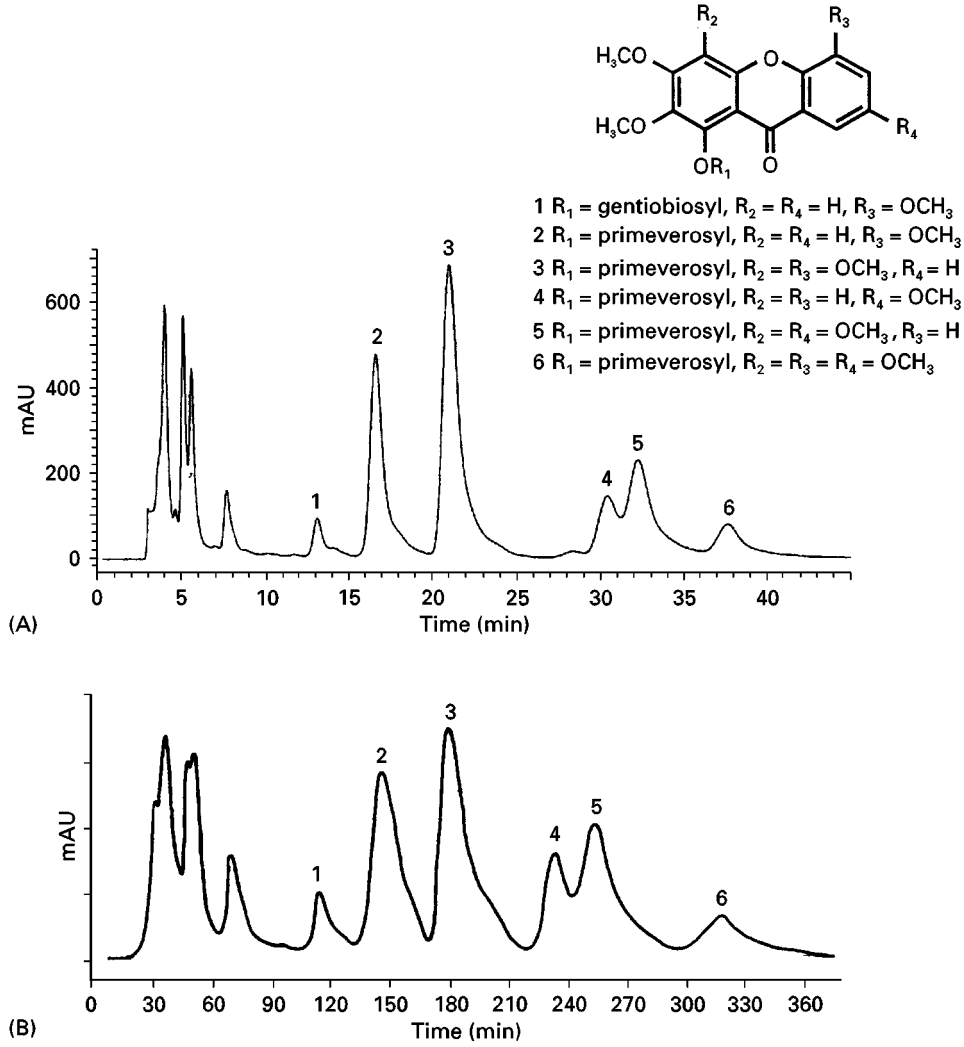

Figure 3 Transposition of conditions for the MPLC separation of xanthone glycosides from *Halenia corniculata* (Gentianaceae). (A) Analytical HPLC on a Lichrosorb 7μm RP-18 (250 mm × 4 mm) column with MeOH/H_2O 40 : 60 (v/v); flow rate 1 mL min^{-1}; (B) MPLC on Lichrosorb RP-18 (15–25 μm) with MeOH/H_2O 40 : 60 (v/v); flow rate 3 mL min^{-1}; column dimensions 460 mm × 12 mm. (Reproduced with permission from Hostettmann *et al.*, 1997.)

fractionated by centrifugal partition chromatography and one fraction was submitted to analytical HPLC with an acetonitrile gradient (**Figure 4A**). Owing to the lower convenience of acetonitrile for preparative purposes (cost, toxicity), conditions were found with methanol on analytical HPLC (Figure 4B). The selected isocratic eluent system was transposed directly to MPLC and 6-h separation led to the isolation of three phenylpropane derivatives (7–9) (Figure 4C).

Conclusion

Since the early 1980s MPLC has been confirmed as an excellent preparative chromatographic tool that is now routinely used in many laboratories. The extended use of various bonded phases in MPLC no longer restricts the use of this technique to the isolation of lipophilic substances with silica gel. For reasons of economy, recycling the stationary phase by simple washing or repacking of the column is of great interest in MPLC. Furthermore, a working experimental setup can be easily and rapidly assembled. The wide range of sample amounts that can be separated with this technique, together with the use of TLC and analytical HPLC in the search for optimal conditions, are also major benefits of this chromatographic method. However, good column packing and adequate sample preparation are prerequisites for successful separations. Further developments in MPLC

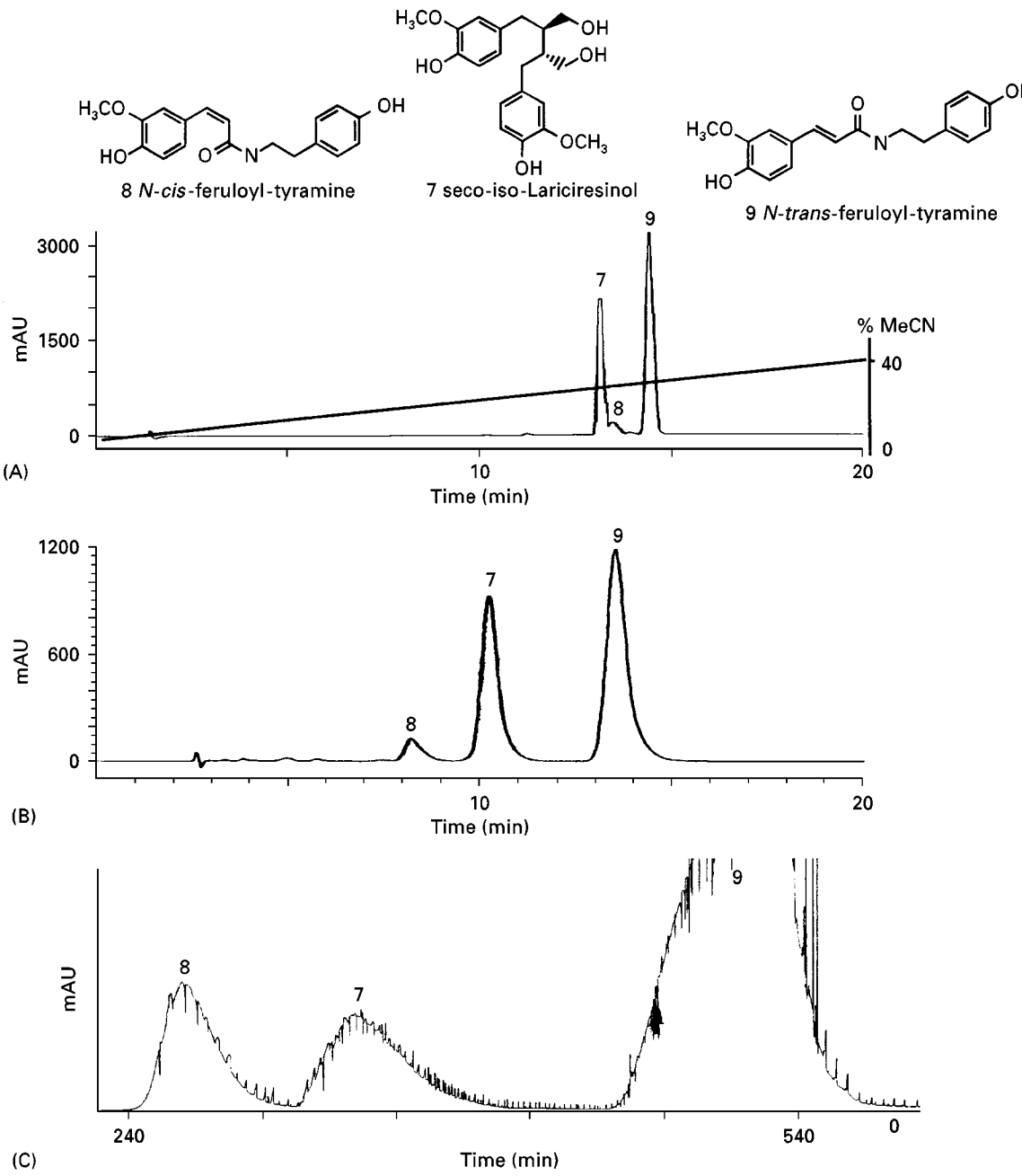

Figure 4 Transposition of analytical HPLC conditions for MPLC separation of phenylpropane derivatives from *Tinospora crispa* (Menispermaceae). (A) Analytical HPLC on a Lichrosorb 7 μm RP-18 (250 mm × 4 mm) column with a MeCN/water gradient 0 : 100 to 40 : 60 (v/v) in 20 min; flow rate 1 mL min^{-1}; (B) analytical HPLC on a Lichrosorb 7 μm RP-18 (250 mm × 4 mm) column with MeOH/water 40 : 60 in 20 min; flow rate 1 mL min^{-1}; (C) MPLC on Lichrosorb RP-18 (15–25 μm) with MeOH/water 30 : 70; flow rate 4 mL min^{-1}; column dimensions 460 mm × 12 mm.

will mainly concern detection problems with the optimization of detectors that can accommodate high sample loads.

See also: **II/Chromatography: Liquid:** Large-Scale Liquid Chromatography. **III/Flash Chromatography. Natural Products:** Liquid Chromatography; Thin-Layer (Planar) Chromatography.

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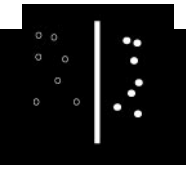
MEMBRANE CONTACTORS: MEMBRANE SEPARATIONS



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Membrane-based processes are receiving recognition for their flexibility and efficiency. Processes like reverse osmosis, ultrafiltration and dialysis are already well developed and recently, membrane application to other separation processes, such as absorption and liquid–liquid extraction, have been gaining considerable attention.

In these latter processes, the porous membrane acts as contacting media for gas–liquid or liquid–liquid phases with comparable advantages to the traditional continuous contact equipment. While in conventional two-phase processes, dispersion of one phase into another immiscible phase is used in order to promote an efficient contact and increase the transport rate, membrane extraction is accomplished without dispersion of the two phases.

Consider a liquid–liquid extraction process and a microporous hydrophobic membrane with the aqueous–organic interface stabilized inside the membrane pores (**Figure 1**). Since the membrane is hydrophobic, the organic phase spontaneously wets the membrane and may permeate through the pores to the aqueous phase. This breakthrough problem can be controlled by applying a higher pressure on the phase that does not wet the pores. This higher pressure must not exceed a critical value, Δp_{cr}, otherwise the nonwetting fluid will penetrate the pores and contaminate the other fluid phase.

If a hydrophilic membrane is used, the procedure is analogous, but in this case it is necessary to impose an organic phase pressure which is higher than that of the aqueous phase.

Although extraction can be conducted using a number of different membrane configurations, including flat-sheet, spiral-wound, rotating annular and hollow fibres, hollow fibres have received the most attention due to their high packing density: typical interfacial areas of contact per unit volume range from 1500 to 7000 $m^2\ m^{-3}$.

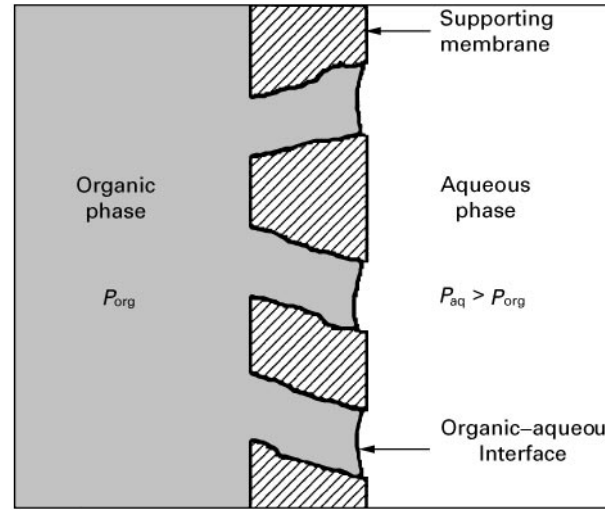

Figure 1 Organic–aqueous interface immobilized in a microporous hydrophobic membrane. P_{aq}, aqueous-phase pressure; P_{org}, organic-phase pressure.