Separation and purification of active TCM components by High Speed Counter Current Chromatography

高速逆流色谱分离纯化中药活性成分

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High Speed Counter-Current Chromatography (HSCCC)

高速逆流色谱 (HSCCC)

General principle of counter-current chromatography (CCC)

逆流色谱 (CCC) 原理

The solutes to be separated partition themselves between two immiscible liquid phases. The partition coefficient for each solute is the ratio between the concentration of the solute in the stationary and mobile phases, respectively:

待分离的溶质在不混溶的两相中分配。每个溶质的分配系数分别是其在固定相和流动相中浓度的比值。

$$K = C_s / C_m$$

分配系数=溶质在固定相中浓度/溶质的流动相中浓度

Thus, K=1 means that the solubility of the solute is the same in both phases. K=10 means that the solute is 10 times more soluble in the stationary phase and will be strongly retarded. Solutes with K=0 is excluded from the stationary phase and will be eluted after one mobile phase volume.

因此，K=1意味着溶质在两相中的溶解度一样。K=10意味溶质在固定相中溶解度高得多，保留很强。溶质的K=0在固定相中没有分配，会在一个流动相体积内被洗脱。

Counter-current extraction
逆流提取

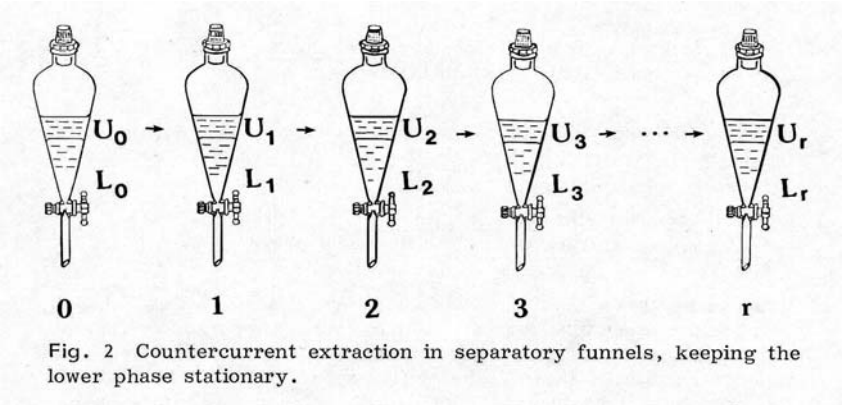


Fig. 2 Countercurrent extraction in separatory funnels, keeping the lower phase stationary.

图. 分液漏斗中的逆流提取，保持下相固定

The great pioneer in counter-current chromatography during the 1940:es and 1950:es was

在二十世纪40-50年代的逆流色谱技术的伟大先锋：

Dr. Lyman C. Craig at the Rockefeller Institute, New York.

Dr. Craig built a very complicated multi-tube glass apparatus that could accomplish several hundred phase transfers. This apparatus was manufactured and sold to many laboratories all over the world and was generally called the ”Craig machine”.

Craig博士制造了一台非常复杂的过个玻璃管组成的仪器，可以完成数百次的相交换。制造了多台这种仪器并销往世界上的很多实验室，通常称为“Craig机器”

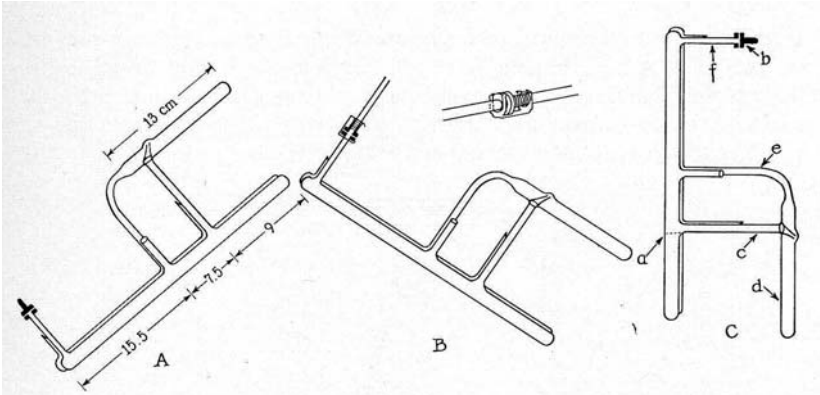


Fig. 5. Schematic drawing of individual units of a countercurrent distribution train. (Taken from *Techniques of Organic Chemistry*, Vol. III, A. WEISSBERGER (Ed.), Interscience, New York, 1950.)

图. 逆流分配的行列中一个独立单元的示意图

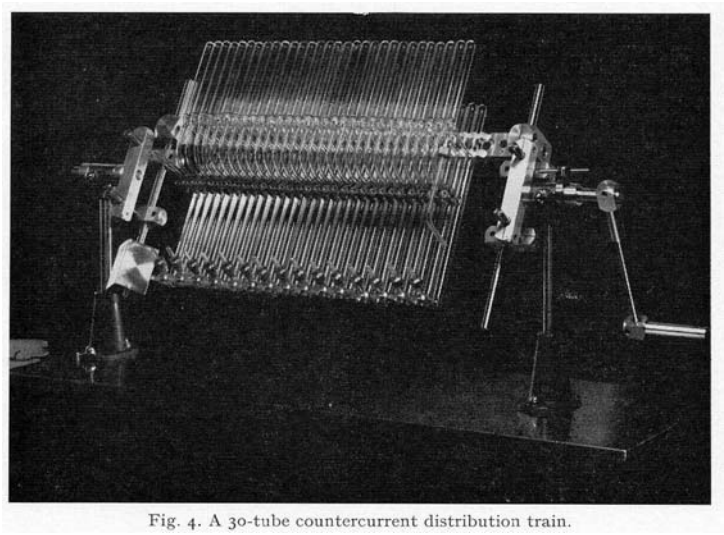


Fig. 4. A 30-tube countercurrent distribution train.

图．一个30支管的逆流分配行列

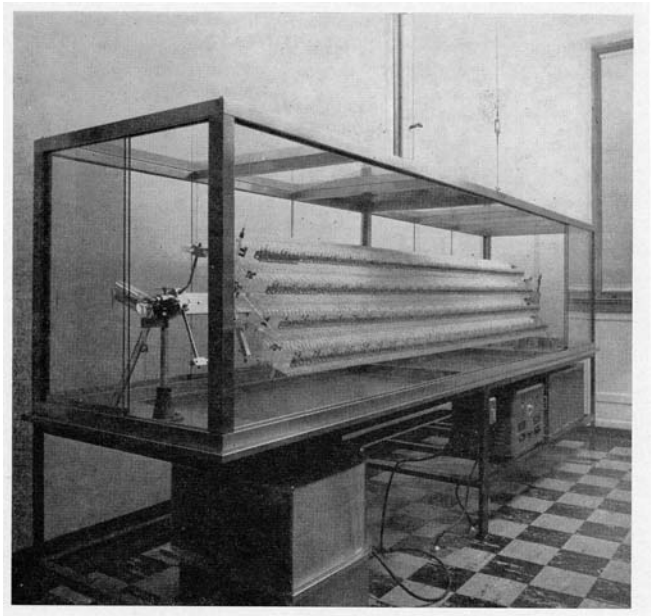


Fig. 13. Photograph of a 1000-tube automatic distribution train.

图．1000支管的自动分配行列

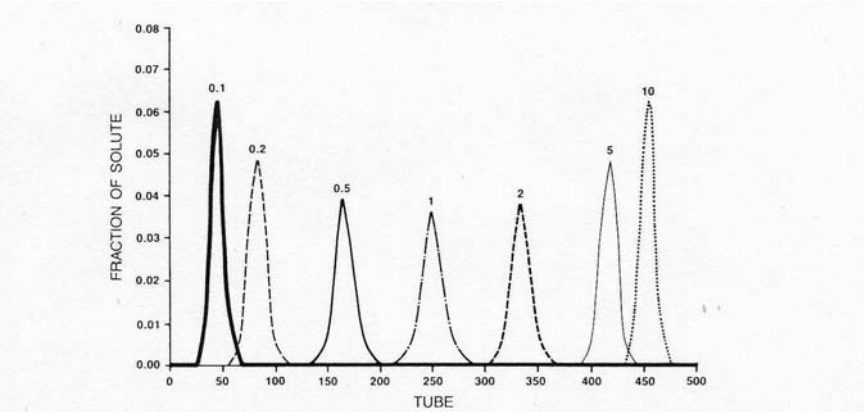


Figure 1·1 Theoretical distribution curves of solutes of various Partition Coefficients from 0.1 to 10 in the tubes of a Craig machine after 500 transfers with equal volumes of each phase

图. 在Craig机器中500支管转移后分配系数从0.1到10的溶质的理论分布曲线

The Craig machine was very tedious and complicated to put together, to run and to maintain so it never became very popular.

这种仪器的组装、运行和维护非常枯燥和复杂，因此并未普及。

Some years later, the Japanese Professor Yoichiro Ito, later active at the NIH in Bethesda, Maryland, USA, developed a new type of counter-current machine based on centrifugation of a rotating coil of Teflon tubing: "The Coil Planet Centrifuge Counter-Current Chromatography Apparatus".

一些年后，日本的Yoichiro Ito先生，在NIH，Bethesda, Maryland, USA, 在旋转聚四氟乙烯螺旋管离心法基础上研制了一种新型的逆流色谱仪器。

High Speed Counter Current Chromatography (HSCCC)

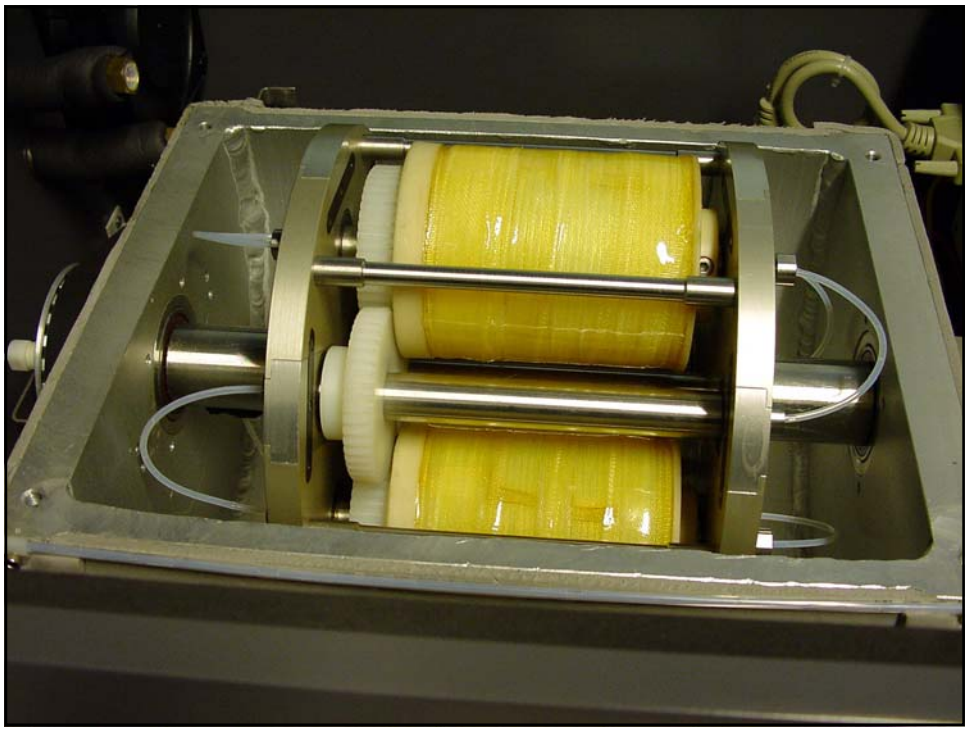
高速逆流色谱 (HSCCC)

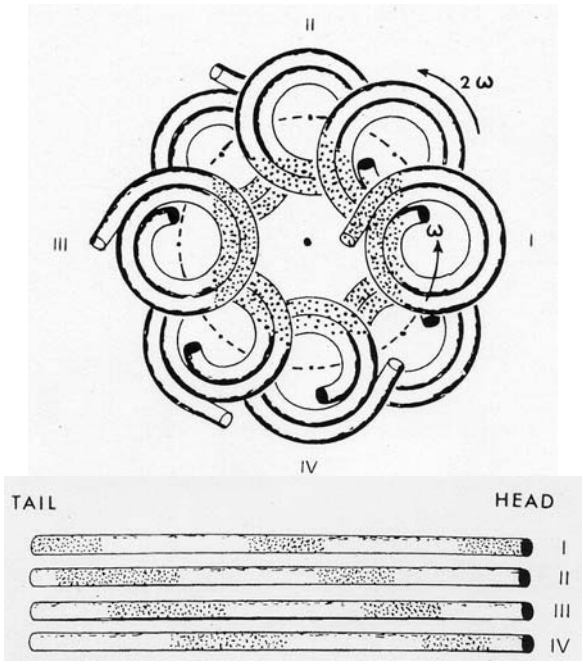
The Ito ”Coil Planet Centrifuge Counter Current Chromatography Apparatus”, also called the ”High Speed Counter Current Chromatography” apparatus (HSCCC) has been further developed by Professor Zhang, Tianyou, at the Beijing Institute of New Technology Application and by Mr. Deng, Qiuyu, at Tauto Biotech Inc., in Shanghai.

Ito的“螺旋管行星式离心仪”，也叫作“高速逆流色谱”仪(HSCCC) 被北京新技术应用研究所的张天佑教授和上海同田生化技术有公司的邓秋云先生进一步改进。

GE Healthcare Biosciences have signed a collaboration agreement with Tauto to link their HSCCC machine to ÄKTA systems for the purpose of TCM separation and purification.

GE Healthcare Biosciences已经与同田签定了合作协议，将GE的ÄKTA 系统与同田的高速逆流色谱仪连接用于中药的分离纯化。




II
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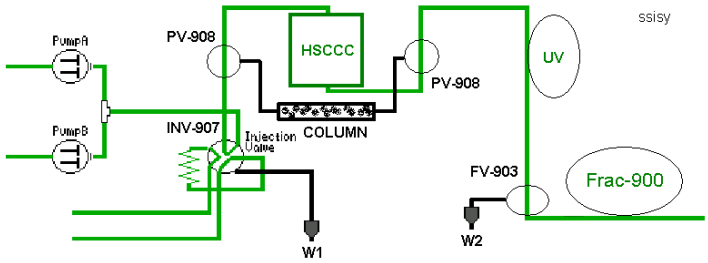
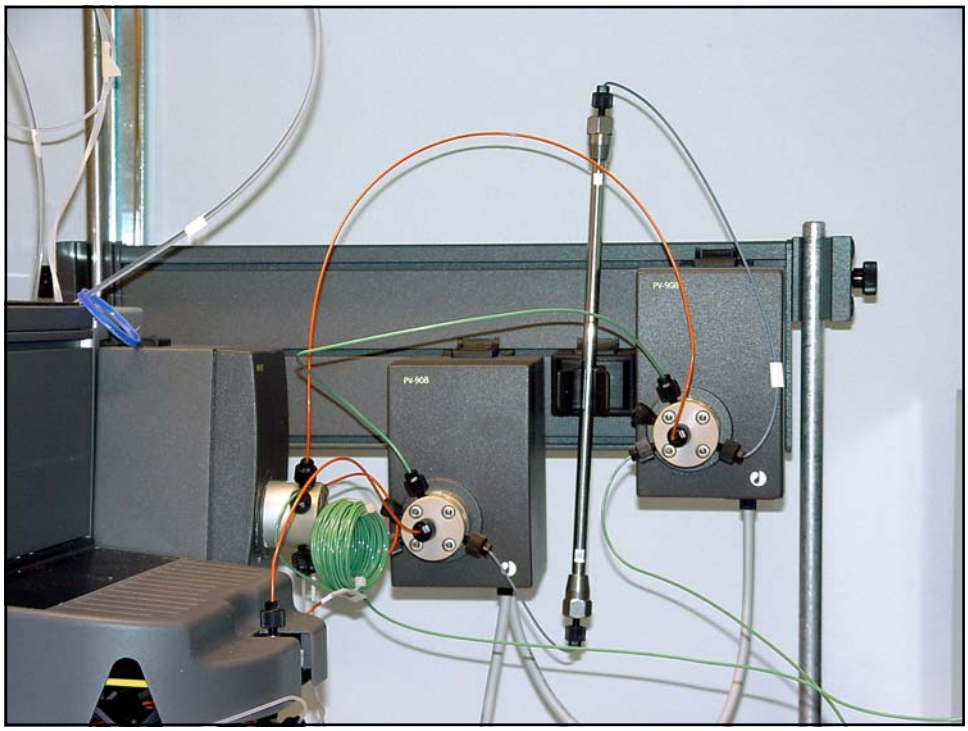
ÄKTAbasic connected to HSCCC TBE-300A via EIC-900



ÄKTA basic与TBE-300A高速逆流色谱以通过EIC-900连接



丹　　参

Salvia miltiorrhiza Bunge, Dan-Shen in Chinese, is one of the herbs that were classified as "Blood-invigorating" in traditional Chinese herbal medicine and were thought by ancient Chinese physicians to make "sluggish" or "stuck" blood flow more freely. Recently, human and animal studies demonstrate that the herb has the effects of vasodilatation, protection of cardiac muscles from anoxia, reduced platelet aggregation and thrombus formation [1]. The major active constitutes of this herb are tanshinones, in-

HSCCC Separation of TCM Components in *Salvia miltiorrhiza* Bunge

Solvent soluble components:

Przewaquinone A

Dihydrotanshinone I

Cryptotanshinone

Tanshinone I

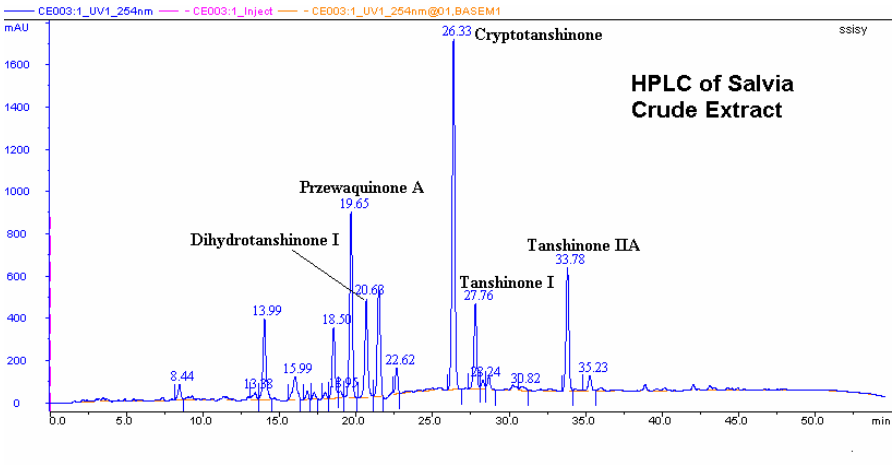
Tanshinone IIA

Water soluble components:

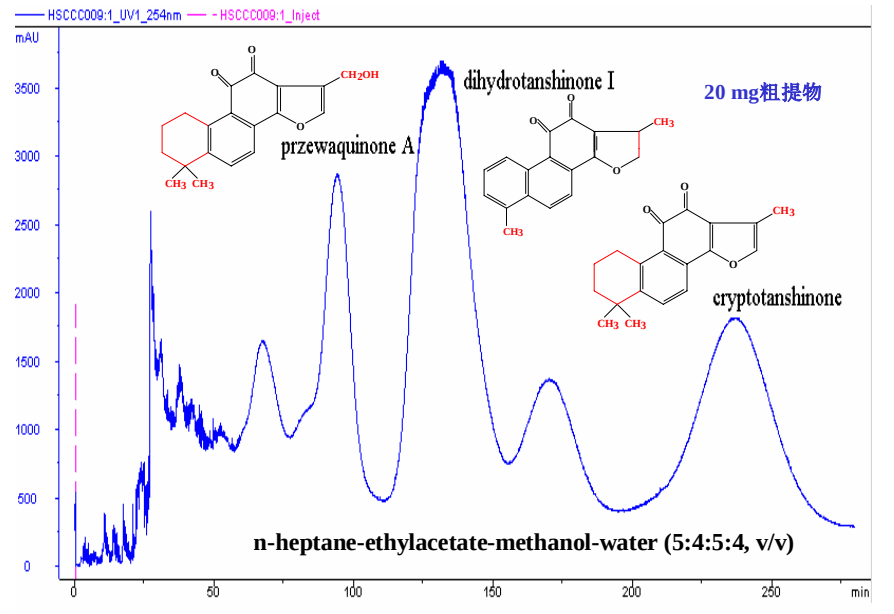
3,4-dihydroxyphenyllactic acid

Salvianolic acid B

Protocatechualdehyde

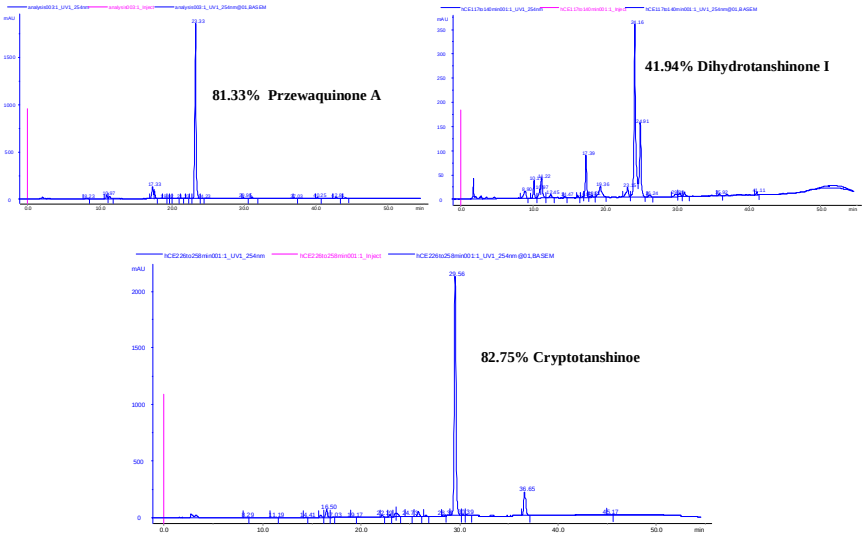


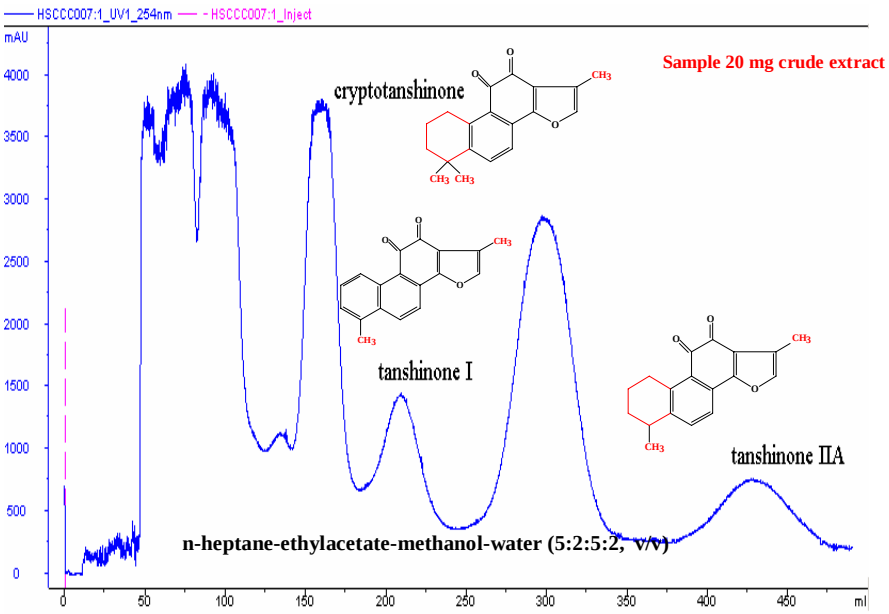
HPLC分析丹参粗提物



正庚烷-乙酸乙酯-甲醇-水（ 5:4:5:4, v/v ）

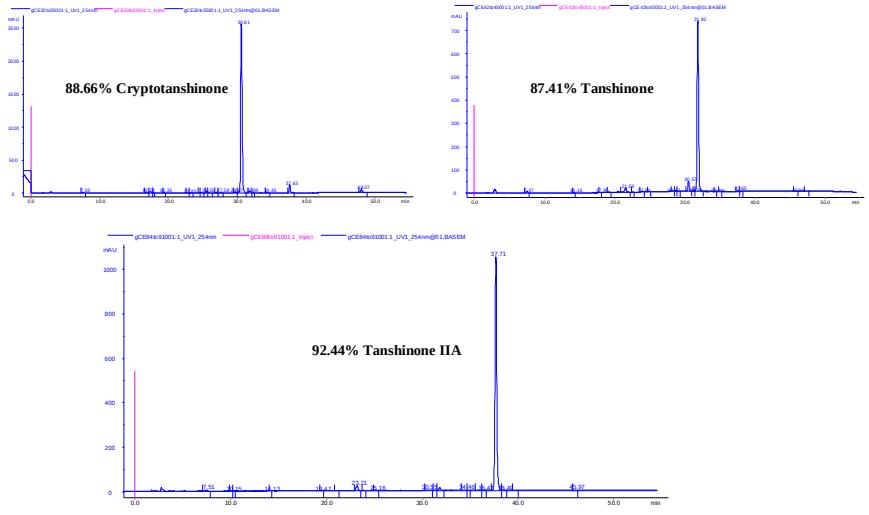
HPLC analysis **HPLC分析**





正庚烷-乙酸乙酯-甲醇-水（ **5:2:5:2, v/v** ）

HPLC analysis **HPLC分析**



One-step separation and purification of 3,4-Dihydroxyphenyllactic acid Salvianolic acid B and Protocatechuic aldehyde from *Salvia miltiorrhiza Bunge* by high-speed counter-current chromatography

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Chinese Academy of Sciences
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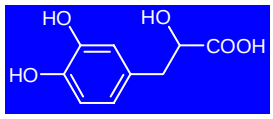
Water soluble components of *Salvia miltiorrhiza* Bunge
丹参水溶性化合物

- **3,4-dihydroxyphenyllactic acid was first isolated from *Salvia miltiorrhiza* Bunge and found to be a coronary vasodilator and to scavenge the free oxygen radicals.**

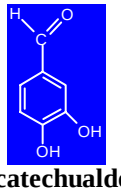
- **Salvianolic acid B has significant scavenging effects on oxygen free radicals and protective effects on heart and brain injuries induced by ischemia-reperfusion.**

- **Protocatechualdehyde (protocatechuic aldehyde) is normally used as a reference standard in analysis of related preparations of *Salvia miltiorrhiza* Bunge.**

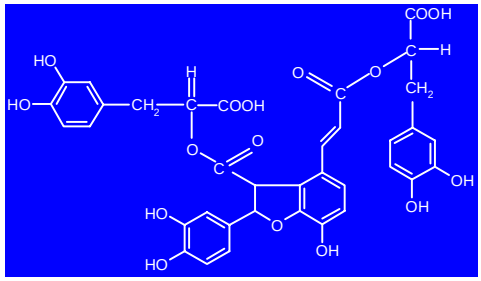
Water soluble components of *Salvia miltiorrhiza* Bunge
丹参水溶性化合物



3,4-dihydroxyphenyllactic acid
丹参素

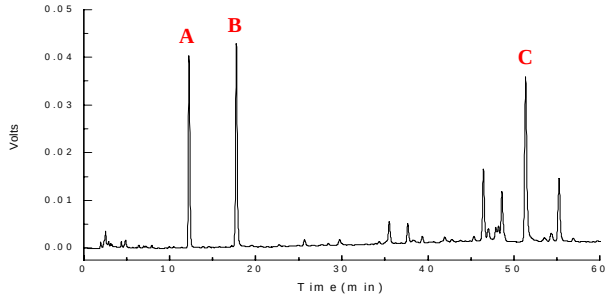


Protocatechualdehyde
原儿茶醛



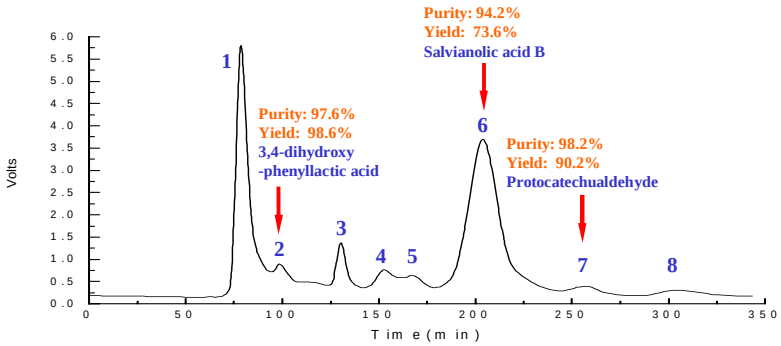
Salvianolic acid B
丹参酚酸B

HPLC analysis of a crude water extract of *Salvia miltiorrhiza* Bunge
高效液相色谱分析丹参水溶性粗样



A. 3,4-dihydroxyphenyllactic acid, 丹参素, 4.0%;
B. Protocatechualdehyde, 原儿茶醛, 6.4%;
C. Salvianolic acid B, 丹参酚酸B, 0.63%.

HSCCC separation and purification of three water soluble components of *Salvia miltiorrhiza* Bunge
高速逆流色谱分离3个丹参水溶性粗制样品



Solvent system: n-hexane-ethyl acetate-methanol-acetic acid-water=1:6:1.5:1.5:8;
正己烷-乙酸乙酯-甲醇-乙酸-水=1:6:1.5:1.5:8
Mobile phase: Lower phase; Flow-rate: 1.5 ml/min; Revolution speed: 850 r/min;
Detection wavelength: 280 nm; Sample load: 100 mg/5 ml.

Separation of twelve water extracted crude samples of *Salvia miltiorrhiza Bunge* on HSCCC

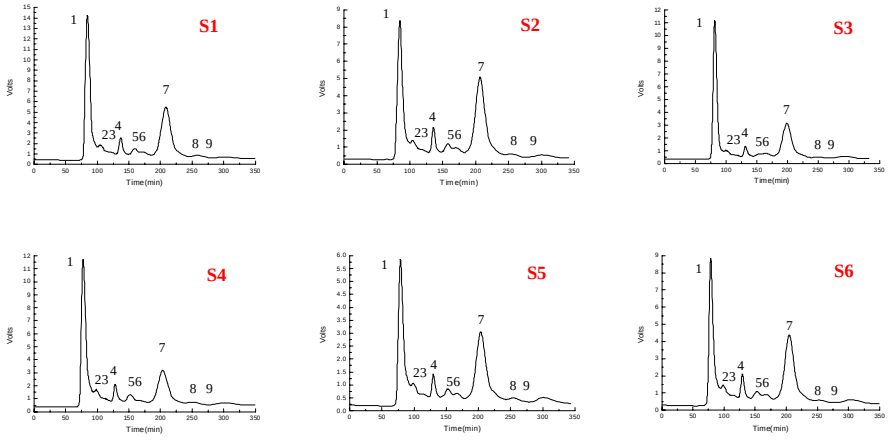
高速逆流色谱分离12个丹参水溶性粗制样品

Nine samples (no. 1 to no. 9) of twelve batches of raw material *Salvia miltiorrhiza* Bunge were supplied by Tianjin TASLY Group Company from different plantations of trueborn plant area, Shangnan, Shanxi province.

Samples no. 10, no. 11 and no. 12 were obtained from Changzhi (Shanxi province), Weifang (Shandong province) and Jiangsu province.

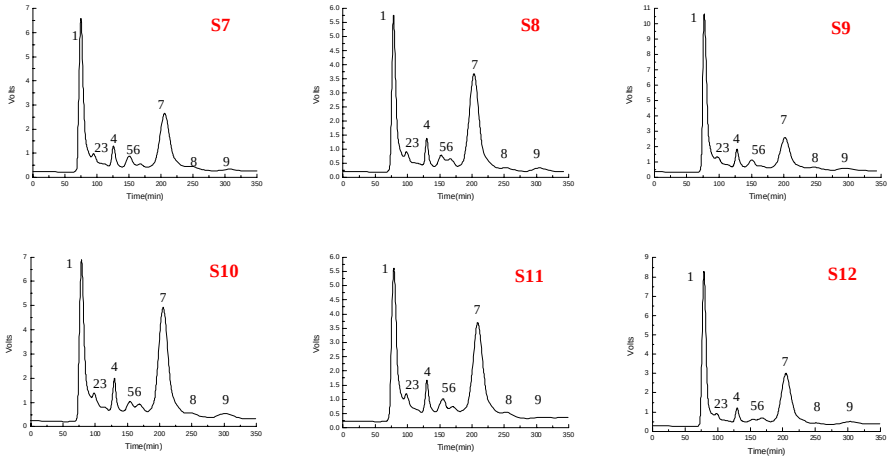
HSCCC separation of twelve crude water extracts of *Salvia miltiorrhiza* Bunge (1/2)

高速逆流色谱分离12个丹参水溶性粗制样品



HSCCC separation of twelve crude water extracts of *Salvia miltiorrhiza* Bunge (2/2)

高速逆流色谱分离12个丹参水溶性粗制样品



高速逆流色谱分离山柚柑的A3化合物

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Short communication

Isolation of high purity 1-[2′,4′-dihydroxy-3′,5′-di-(3″-methylbut-2″-enyl)-6′-methoxy] phenylethanone from *Acronychia pedunculata* (L.) Miq. by high-speed counter-current chromatography

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Acronychia pedunculata (L.) Miq.

山柚柑

***Acronychia pedunculata* (L.) Miq. (Shan You Gan or Jiang Zhen Xiang) is a small evergreen shrub widely distributed in Indo-Malayan and Southern China. The roots, stems, leaves, and fruits of this plant have been used in folk medicine for the treatment of diarrhoea, tussis, asthma, ulcers, itchy skin, scales, pain, and rheumatism, and as an antipyretic and antihemorrhagic agent as well as an aphrodisiac.**

山柚柑是一种常绿灌木，广泛分布于印度-马来半岛和中国南部。该植物的根、茎、叶和果实都可作为民间药使用，用于治疗腹泻、咳嗽、哮喘、溃疡、皮肤瘙痒、鳞屑、疼痛、风湿，并作为退热剂和催欲剂。

Cyclooxygenase (COX) enzymes catalyze the conversion of arachidonic acid into prostaglandins. There are two isoforms, COX-1 and COX-2. COX-2 is not detected in most normal tissues and is induced by mitogenic and inflammatory stimuli and produces prostaglandins in inflamed and neoplastic tissues.

环加氧酶（ COX ）催化花生四烯酸转化为前列腺素。有2个对碘氧基苯甲醚COX-1和COX-2。COX-2在大多数组织中无法检测，且是由促有丝分裂的和炎性的刺激物诱发，在红肿的和肿瘤组织中产生前列腺。

Consequently, selective COX-2 inhibition is necessary in order to minimize side effects caused by the inhibition of COX-1 by common non-steroidal anti-inflammatory drugs used to treat different inflammatory disorders.

因此，选择性的COX-2抑制是非常必须的以减小由COX-1抑制引起的副作用，COX-1抑制由常用于治疗不同炎性紊乱的非-类固醇抗炎药物引起的。

A dichloromethane extract of *Acronychia pedunculata* stem bark showing high cyclooxygenase-2 (COX-2) inhibitor activity was chosen as starting material for its isolation and purification.

山柚柑树皮的二氯甲烷提取物表现了很强的环加氧酶-2（ COX-2 ）抑制剂活性，将其作为分离和纯化的起始的原材料。

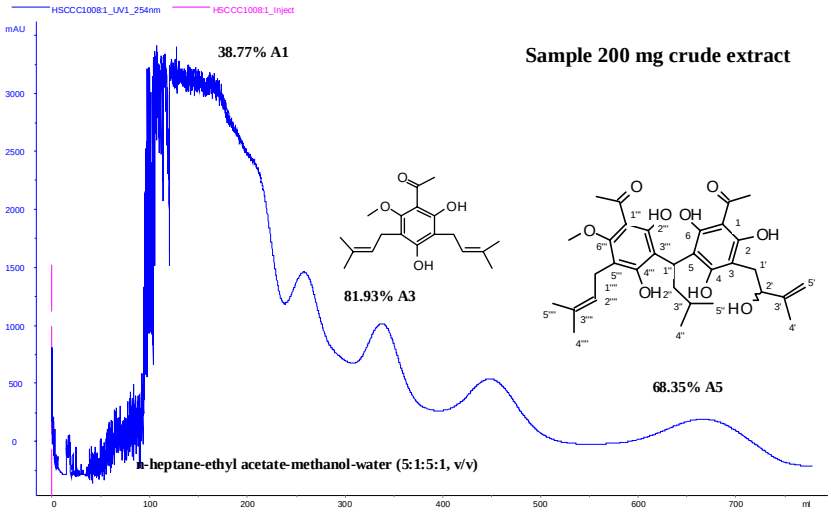
The purpose of this work was to determine the chemical structure and biological activity of the inhibitor.

本研究的目的在于确定抑制剂化学结构和生物学活性。

The dichloromethane extract was first subjected to a silica column clean-up step. It was then applied to the TBE-300A HSCCC column from Tauto Biotech, Shanghai, connected to a ÄKTAbasic chromatography system from GE Healthcare Biosciences.

二氯甲烷提取物先经硅胶柱粗分离。然后使用TBE-300A型HSCCC（同田，上海)与GE Healthcare Biosciences 的ÄKTAbasic系统连接进行分离。

HSCCC on a TBE-300A connected to ÄKTAbasic system



TBE-300A型HSCCC与ÄKTAbasic系统连接进行分离

Preparative purification of A3 to high purity
制备级纯化高纯度A3

A3 (1-[2', 4'-dihydroxy-3', 5'-di-(3''-methylbut-2''-enyl)-6'-methoxy] phenylethanone) is one of the main components in solvent extracts of *Acronychia pedunculata* and shows a high COX-2 inhibitory activity.

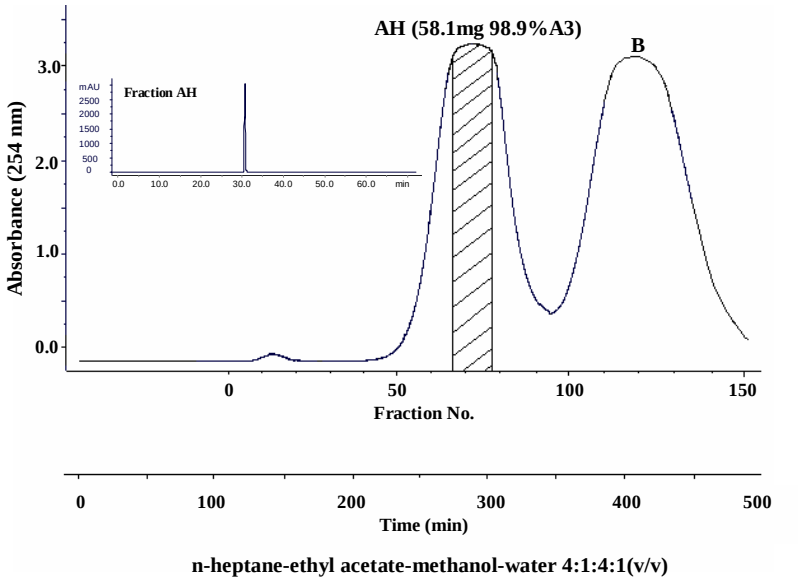
A3（ 1-[2',4'-二羟基-3',5'-二-(3''-甲基丁酰-2''-烯基)-6'-甲氧基] 苯乙酮 ）是山柚柑溶剂提取物的主要成分之一，具有很高的COX-2抑制活性。

In the large scale purification of A3 the silica column clean-up step gave an A3 purity of 35.7%. Using the optimized n-heptane-ethyl acetate-methanol-water solvent system (4:1:4:1, v/v), 58.1 mg A3 with a purity of 98.9% could be obtained after the HSCCC step.

硅胶柱上A3的规模纯化中获得了35.7%纯度的样品。在HSCCC上使用优化的溶剂体系正庚烷-乙酸乙酯-甲醇-水（ 4:1:4:1, v/v ）获得了58.1 mg的98.9% A3。

HSCCC on a TBE-300A connected to ÄKTAbasic system

TBE-300A型HSCCC与ÄKTAbasic系统连接进行分离



n-heptane-ethyl acetate-methanol-water 4:1:4:1(v/v)

正庚烷-乙酸乙酯-甲醇-水（ 4:1:4:1, v/v ）

One-step separation and purification of

the alkaloid dl-tetrahydropalmatine

from *Corydalis yanhusuo* W. T. Wang

by high-speed counter-current chromatography

Ming Gu, Zhiguo Su and Fan Ouyang

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Institute of Process Engineering
Chinese Academy of Sciences
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***Corydalis yanhusuo* W. T. Wang**

延胡索生物碱样品

Sample: Crude extract of *Corydalis yanhusuo* W. T. Wang
样品：罂粟科紫堇属植物延胡索干燥块茎的醇提物

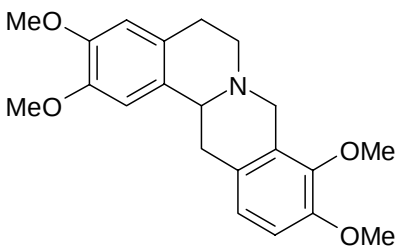
Function: Promotes blood circulation, reinforces vital energy and alleviates pain
功能：行气活血，散瘀止痛

Major component: Alkaloids
主要化合物类型：生物碱类化合物

Representative component: dl-Tetrahydropalmatine
代表性化合物：延胡索乙素

Representative component of *Corydalis yanhusuo* W. T. Wang

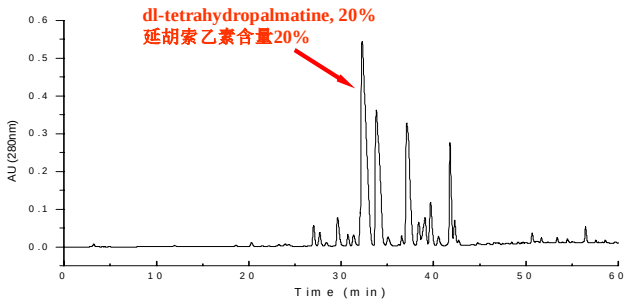
延胡索生物碱代表性化合物



dl-tetrahydropalmatine
延胡索乙素

Analysis of *Corydalis yanhusuo* W. T. Wang by HPLC

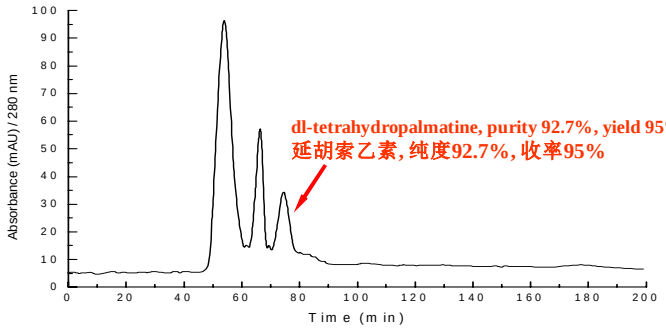
高效液相色谱分析延胡索生物碱样品



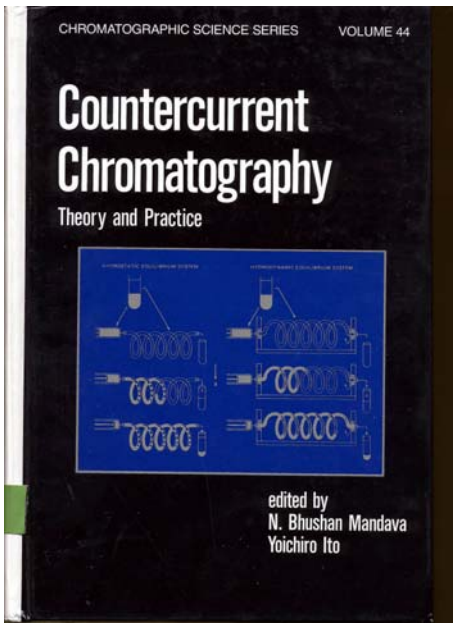
Column: Agilent 4.6×250 mm; Crude extract: 5 mg/ml, 20 μl;
Mobile phase: methanol-water-acetic acid-triethylamine,
Phase A: 10:89.1:0.8:0.1, Phase B: 10:89.1:0.8:0.1,
Gradient: 0-10 min, 1-16% B, 10-35 min, 16-40% B,
35-60 min, 40-100% B;
Flow rate: 0.9 ml/min; UV detection: 280 nm

Separation of *Corydalis yanhusuo* W. T. Wang by HSCCC

高速逆流色谱分离延胡索生物碱样品



Solvent system: n-hexane-ethyl acetate-methanol-water=4:6:5:5;
正己烷-乙酸乙酯-甲醇-水= 4:6:5:5;
Sample: 25 mg/5 ml;
1.2 ml/min; 850 r/min; 280 nm



Thank you very much

for your attention!