High Speed Countercurrent Chromatography

Non-consumable Preparative Chromatographic Technique

TAUTO BIOTECH

Company Introduction

Shanghai Tauto Biotech Co., Ltd. is a professional manufacturer of HSCCC (High-speed counter current chromatography) instruments. We possess the patented HSCCC techniques and have manufactured a series of new-type HSCCC equipments all by ourselves, which named TBE series in analysis style, semipreparative style and preparative style. TBE-series HSCCC products have already been CE certified. The manufacture techniques have reached the first class level in the world.

Backed up by advanced techniques, the company developed the self-patented new-type HSCCC which boasts reliable performance and takes a leading role in the application of counter-current chromatography techniques worldwide. The TBE Series High speed counter-current chromatography instruments integrated the functions of extraction, separation, purification and manufacturing. They can provide advanced separation and purification performance for antibiotics and valid monomer of herbals to the higher colleges, research institutes and pharmaceutical enterprises. From the year of 2005 to 2007, over 43.2% of HSCCC research articles are published by Tauto's customers.

Using HSCCC, Tauto has produced about 100 kinds of APIs of natural products with purities above 99%. In addition, on industrialization of HSCCC technology we build a bulk drug production line of Huperzine A, of which the production scale is able to be extended according to the market requirement.

Tauto has been ISO 9001:2000 certified by DNV. it has established long-term cooperation relationship with GE Healthcare Bio-sciences Co., Ltd. Both two parties are devoted to the technology and marketing development which brings a more brilliant prospect to the development of HSCCC. Tauto's HSCCC instruments have been sold to many districts and countries around the world such as the USA, Switzerland, German, Japan, South Korea, Taiwan, Hong Kong, Singapore, Vietnam, Thailand, etc.

With pioneering spirit, Tauto is marching towards a higher level of biotechnology.

CE

ISO 9001:2000
Certified by DNV

Principle of HSCCC

1. Counter-current Distribution

In the 1940s Lyman C. Craig (1906-1974) invented the first apparatus (besides a separation funnel!) to conduct counter-current partitioning; he called this Countercurrent Distribution (CCD).

CCD can achieve continuous extraction. However, the operation process is incontinuous. The CCD apparatus is huge and fragile due to its complicated structure. It costs a mass of solvent and is low in efficiency. There is a danger of emulsification which can't be avoided.

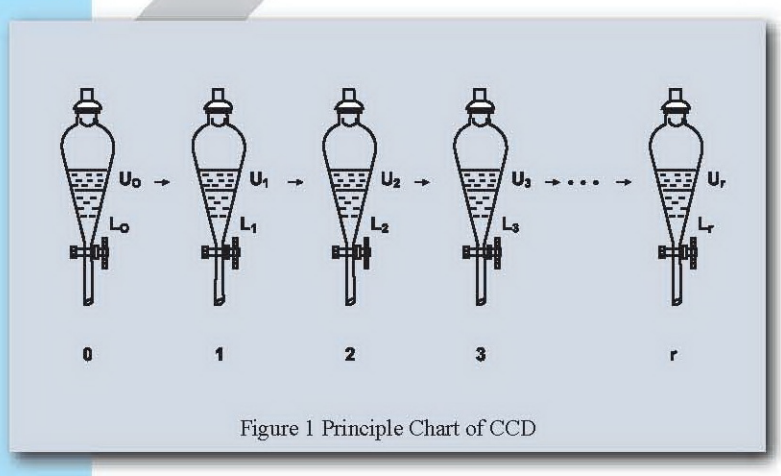


Figure 1 Principle Chart of CCD

2. Droplet CCC (DCCC)

Droplet CCC is the oldest form of CCC. It uses only gravity to move the mobile phase through the stationary phase.

The disadvantage of DCCC is that flow rates are low, and poor mixing is achieved for most binary solvent systems, which makes this technique both time-consuming and inefficient.

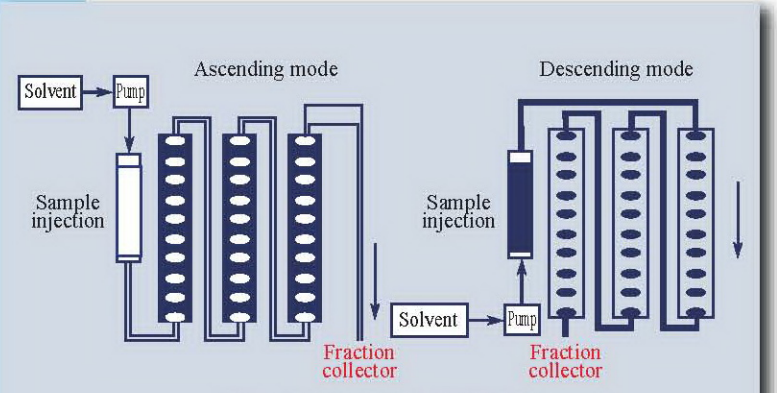


Figure 2 Principle Chart of DCCC

3. Centrifugal Partition Chromatography (CPC)

CPC is invited in 1970s. Basically, CPC is hydrostatic like DCCC, but with improved mixing and more theoretical plates.

The columns are cut into a rotor, oriented out from the middle, and connected by channels. The rotor is filled with the stationary phase, and the mobile phase is pumped through it as the rotor spins.

CPC instruments had the disadvantage of requiring a rotary seal that frequently needed to be replaced.

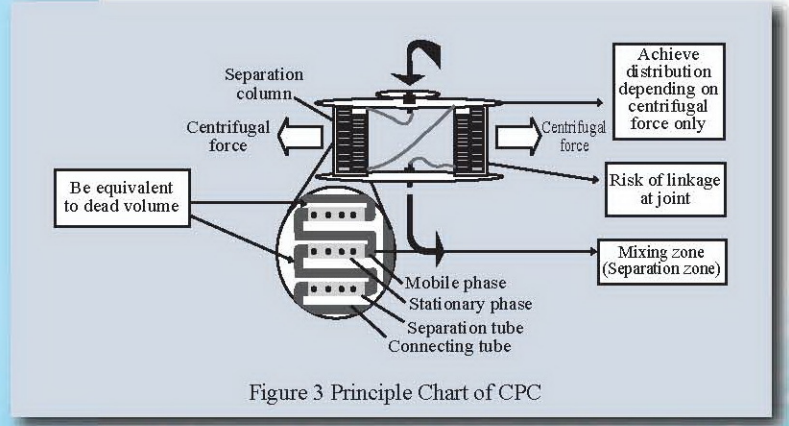


Figure 3 Principle Chart of CPC

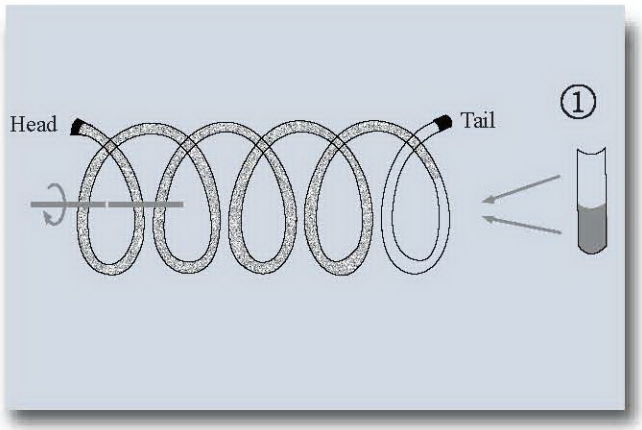
Principle of HSCCC

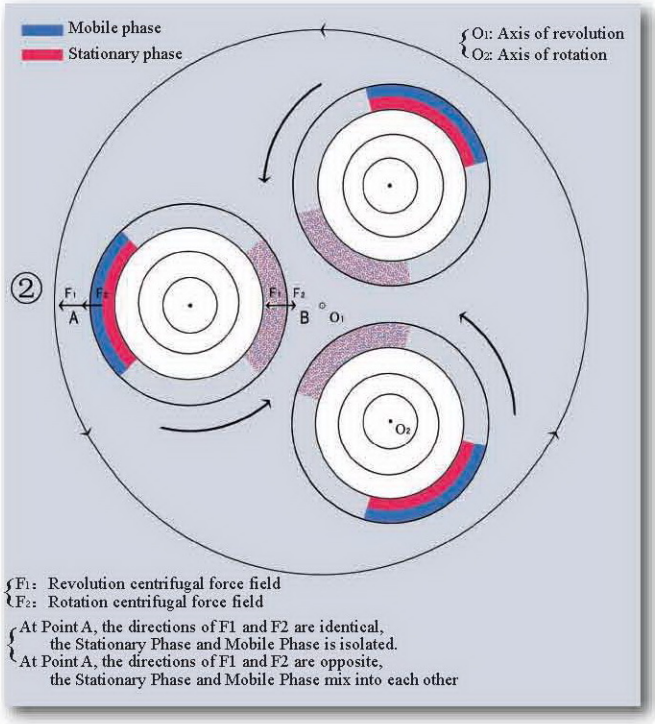
4. High Speed Countercurrent Chromatography (HSCCC) ---The most advenced CCC technology

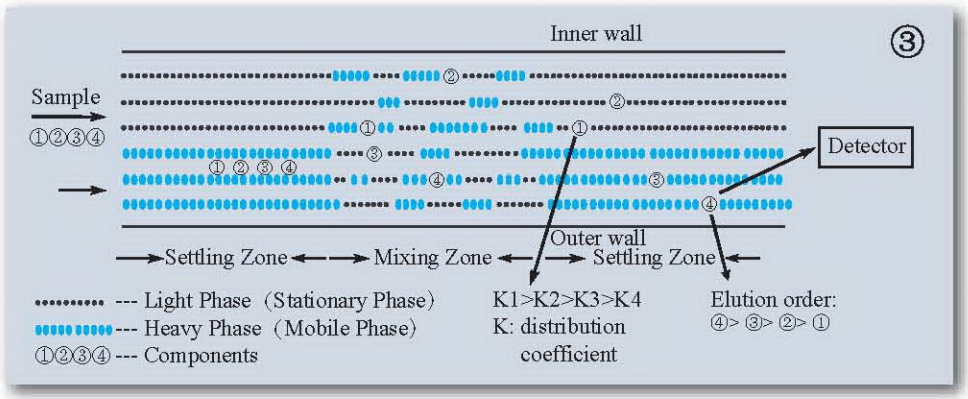
Features:

1) Based on the Hydrodynamic Equilibrium System (HDES)

2) The two-dimensional centrifugal force field caused by the simultaneous rotation and revolution holds one liquid phase as stationary phase in the separation tube.

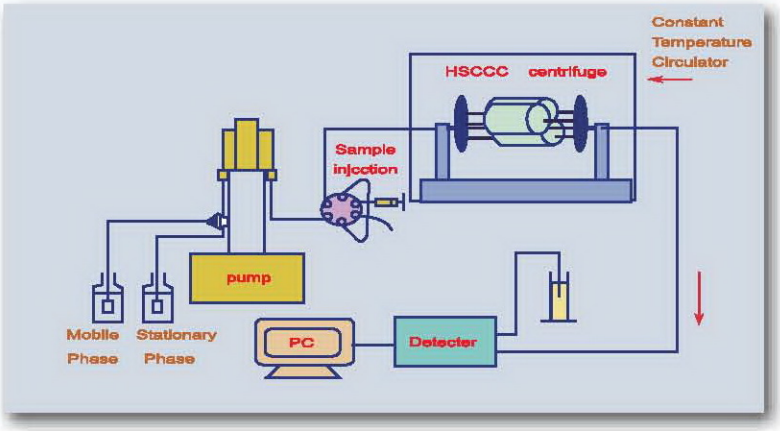






3) HSCCC actualizes high frequency extraction between two phases by high speed planetary centrifuge. When 1000 rpm, the frequency of mixture and distribution can achieve 17 times per second. Finally different components in the sample will be eluted due to their respective distribution coefficient.

5. HSCCC Working Flow Chart



Features of HSCCC

High Performance -- High speed continuous liquid-liquid extraction leads to high throughput preparative separations. Target components can be isolated to purity over 99% by HSCCC according to their different distribution coefficients in the two immiscible phases.

100% Sample Recovery -- Since HSCCC does not use a solid support, irreversible adsorption, degradation of components and contamination onto the column is avoided, and a 100% recovery can be achieved in practice.

Scaleable -- HSCCC is able to range from milligrams to tens of grams on the same instrument. HSCCC separation technologies on small-scale instrument can be scaled up on large-scale one as well. The repeatability is excellent.

Cost-efficient -- HSCCC is a non-consumable chromatographic technique. The only running costs are that of the solvent. In addition, solvent usage in HSCCC is significantly lower (by 10-50%) than that of other preparative chromatography techniques, such as HPLC.

Versatile Selectivity -- Separation of virtually every compound class has been demonstrated with CCC. Selectivity over a full range of polarities is achieved through the use of appropriate HSCCC Solvent Systems.

Safe Operation -- Volatilization can be eliminated as the separation is performed in close coiled tubes

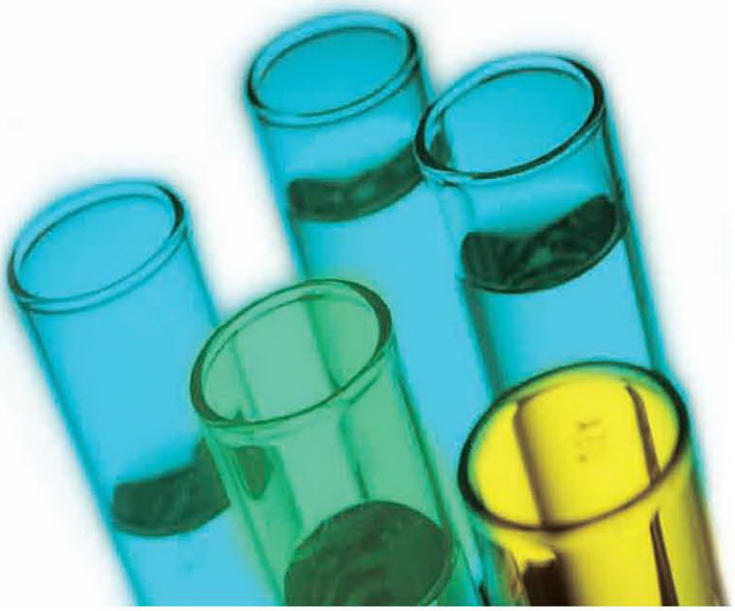
In comparison to HPLC

Larger Preparation Capacity
The semi-preparative scale HSCCC can be competent for the work of preparative-scale HPLC.

Nice Repeatability
The repeatability of experiments can archive 100%.

No Consumables & Low in Operation Cost
HSCCC is a support-free liquid liquid partition chromatographic technique, no columns to buy or replace and 10-50% reduction in solvent use compared to HPLC

No Irreversible Adsorption & Simplified Sample Pre-Preparation
The irreversible adsorbing effects on stationary phase material and sample decomposition can be eliminated. The sample pre-preparation is greatly simplified as well.



Application Fields

By now HSCCC has been applied to different researches such as biochemistry, pharmacy, phytochemistry, agriculture, environmental science, material research, chemistry, marine biology and inorganic ion. It is an extremely effective technique for the separation of natural products, antibiotics, proteins and noble metals.

(1) Separation of APIs from natural products
(2) Separation of large biological molecules such as proteins, polypeptides and polysaccharides
(3) Separation of antibiotics
(4) Separation of rare earth elements and noble metal elements
(5) Purification of chemical synthetic products
(6) Separation of functional food component
(7) Separation and analysis of pesticide residues
(8) Quality control research of herbals
(9) Separation of active marine ingredients

Analytical HSCCC TBE-20A

Explorer of Non-Consumable Preparative Purification Technologies for Natural Product

Swift and efficient separation.

Low solvents consumption.

This model is dedicated to the researches of separation techniques at early stage, fingerprint research and sample analysis research.

Model	TBE-20A
Separation Column	Triple-column
Total Column Volume	20ml
Sample Loop Volume	20μl
Max Loading	Micrograms-milligrams
Max Revolution Speed	2000rpm
Working Revolution Speed	1400-2000 rpm
Stationary Phase Flow Rate	5.0ml/min
Mobile Phase Flow Rate	0.5-1.5ml/min
Purging Revolution rate	500 rpm reverse
Purging Flow Rate	5.0ml/min
Max System Pressure	2MPa
Dimension	330×600×550mm
Power Requirements	220 V ± 10%, 50/60 Hz•300W
Net Weight	75KG

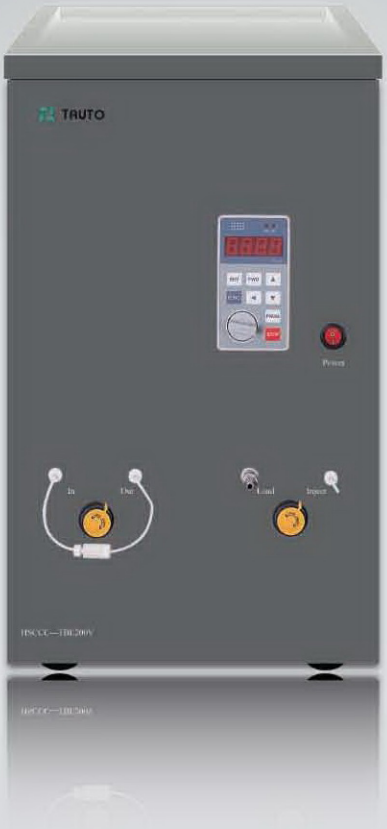
Products Features

- **Triple-Separation-Column**
  When revolving at high speed, the machine can reach a perfect balance. The retention rate of the solvent system is satisfactory. Meanwhile the stability of the machine is guaranteed and the noise is effectively lowered.
- **Precise temperature control**
  The temperature of separation process can be controlled precisely. The separation process will avoid to be impacted by the environment temperature. So the repeatability of the experiment is guaranteed. By adjusting the temperature setting, the separation degree can be improved and the range of optional. solvent system is significantly widened
- **High separation degree**
  The target components in samples can be separated to 99% purity one-steply.
- **High recovery rate**
  There is no absorptive loss and sample denaturalization caused by solid support. As so, the recovery rate is nearly 100%.
- **Cost-efficiency**
  As no column filling is used there is no need to replace the column. Also it requires only analytical grade solvents. The cost of running and maintenance is quite low.

Semi-Preparative HSCCC TBE-200V

An Expert System for Purification of Proteins

Model	TBE-200V
Separation Column	Triple-column
Valve Switching	Four flow routes switch function, dual sixway-valve design
Total Column Volume	200ml
Sample Loop Volume	20ml
Max Loading	Milligrams-Grams
Max Revolution Speed	1000 rpm
Working Revolution Speed	700-900 rpm
Stationary Phase Flow Rate	30ml/min
Mobile Phase Flow Rate	0.8-2.0ml/min
Purging Revolution rate	300 rpm reverse
Purging Flow Rate	30ml/min
Max System Pressure	2MPa
Dimension	330×600×550mm
Power Requirements	220 V ± 10%, 50/60 Hz•300W
Net Weight	80KG



TBE-200V is dedicated for purifying large molecules like proteins, polypeptides and polysaccharides. It is suitable for separation of the active ingredients sensitive to the environment temperature.

Products Features

- **riple-Separation-Column**
  When revolving at high speed the machine can reach a perfect balance. The retention rate of the solvent system is satisfactory. Meanwhile the stability of the machine is guaranteed and the noise is effectively lowered.
- **Dual sixway–valve design**
  Four flow routes switch function. One valve is the sampling valve which enables one operator to finish the sampling process by him or herself. Another valve is the flow route control valve. Without stop the machine the operator can switch from forward revolution to inverse revolution and vice versa. So the machine can accomplish elution forwardly and reversely.
- **Precise temperature control**
  The temperature of separation process can be controlled precisely. The separation process will avoid to be impacted by the environment temperature. So the repeatability of the experiment is guaranteed. By adjusting the temperature setting, the separation degree can be improved and the range of optional solvent system is significantly widened.
- **Combination with Aqueous two-phase systems**
  - ■ Aqueous two-phase systems provide mild conditions that do not harm or denature proteins or polypeptides.
  - ■ The polymer layer stabilizes the extracted protein molecules, favoring a higher concentration of the desired protein in one of the layers, resulting in an effective extraction.
  - ■ These systems are amenable to scale-ups, from laboratory-sized setups to those that can handle the requirements of industrial production. They may be employed in continuous protein-extraction proceses.
  - ■ The continuous high performance countercurrent distribution and optimized retention rate of stationary phase in HSCCC instrument improves the separation degree and shortens separation time.

Other Models

Semi-Preparative HSCCC TBE-300B

Model	TBE-300B
Separation Column	Triple-column
Valve Switching	Four flow routes switch function, dual sixway-valve design
Total Column Volume	280ml
Sample Loop Volume	20m
Max Loading	Milligrams-Grams
Max Revolution Speed	1000 rpm
Working Revolution Speed	800-1000 rpm
Stationary Phase Flow Rate	30ml/min
Mobile Phase Flow Rate	2.0-4.0ml/min
Purging Revolution rate	300 rpm reverse
Purging Flow Rate	30ml/min
Max System Pressure	2MPa
Dimension	330×600×550mm
Power Requirements	220 V ± 10%, 50/60 Hz•300W
Net Weight	80KG

This Model is dedicated for the separation of middle and small molecules like herbal ingredients, chemical synthetic compounds, antibiotics, etc. It is suitable for separating the active ingredients sensitive to the environment temperature.

Preparative HSCCC TBE-1000A/ TBE-5000A

These two models are dedicated for separating the active ingredients sensitive to the environment temperature.

- High preparation capacity;
- Satisfying separation degree;
- Suitable for scale up of HSCCC separation technologies;
- Stable running.

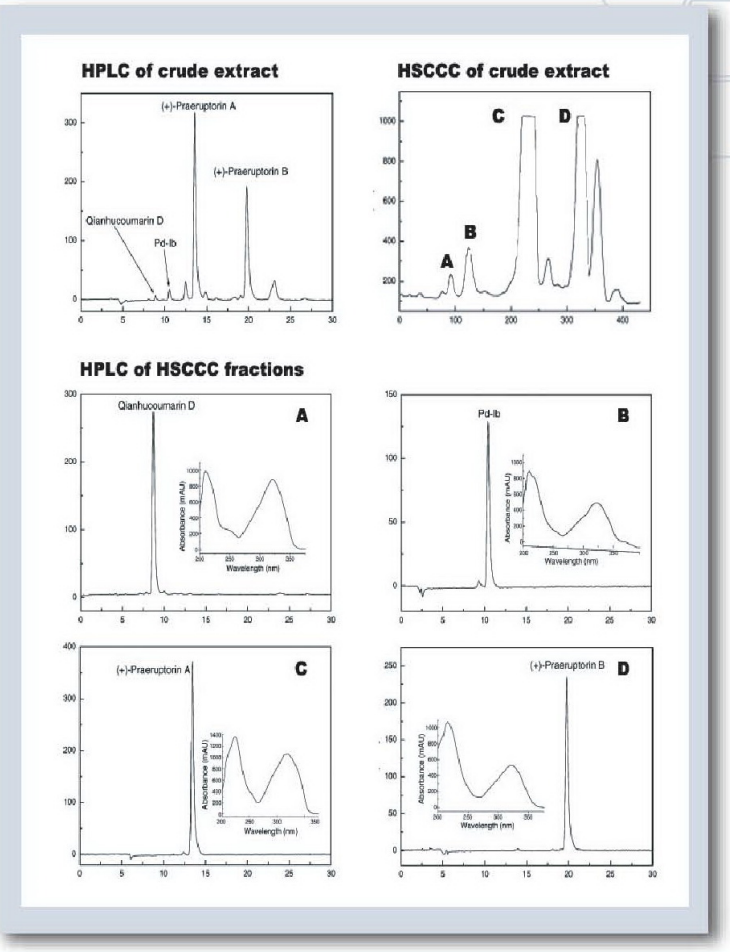
Model	TBE-1000A	TBE-5000A
Separation Column	Triple-column	Triple-column
Valve Switching	Four flow routes switch function, dual sixway-valve design	Four flow routes switch function, dual sixway-valve design
Total Column Volume	1000ml	4800ml
Sample Loop Volume	80ml	200ml
Max Loading	Grams -10 Grams	Grams-100 Grams
Max Revolution Speed	600 rpm	600 rpm
Working Revolution Speed	400-550 rpm	400-550 rpm
Stationary Phase Flow Rate	90ml/min	180ml/min
Mobile Phase Flow Rate	5.0-10ml/min	20-50ml/min
Purging Revolution rate	200 rpm reverse	200 rpm reverse
Purging Flow Rate	90ml/min	180ml/min
Max System Pressure	2MPa	2MPa
Dimension	628×918×1048mm	730×1100×1122mm
Power Requirements	220 V ± 10%, 50/60 Hz•900W	220 V ± 10%, 50/60 Hz•1800W
Net Weight	400KG	540KG

Sample

Preparative separation of coumarins in HSCCC

Sample: Crude extract of Peucedanum praeruptorum
Apparatus: TBE-300B
HSCCC experimental conditions:
Solvent system: system A light petroleum/ethyl acetate/methanol/water (5 : 5 : 5 : 5, V/V) system B light petroleum/ethyl acetate/methanol/water(5 : 5 : 6.5 : 3.5, V/V)
Mobile phase: upper phase of system A
Elution: gradient: 0-150min elute with the lower phase of system A, 150-300min elute in linear gradient with the lower phase of system A and B. Decrease the concentration of lower phase of system A from 100% to 0%

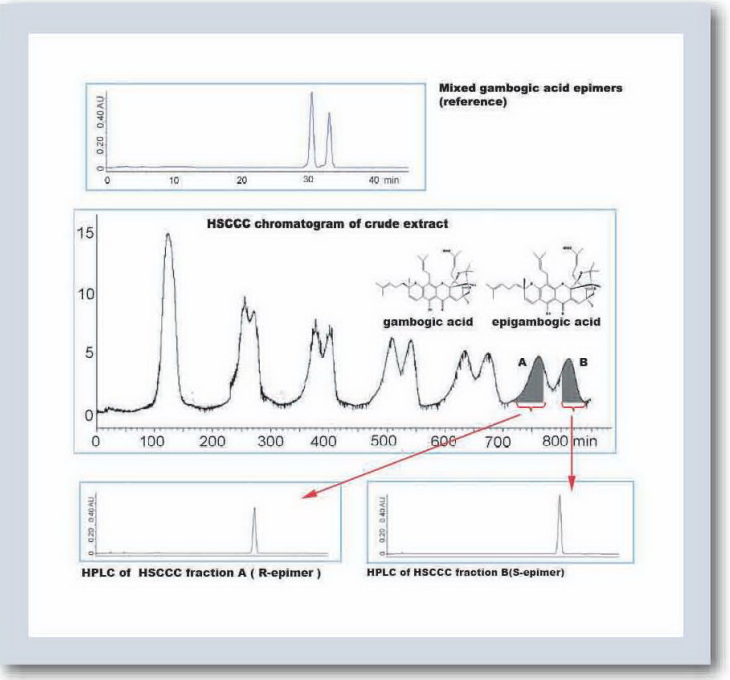
Flow rate:	2.0ml/min
Revolution speed:	900r/min
UV detection wavelength:	254nm
Injected mass:	110mg
Product purity: A. qianhucoumarin D	98.6%
B. Pd-Ib	92.8%
C. (+)-praeruptorin A	99.5%
D. (+)-praeruptorin B	99.4%



Application of HSCCC in isomers separation

Sample: Crude extract of gambolic acid isomers
Apparatus: TBE-300B
HSCCC experimental conditions:
Solvent system: n-hexane/methanol/water (5 : 4 : 1, V/V)

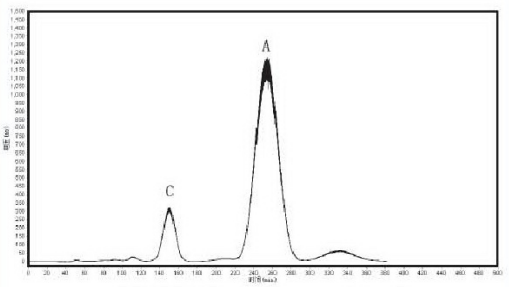
Stationary phase:	upper phase
Flow rate:	2.0ml/min
Revolution speed:	800r/min
UV detection wavelength:	360nm
Injected mass:	50mg
Product purity: A. gambolic acid	97.2%
B. epigambolic acid	97.5%



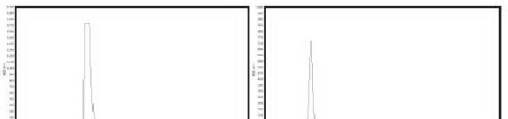
Sample

Preparative separation of antibiotics in HSCCC

Sample: Crude erythromycin
Apparatus: TBE-300B
HSCCC experimental conditions:
Solvent system: hexane/ethyl acetate/methanol/0.5% ammonia (3 : 7 : 5 : 6, V/V)
Stationary phase: upper phase
Flow rate: 1.5ml/min
Revolution speed: 850 r/min
Detection: ELSD
Injected mass: 300mg
Product purity: Erythromycin A over 98.6%
Erythromycin C 98.8%

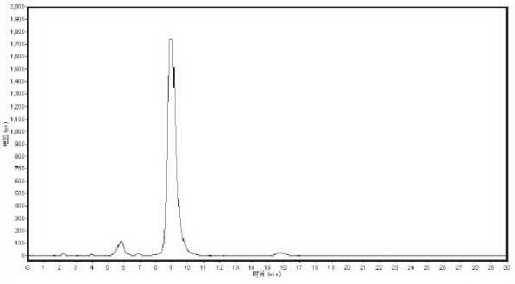


HSCCC chromatogram of crude erythromycin determined by ELSD



ELSD detection of erythromycin A

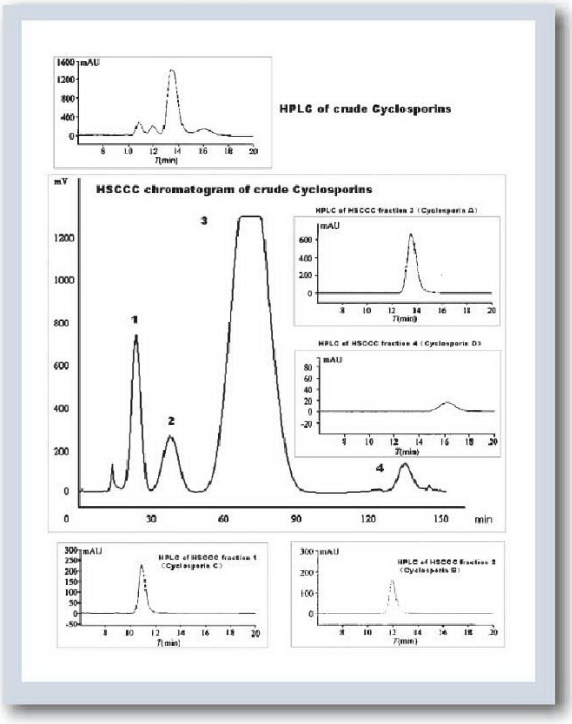
ELSD detection of erythromycin C



ELSD detection of crude erythromycin

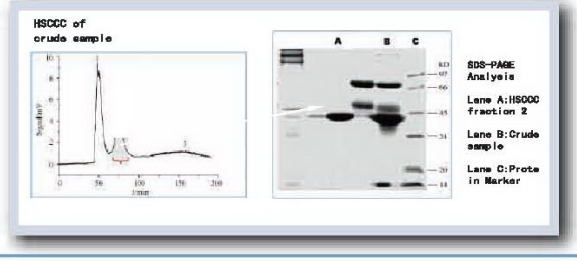
Preparative separation of antibiotics in HSCCC

Sample: Crude cyclosporine
Apparatus: TBE-20A
HSCCC experimental conditions:
Solvent system: light petroleum/ acetone/water (3 : 3 : 2, V/V)
Stationary phase: upper phase
Flow rate: 0.5ml/min
Revolution speed: 1600 r/min
Detection: ELSD
Product purity: cyclosporine A 98.6%
cyclosporine B 98.7%
cyclosporine C 99.3%
cyclosporine D 98.5%



Preparative separation of proteins in HSCCC

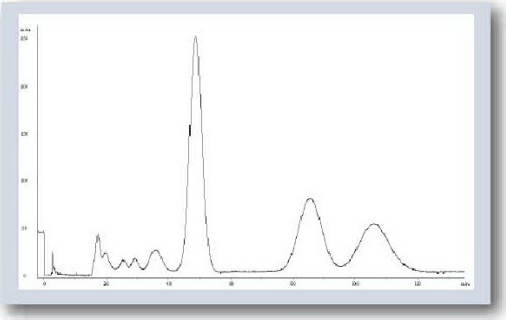
Sample: 5ml albumen of hen egg (240mg, containing 132mg of ovalbumin approximately)
Apparatus: TBE-200V
HSCCC experimental conditions:
Aqueous two-phase System: PEG1000/KDP/water (16 : 17 : 67, W/W)
Stationary phase: upper phase
Flow rate: 1.8ml/min
Revolution speed: 850r/min
UV detector wavelength: 280nm
Peak 2 is the ovalbumin peak, purity 99%, recovery rate 95%



Technology Scale Up & Separation Production

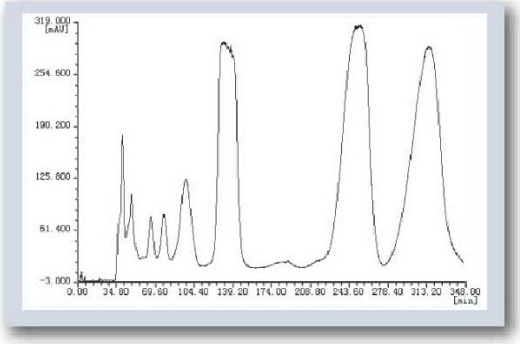
Sample: Chinese aloe extracts

Apparatus: Analytical HSCCC TBE-20A



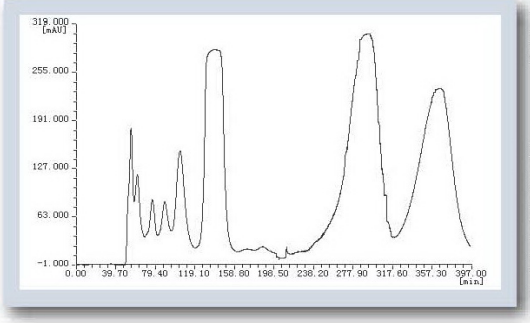
HSCCC experimental condition
Solvent system:hloroform/isobutanol/
methanol/water (4 : 0.25 : 3 : 2, V/V)
Flow rate: 0.5 ml/min
Revolution rate: 1600 r/min
UV detection wavelength: 254 nm
Injected mass: 3 mg
Retention rate: 71.8%

Apparatus: Semi-preparative HSCCC TBE-300B



HSCCC experimental condition
Solvent system: chloroform/isobutanol/
methanol/water (4 : 0.25 : 3 : 2, V/V)
Flow rate: 2 ml/min
Revolution rate: 880 r/min
UV detection wavelength: 254 nm
Injected mass: 300 mg
Retention rate: 74.4%

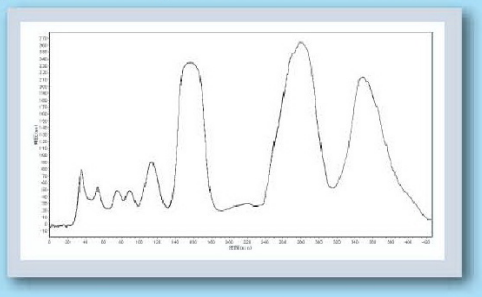
Apparatus: Preparative HSCCC TBE-1000A



HSCCC experimental condition
Solvent system: chloroform/
isobutanol/methanol/water
(4 : 0.25 : 3 : 2, V/V)
Flow rate: 10 ml/min
Revolution rate: 480 r/min
UV detection wavelength: 254nm
Injected mass: 1000 mg
Retention rate: 71.0%

Antibiotics	Altertoxin I, Cyclosporine A, Cyclosporine B, Cyclosporine C, Cyclosporine D, Cytochalasin N, Erythromycin A, Erythromycin B, Erythromycin C, Isovaleryl spiramycin III,
Proteins	α- Amylase, Cytochrome C, Hemoglobin, Lysozyme, Ovalbumin, Ovotransferrin, rhCIFN-α
Phenylpropanoids	Angelica coumarin, Artemisiae capillaris scoparone, Cnidii coumarin, Cortex fraxini coumarin, Costus alctone, Curdione, Daphnoretin, Dehydro costus alctone, Deoxyschizandrin, Edgeworoside C, Germacrone, Pinoresinol diglucoside, Isopsoralen, Liriodendrin, Honokiol, Magnolol, Peucedanum decursivum coumarin, Peucedanum praeruptorum dunn coumarin, Psoralen, γ-Schizadrin, Syringin, Thyme coumarin, Umbelliferone
Flavonoids	Baicalein, Baicalin, Chrysin, Daidzin, Daidzein, Didymin, Genistin, Glycitein, Glycitin, Hyperoside, Isoliquiritigenin, Isorhamnetin, Isovitexin, Isoorientin, Kaempferol, Kuyayinone, Liquiritigenin, Nairutin, Quercetin, Scutellarin, Trollius ledebouri flavonoid glycosides, Wogonoside,
Terpenoids	Atractyloside, Atractylenolide III, Bilobalide, Gentiopicrin, Ginkgolide A, Ginkgolide B, Ginkgolide C, Ginkgolide J, Harpagoside, Jasminoidin, Paclitaxel, Ursolic acid
Quinones	Cryptotanshinone, Tanshinone I, Tanshinone IIA,
Steroids	Androstenedione, β- Ecdysterone, Sitosterol, Spinasterol
Alkaloids	Berberine, Camptothecin, Coptisine, Evodiamine, Heteratisine, Huperzine A, Huperzine B, Matrine, 15-a-Neoline, l-Tetrahydropalmatine, Oxymatrine, Oxysophocarpine, Rutacarpine, Palmatine, Verticine, Verticinone,
Isomerides	Barbinervic Acid stereisomer, Gambogic acid stereisomer
Saponins	Astragaloside, Clinopodiside A, Ginsenoside-Re, Ginsenoside-Rg1, Glycyrrhizic acid
Organic acid, acid anhydride and esters	Cinnamic acid, 3,4--Dihydroxyphenyllactic acid, Eicosanoic acid, Ferulic acid, Gallic acid, Ginkgolic acid (C13: 0), Ginkgolic acid (C15: 1), Ginkgolic acid (C17: 1)
Polyphenols	EGCG, Salvianolic acid B, Theaflavin
Other substances	Amygdalin, Aurentiamide acetate, Chebulagic acid, Chebulinic acid, Curculigoside, Curculigoside B, 10-Hydroxynonacosane Z-Ligustilide, Lindenenol, Linderalactone, Pseudostellarin, Senkyunolide A,

Apparatus: Preparative HSCCC TBE-5000A



HSCCC experimental condition
Solvent system: chloroform/isobutanol/methanol/
water (4 : 0.25 : 3 : 2, V/V)
Flow rate: 40 ml/min
Revolution rate: 480 r/min
UV detection wavelength: 254 nm
Injected mass: 5000 mg
Retention rate: 70.6%

TAUTO BIOTECH

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