Technological Innovation

High Performance Centrifugal Partition Chromatography

Automatic Operation Offers Simple Applications To Everyone

Model CPC240

Without A Solid Stationary Phase Support, Effectively Separate, Isolate And Purify Milligrams to Multigrams

- ◆Bio Polymers
- ◆Fermentation Products
- ◆Foods & Additives
- ◆Fine Chemicals
- ◆Genetically Engineered Substances
- ◆Natural Organic Compounds
- ◆Physiological Activated Substances
- ◆Pharmaceuticals
- ◆Petrochemicals
- ◆Rare Metal & Earth
- ◆And much more

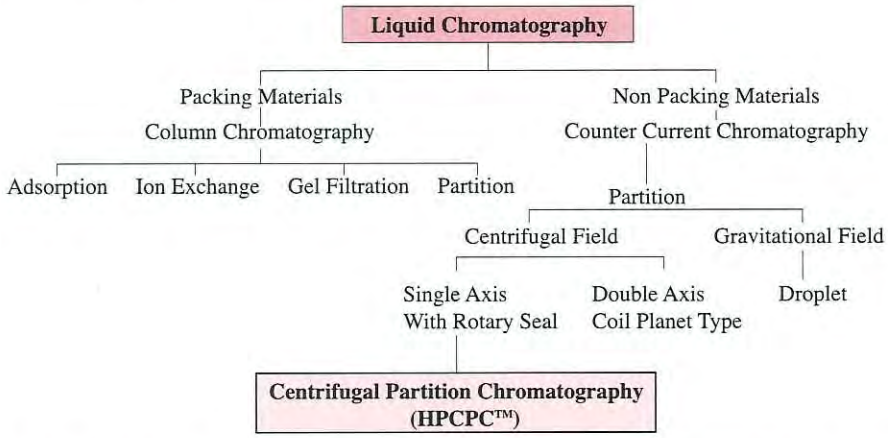
With

- ▼High Efficiency
- ▼Ultra-selectivity
- ▼ Short Run Times...and···
- ▼Retention of Virtually all
- ▼Biological Activity and
- ▼Molecular Integrity

EverSeiko Corporation

What is HPCPC™

High Performance Centrifugal Partition Chromatography, HPCPC™, is a new technique of Liquid Chromatography, LC, which is support free, and which finds its place among the several Liquid Chromatographic methods as shown on the following chart:



Around fifty years ago, the concept of partitioning solutes between two liquids gave birth to two cognate methods, one was the counter-current distribution, and another was the liquid- liquid partition chromatography. Thirty years ago, Sanki Engineering Ltd. (now absorbed by System Instruments Company Ltd.) opened the way to high performance centrifugal partition chromatography (HPCPC™), which was taking the best of the two first techniques, namely the versatility of a true liquid-liquid process combined with the quickness and advanced technology of chromatography. The HPCPC™ is gaining more and more interest as a semi-prep and preparative scale chromatographic method. The four main advantages of the HPCPC™ over its parent prepscale column chromatography are:

■**No loss of sample** since both mobile and stationary phases are liquids and can be collected for total recovery.

■The volume ratio of stationary to mobile phase is definitely much higher, which leads to **higher capacities and better resolution** with no need of a high number of theoretical plates.

■The **extreme flexibility of biphasic system** (mixtures of two or three or four solvents), which allows to modify the selectivity of a system in order to get a pure compound, in the HPCPC™ polarities of both phases can be smoothly modified.

■The reduced **solvent consumption, ten times less** than for preparative scale chromatography for the same throughput, which is of interest for environmental considerations. Separation accomplished with laboratory HPCPC™ can be directly scaled up to production scale HPCPC™.

■**Another major advantage** is the extremely low price of the stationary phase (solvents) compared to that of column packings. Moreover, stationary can be refreshed easily, and added materials like chiral selectors or complexing agents can be recovered with no loss. Several publications in international journals bring valuable information.

The new innovated HPCPC™ is getting more involved in many fields of chemistry, for purification of antibiotics, peptides, tannins, saponins, lipids, drugs,…… Its future development will see the production of bigger HPCPC™ units, and it will incorporate crucial fields of chemistry, such as chiral separations.

Superior To Conventional Preparative LC

◆ HPCPC™ is fast !

Since stationary phase solvents are retained in the partition channels by centrifugal force, high mobile phase flow rates may be used without appreciable loss of resolution.

◆ Great advantages over conventional preparative LC

Since a solid support is not used with HPCPC™ , irreversible retention of valuable sample components is completely eliminated, denaturation and decomposition, often encountered with conventional LC column packings, are virtually eliminated. And this is generally accomplished with retention of biological activity. Moreover, the capacity of an HPCPC™ column (rotor) is significantly greater than an HPLC column of the same total volume. Consequently, overload effects are rarely encountered with HPCPC. Purification of large quantities is routine, always with 100% material balance···there is no adsorption or irreversible retention.

◆ No columns or Packings to replace

The problems of formation of voids, contamination of fractions with silica and with components of previous runs, and the cost of replacing expensive HPLC columns are gone.

◆ Fewer Theoretical Plates are Needed

To achieve a given level of resolution between two peaks with HPCPC™ than with HPLC, for instance, for a value of α (selectivity) =1.2 and K (partition coefficient) = 1···
185,000 T.P are needed to achieve baseline resolution (Rs of 1.5) with HPLC···.whereas only 2,200 plates are needed to do the job with HPCPC™ This is a direct consequence of the standard resolution equation for liquid chromatography.

◆ Normal phase and reversed phase chromatography can be done in the same run

Use dual mode HPCPC™ to accomplish even the most demanding separations of samples containing complex mixtures of polar/non-polar, hydrophilic/hydrophobic substances. Even chiral substances are resolved with suitable chiral HPCPC™ Phases.

◆Work at any pH

With HPCPC™ , there is no need to concern yourself with unwieldy pH limitations that are often associated with solid stationary phase supports.

●Innovations overcome previous weakness of the CPC

Weak Point (1)

Separation tasks are intricate and need to attend all the time.
Overcome the above by innovated automation system.
1.Separation tasks can be preset on the 10 executed files.
2.Capable to link multiple files which have different separation conditions among the separation-executed files (solvents, rotations, flow rates, etc.) so that the most suitable conditions can be considered sequentially.
3.Sequential separations with same executed file and sequential injections into same stationary phase (reproducibility is very good by means of using same condition for separation of samples) can be performed easily. Automation can be materialized by connection of Autosampler and Fraction Collector.
4.Data and File handlings will become easy by linking with computer in which data acquisition program was pre-installed.

Weak Point (2)

Take long time to decide the most suitable separation conditions.
Overcome by improved small rotor which is just 1/3 volume of the previous model LLB-M.

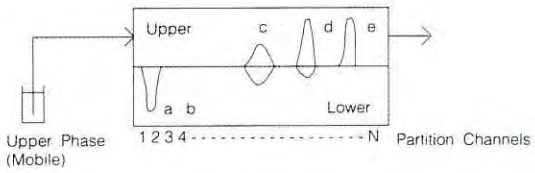
Principle of HPCPC™

The HPCPC™ is a new liquid chromatographic technique that utilizes liquid-liquid partition, counter current distribution, in the absence of a solid support, to perform separations of complex mixtures of chemical substances. The HPCPC™ is an alternative to packed-bed columns for preparative HPLC and operates by classical liquid-liquid partitioning in a high performance centrifugal system. A solid stationary phase is not used. Instead, stationary phase liquid is retained by centrifugal force in discrete partition channels within a unique patented circular Partition Disk Pack. A packed column generally contains only 2 to 7 percent of stationary phase, severely limits its capacity. In an HPCPC™ system, the column contains between 50 and 80 percent stationary phase. The stationary phase is held in numerous discrete partition cells. Microdroplets of mobile phase liquid pass continuously through the stationary phase liquid. Any two-phase solvent mixture can be used, at any pH, to perform normal and reversed phase chromatographic separations.
An injected sample, carried by the mobile phase, moves sequentially through the partition channels, where components are partitioned between the mobile and stationary liquid phases, separated from each other on the basis of differences in their partition coefficients, and eluted.

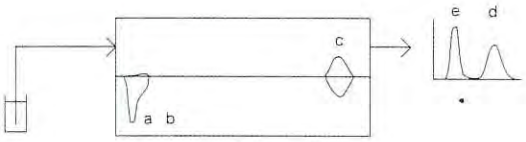
Single HPCPC™ Unit Introduces Dual Applications

Separation by either normal phase or reversed phase elution is accomplished with a single two-phase solvent system. Dual-mode HPCPC™ is illustrated for a hypothetical mixture of five components (a, b, c, d and e) as below. Assuming their "partition coefficients" (ratio of concentrations in stationary/mobile phase) are in the order a>b>c>d>e.

①Normal Phase Separation

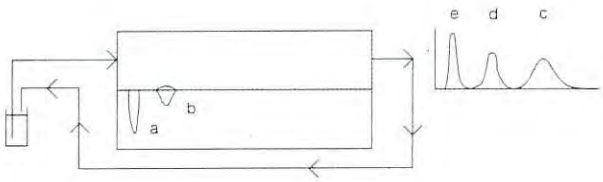


②Normal Phase Elution

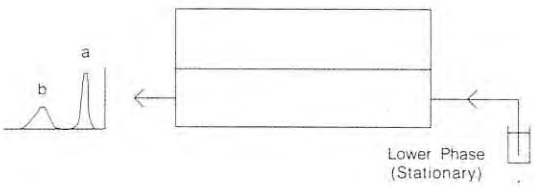


①The components with larger partition coefficients (a & b) are primarily remained in the "Lower Stationary Phase" due to their strong affinity for the Lower.
② The components with smaller partition coefficients (c, d & e) are separated from each other via "Upper Mobile Phase Elution". During upper phase elution, however, components a and b may migrate, very slowly and actually separated from each other within the stationary phase.

③Recycle

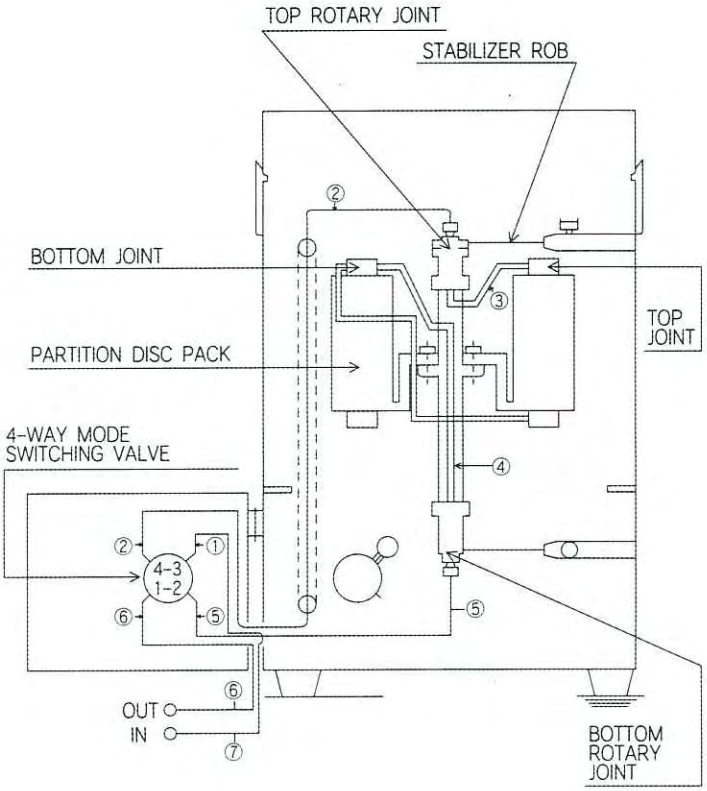


④Reversed Phase Elution



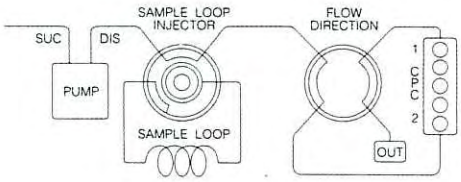
③After c, d & e have eluted, the Upper Mobile Phase is recirculated for a time sufficient for compete partition of components a & b.
④ The phases and the flow direction are reversed at this point and components a & b are eluted via "Lower Phase Elution".

Flow Diagram

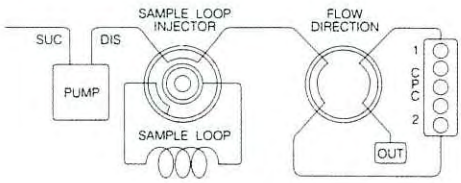


Pilot-scale Model CPC5400

1.Washing

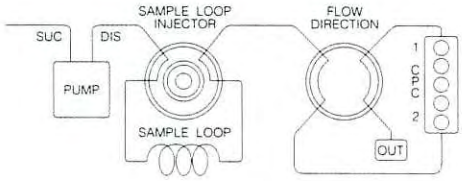


2.Injection



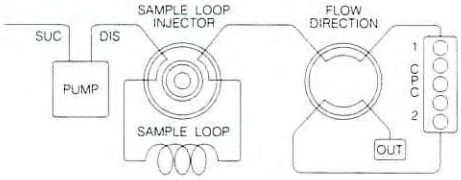
3.Ascending Mode

Mobile Phase: Upper



4.Descending Mode

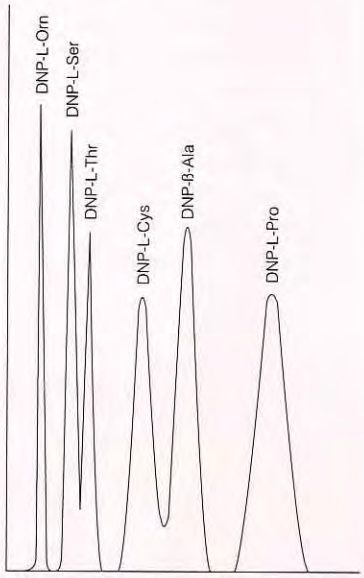
Mobile Phase: Lower



Rotor

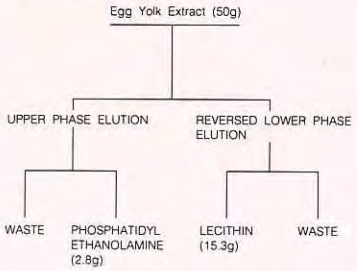
Applications

Amino Acids



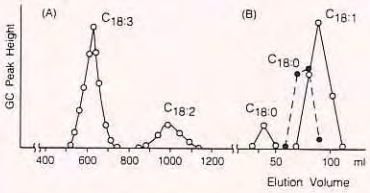
Reversed phase elution of DNP derivatives of amino acids.

PHOSPHOLIPIDS



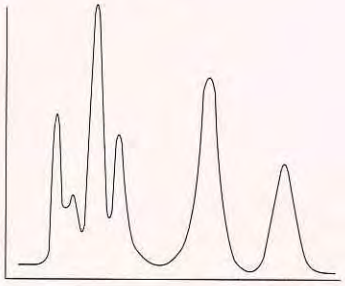
Phosphatidylcholine (lecithin) may be readily isolated and purified in preparative scale with excellent yield and purity by CPC.

Fatty Acids

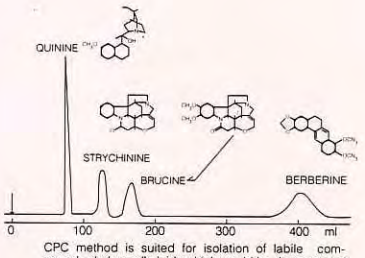


C_{18} series unsaturated fatty acids esters were isolated from vegetable oil preparation. Linolenic ($C_{18\ 3}$, purity 98.3%) and linoleic ($C_{18\ 2}$, purity 99.8%) acids esters were obtained in preparative scale.

Steroidal Saponins

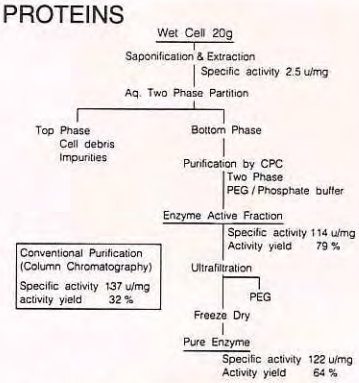


ALKALOIDS

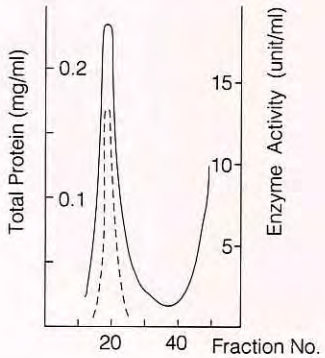


CPC method is suited for isolation of labile compounds shch as alkaloids which would be decomposed or lost upon passage through column packing resins.

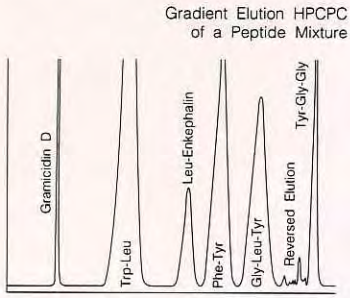
PROTEINS



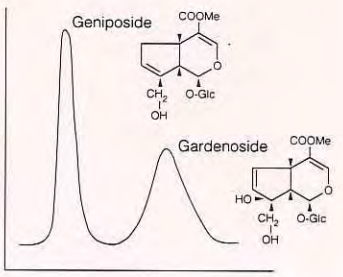
Enzyme, L-leucine dehydrogenase from B. sphaericus, was purified by CPC using aqueous polymer two phase. The procedure is simple and activity yield is better than the conventional separation method.



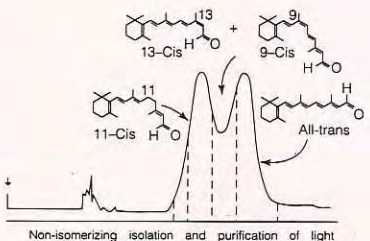
Peptides



Iridoid Glucosides

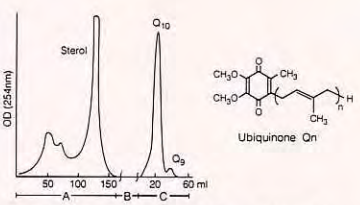


NATURAL PRODUCTS



Non-isomerizing isolation and purification of light and heat- sensitive retinals by CPC.For these substance, HPLC was unsuccessful for the isolation of more than sub-milligram quantities. (Bruening, R.C., Derguini, F., Nakanishi, K., J. Chrom., 357:340-343 (1986)

Co-Enzymes



High-grade co-enzyme Q_{10} (99.5 %) was obtained with CPC by normal phase elution after reversed phase development. Resolution between Q_9 and Q_{10} was almost identical with that of analytical HPLC.

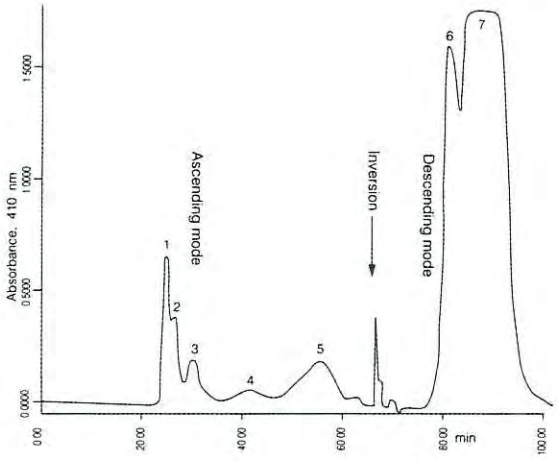
A: Reversed phase elution
B: Reversed phase development (recycle)
C: Normal phase elution

Selection of Two-Phase Partition Solvents

A two-phase solvent system is used as separation medium in Centrifugal Partition Chromatography. One serves as the stationary phase, the other serves as the mobile phase. The solvents may be selected from an infinite variety of possible combinations. Followings are some frequently used solvent combinations.

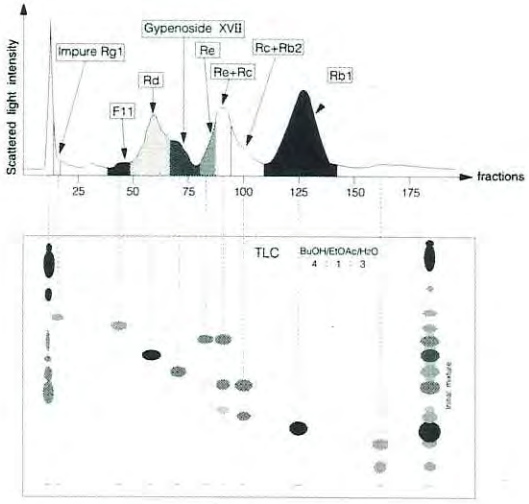
Samples			Two-Phase Parition Solvents
Water-Soluble MW>2000	Bio high polymers (Proteins)	Denaturalized/sluggish in Organic solvents	Dextran/PEG
		No denaturalization/sluggishness in high temperature solvents	PEG/Aqueous salt solution EtOH/Aqueous salt solution Acetonitrile/Aqueous salt solution
	Bio high polymers (Nucleic acid, Sugars)		
	Oligomers (Peptides, Sugars)		Butanol/Acetic Acid/H_2O Butanol/Methanol/H_2O Butanol/Ethanol/H_2O
Water-Soluble MW>2000	Ionic substances		Butanol/Acetic Acid/H_2O Butanol/Methanol/Buffer Butanol/Ethanol/Buffer Ethyl Acetate/Buffer
	Non-ionics		Butanol/Acetic Acid/H_2O Butanol/Methanol/H_2O Butanol/Ethanol/H_2O Butanol/Ethyl acetate/H_2O
Water-Insoluble	High Polars (Soluble in Methanol, Acetone)		Butanol/Acetic Acid/H_2O Butanol/Methanol/H_2O Butanol/Ethanol/H_2O Butanol/Ethyl acetate/H_2O
	Normal Polars (Soluble in Chloroform)		Chloroform/Methanol/H_2O Methyl-tert. butyl ether/Acetonitrile/H_2O Methyl-isobutyl ketone/Acetone/H_2O Heptane/Ethyl acetate/Methanol/H_2O
	Low Polars (Soluble in Hexane)		Hexane/Acetonitrile Hexane/Methanol/H_2O Hexane/Ethanol/H_2O Heptane/Ethyl acetate/Methanol/H_2O

Amphotericin B (ClO4 Salt) Separation



Reference : A.P Foucault, P, Durand, E.Canacho-frias F. Le Goffic, AnalyticalChemistry, 65(1993)2150

Ginsenosides from Panax Quinquefolium L Separationi



L.Le Men Olvier, J.H. Renault P. Thepenier, M.J. Jacquier, M. Zeches Hanrot. A.P. Foucault Journel of Liquid Chromtography 18(1995)1655

[Recommended reference : Centrifugal Partition Chromatography edited by Dr. Alain P. Foucault.]

Specifications

Model	CPC80	CPC240	CPC1400
Rotational Speed	0 ~ 2000 rpm(100rpm steps)		0 ~ 1500 rpm (100rpm steps)
Washing Speed	300 rpm		300 rpm
Separation Speed	1500 rpm		1100 rpm
Flow Rate	0.1 ~ 30ml/min		1 ~ 200ml/min
Washing Flow	20ml/min		80ml/mi
Separation Flow	5ml/min		25ml/min
Standard Valves	4-way Mode Switching Valve	4-way Mode Switching Valve	4-way Mode Switching Valve, 6-way Sample Injection Valve
Display	LCD Display		
Rotor Material	Poly-phenylenesulfide(PPS) Stainless Steel		Poly-chlorotrifluoroethylene (DAIFLON), Stainless Steel
Number of Disk	4 pcs x 1 Pack	12 pcs x 1 Pack	18 pcs x 1 Pack
Rotor Dimension	200φ x 20H mm	200φ x 80H mm	300φ x 150H mm
Rotor Volume	80 ml	240 ml	1400 ml
Partition Cells	712	2136	1296
Cell Length	15 mm	15 mm	29 mm
Centrifugal Radius	82.5 mm (average)		120 mm (average)
Estimated Separation/Run	8ml/run	24ml/run	140ml/run
Max Pressure	60 kg/cm²		
Safety Device	The rotor is automatically stopped when its lip is opened		
In/Out Line Tubing Size	1/16" PEEK (0.75 ID x 1mm OD)		
Rotor Net Weight	5 kg	7 kg	25 kg
Overall Dimension	330W x 475D x 480H mm		420W x 610D x 500H mm
Net weight	40 kg	43kg	70 kg
Power	AC100~240V, 3A, φ, 50/60Hz		

Options

Dedicated Controller, Semi-preparative Pump
Injector, UV-Vis Detector, Fraction Collector

Specifications of the CPC Controller (Option)

Executed Files	10 Files (the 10th File is exclusively used for washing only) ◎Sequential separation by means of a same file ◎Most suitable conditions can be determined by using linkage of multiple files. ◎Sequential injections into same stationary phase.
Valves	Valve 1: Max 6 different solvents can be inter-switched Valve 2: 6-way valve for injection and loading Valve 3: Ascend and Descend modes can be switched
Display	LCD, 40 characters x 4 lines Function Keys: F1, F2, F3 and F4
Net Weight	17 kg
Dimension	380W x 350D x 500H mm

Specification of the Solvent Delivery Pump (Option)

Flow Rate	0.1 ~ 50ml/min
Max. Pressure	7.0Mpa (during connecting with CPC)
Plunger Diameter	7.0φ
Display	Pressure, Flow Rate, Upper/Lower Pressure Limit (pre-settable)
Net Weight	5 kg
Dimension	80W x 310D x 140H mm

◎Specifications are subject to change without prior notice.
◎Patents for Rotor Joint in Japan and for Rotor in U.S.A.

Manufacturer

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