Separation and purification of Z-Ligustilide and Senkyunolide A from *Ligusticum Chuanxiong* Hort. by High-speed counter-current chromatography

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Abstract High-speed counter-current chromatography was applied to the separation and purification of Z-Ligustilide and Senkyunolide A from the essential oils of *Ligusticum chuanxiong* Hort.. Essential oils which were extracted from dried roots of *Ligusticum chuanxion* by supercritical carbon dioxide were separated by HSCCC using a two-phase solvent system composed of nhexane-ethyl acetate-methanol-water (7:2:5:4 v/v). Z-Ligustilide and Senkyunolide A were yielded at over 98% purity determined by GC.

Keywords: Counter-current chromatography; Z-Ligustilide; Senkyunolide A; *Ligusticum chuanxiong*

1. Introduction

Ligusticum chuanxiong Hort.(Chuanxiong) is well known in Chinese traditional medicine for it's extensive application in treating headaches, ischemic stroke, anemia, and cerebral vascular[1,2]. The essential oils of *Chuanxiong* constited of Z-Ligustilide, Senkyunolide A and other phthalides. Z-Ligustilide was proved to be the main pharmaceutical component[3,4]. Z-Ligustilide and Senkyunolide Awith high purity which can be used as markers are needed for the quality control of *Ligusticum Chuanxiong medicinal materials* or any products from *Ligusticum Chuanxiong*.

On the other hands, these phthalides are proved to be unstable[5], so it's difficult to get the purified products by conventional silica gel column chromatography. Furthermore these methods were time-consuming and used large amounts of reagent[4]. High-speed counter-current chromatography (HSCCC) has gained excellent achievements in the development of natural medicines from natural plant, many compounds with high purity have been separated from herb. However only little literature have been reported about the appliance of HSCCC to separate the essential oils from plant.

The present paper describes the method to separate Z-Ligustilide and Senkyunolide A from a crude extract of *Chuanxiong* by HSCCC.

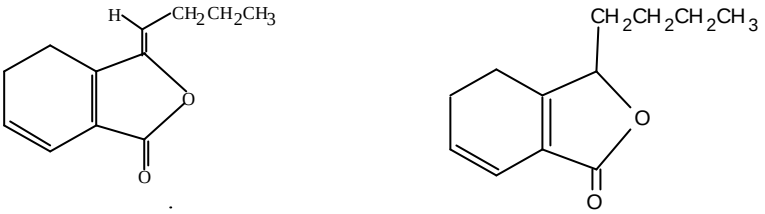

Fig. 1. The chemical structures of （Z）-Ligustilide and Senkyunolide A

2. Experimental

2.1 Apparatus

A Supercritical carbon dioxide apparatus (Nantong Huaan supercritical Extraction Co. Ltd.) was used.

A TBE-300A Semi-Preparative HSCCC (Shanghai Tauto Biotech Co., LTD, Shanghai, China) was performed equipped with three PTFE multilayer coils separation column of 150m×1.5mm I.D. with a total capacity of 280 ml. The β value varied from 0.5 at the internal terminal to 0.8 at the external terminal (β=r/R where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge).

The solvent was pumped into the column with a Model S-1007 constant-flow pump. A Model 8823B UV detector was used to continuously monitor the effluent at 280nm. A manual sample injection valve with a 20 ml loop was used to introduce the sample into the column.

A Shimadzu gas chromatograph, Model GC-14B (Japan), was employed equipped with a flame ionization detector (FID) and a capillary column: length 50 m, id 0.2mm, SE-54 type bonded phase, film thickness 0.5 μm, (Dalian Elite Analytical Instruments Co., Ltd., Dalian, China.)

2.2 Regents

All organic solvents used for HSCCC were of analytical grade and purchased from Tianjin Shield Company, Tianjin, China.

2.3 Preparation of two-phase solvent system

The two-phase solvent used for HSCCC was composed of nhexane - ethyl acetate - methanol-water. After thoroughly equilibrating the mixtures in a separatory funnel at room temperature, two phases were separated before use where the organic phase was used as the stationary phase and the aqueous phase as the mobile phase.

2.4 Preparation of sample and sample solutions

About 1.0 Kg of dried and powered roots of *Chuanxiong* was extracted with supercritical carbon dioxide under the pressure of 25 MPa and the temperature of 40℃ which yielded 12 g of essential oil. A 120-mg amount of this oil was dissolved with 4 ml of mixture of upper and lower phases for preparative separation.

2.5 Separation procedure

The coiled column was first entirely filled with the upper organic phase. Then the apparatus was rotated at an optimum speed of 800 $r*min^{-1}$, while the lower aqueous phase was pumped into the head end of the column at a suitable flow-rate of 2.0 ml/min. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, 4 ml of the sample solution containing 120mg of the essential oils was injected through the injection value. The effluent from the tail end of the column was continuously monitored with UV detection at 280 nm and peak fractions were each collected according to the elution profile.

2.6 TLC analysis

TLC analysis was carried out on Silica gel 60 F_{254} and Silica gel. The solvent consisted of nhexane-ethyl acetate (10:1), and the simple were dissolved in methanol. Direct detection was in UV light at 254 nm and 365 nm.

2.7 GC analysis and identification of CCC peak fractions

Each purified peak fraction from the preparative HSCCC separation was analyzed by GC with a capillary column (50m, 0.25mm i.d., 20μm film thickness) and FID detection. The oven temperature was 240℃, the temperature of the on-column injector and detector was 280℃. The pressure of the carrier gas (N_2) was 210KPa with a split ratio of 30:1. Samples of 1 μL were injected manually.

3. Results and discussion

The extracts of supercritical carbon dioxide of *Chuanxiong* dissolved in methanol were injected into GC. The chromatogram is shown in Fig.2. It shows that the main components of *Chuanxiong* are Senkyunolide A and Z-ligustide.

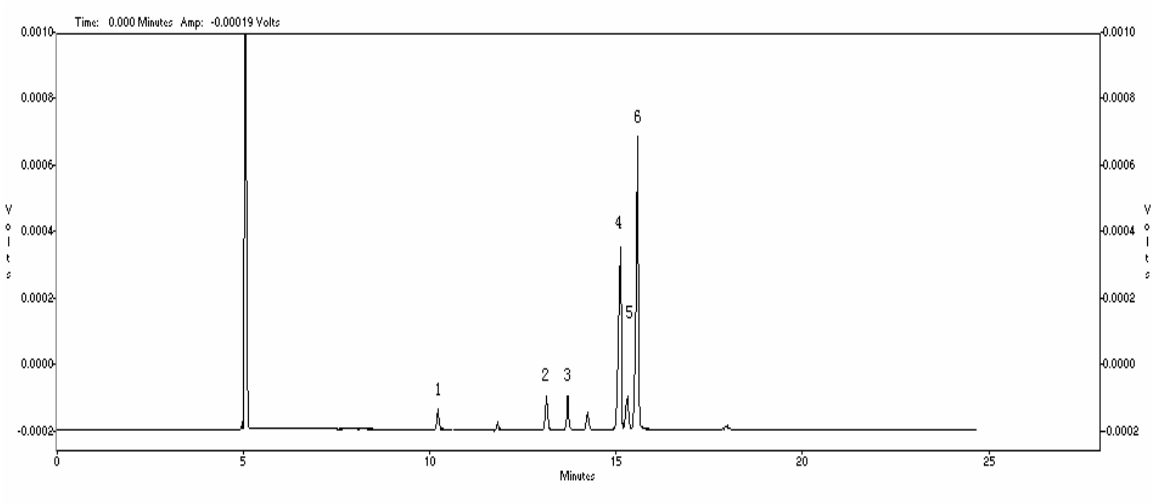

Fig. 2. GC analysis of ***Chuanxiong*** essential oils by supercritical carbon dioxide (Simple in methanol)

1.decahydro-4α-methyl-1-methylene-7-naphthalene; 2. 3-butylphthalide; 3. 3-butylidenephthalide; 4. senkyunolide A; 5. neocnidilide; 6. (Z)-Ligustilide.

In order to achieve an effective separation by HSCCC, the most important thing is to determine a suitable solvent system which provides an optimum range of partition coefficient (2>K>5) for the target compounds. We selected the two-phase solvent system composed of nhexane - ethyl acetate - methanol- water after numerous series of experiments. Then we modified the system, monitored the separating result by using the TLC and GC. Fig. 3 shows the results of the preparative separation of 120 mg of the oils from Chuanxiong by HSCCC which yielded 18 mg of Z-ligustide and 10 mg of Senkyunolide A. Two target compounds can be separated by the solvent system composed of nhexane - ethyl acetate - methanol –water (7:2:5:4,v/v).

Fig 4 shows the results of GC analyses of Senkyunolide A and Z-ligustide purified by HSCCC which indicated that the purity of the two target compounds were over 98%. Electrospray ionization (ESI) MS was adopted to analyze Senkyunolide A and Z-ligustide. The mass data of Z-ligustide were formed as m/z (rel. int.), 190(M^+, 66), 161(100), 148(75), 133(18), 105(51), 77（28）, 55(41) and the mass data of Senkyunolide A were formed as m/z (rel. int.), 192（M^+,23）, 135（5）, 107（100）, 79（22）. Both of the mass data of Senkyunolide A and Z-ligustide were in agreement with the literature[3].

The results of our studies indicate that HSCCC was successfully used for the separation and purification of Senkyunolide A and Z-ligustide.