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Recycling of Sclareolide in the Crystallization Mother Liquid of Sclareolide by Adsorption and Chromatography

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***Abstract*—**Sclareolide is made from sclareol by oxidiative synthesis and subsequent crystallization, while the crystallization mother liquor still contains 15%~30%wt of sclareolide to be reclaimed. With the reaction material of sclareol is provided as plant extract, many sorts of complex impurities exist in the mother liquor. Due to the difficulty in recycling sclareolide after solvent recovery, it is common practice for the factories to discard the mother liquor, which not only results in loss of sclareolide, but also contributes extra environmental burden. In this paper, a process based on adsorption and elution has been presented for recycling of sclareolide from mother liquor. After pretreatment of the crystallization mother liquor by HZ-845 resin to remove parts of impurities, sclareolide is adsorbed by HZ-816 resin. The HZ-816 resin loaded with sclareolide is then eluted by elution solvent. Finally, the eluent containing sclareolide is concentrated and fed into the crystallization step in the process. By adoption of the recycle from mother liquor, total yield of sclareolide increases from 86% to 90% with a stable purity of the final sclareolide products maintained.

***Keywords*—**Sclareolide, resin, adsorption, chromatography.

I. Introduction

SCLAREOLIDE is a special-purpose substance. It can be used for the treatment of infectious diseases caused by microorganisms (such as fungi, bacteria) [1]. In the blended cigarettes it can be used as flavoring agents of tobacco, adding amber-like aroma to the tobacco. It is also available in foods containing sweetener as a flavoring agent to increase the olfactory effect of food.

Natural sclareolide exists in the oil of clary sage and its mass fraction is about 35% [2]. The synthetic sclareolide is mainly obtained by oxidation of sclareol. Pure sclareolide is white crystal with a lasting amber aroma. It does not dissolve in water, but is soluble in methanol, ethanol, acetone, petroleum ether and other organic solvents. At present there are mainly two preparation methods of sclareolide. The first method is the chemical synthesis, such as U.S. Pat. No. 5,525,728 [3] and U.S. Pat. No.5,247,100 [4]. This method uses natural sclareol as raw material and the crude product of sclareolide is obtained by oxidation reaction with a ruthenium catalyst. The second method is biological fermentation, turning the sclareol into the crude product of sclareolide via *Cryptococcus albidus* and *Bensigntonia ciliata*. Methods of sclareolide purification are mainly molecular distillation and crystallization. The system of molecular distillation [5] requires high vacuum and excellent air tight equipment should be employed. The distance between the evaporation surface and the condensation surface must be proper so that the equipment is difficult to be manufactured. What's more, its processing capacity is small and its production cost is high. Crystallization is an effective way to separate impurities. But, sclareolide is made from natural material and various impurities with similar structure exist in the mixture. Single crystallization cannot meet the requirement of quality and yield.

In recent years, column chromatography technology has gradually matured. It has achieved satisfactory results to separate substances with close boiling points and similar structure. Chromatography, relying on the difference of material distribution ratio between the stationary and mobile phases, achieves the goal of separation and purification of substance [6]. The distribution coefficient, the column length and the retention time are main factors affecting the chromatography effect. Eddy diffusion, longitudinal diffusion and mass transfer impedance also have some influence on the chromatographic separation [7]. Therefore，it is important for chromatography to choose proper chromatography media and operation condition, such as flow rate, feed concentration, elution solvent and flow rate of elution.

There are a lot of reports on research about the application of adsorption resins. By studying four kinds of resins, [8] found that NK-S3 resin could adsorb much tea polyphenol, up to 81.57mg/ml. The elution ratio was close to 100% in the proper elution condition. With the comparison of 15 kinds of resin in static adsorption, dynamic adsorption and elution properties, [9] developed the process that could continuously get three kinds of effective components: polysaccharide, polyphenol and caffeine from tea. Shengnan Sun [10] separated and extracted the aroma components of honeysuckle via water extraction and macroporous resin chromatography. Libo Wang [11] chose D101-1 resin from 9 kinds of macroporous resin to purify pumpkin polysaccharide. The purity of pumpkin polysaccharide increased from 25.68% to 61.39%. But the purification of sclareolide by resin has not been reported.

Sclareol is oxidized to sclareolide via the catalyzation of potassium permanganate and sclareolide product with high

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purity can be obtained by crystallization process. But the crystallization mother liquor in the process still contains 15%~30%wt of sclareolide. Sclareol as the reaction material is provided in the form of a natural plant extract, so miscellaneous impurities are introduced into the reaction system and finally left in the crystallization mother liquor. Some of impurities are quite similar to sclareolide in molecular structure and properties. The mother liquid is usually abandoned after solvent recovery in industrial processes, which not only leads to product loss, but also exerts environmental burden to deal with the wastes. This study plans to recycle sclareolide in the crystallization mother liquor via adsorption and chromatography, aiming to obtain higher product yield with required purity. As shown in Fig. 1, mother liquor is firstly pretreated by HZ-845 resin to remove part of impurity and reduce the difficulty of subsequent separation. Then sclareolide in the mother liquid is adsorbed by HZ-816 resin. The sclareolide loaded in the resin is then eluted by elution solvent. The collected eluent containing sclareolide is finally concentrated and fed back into the crystallization step to increase sclareolide yield.

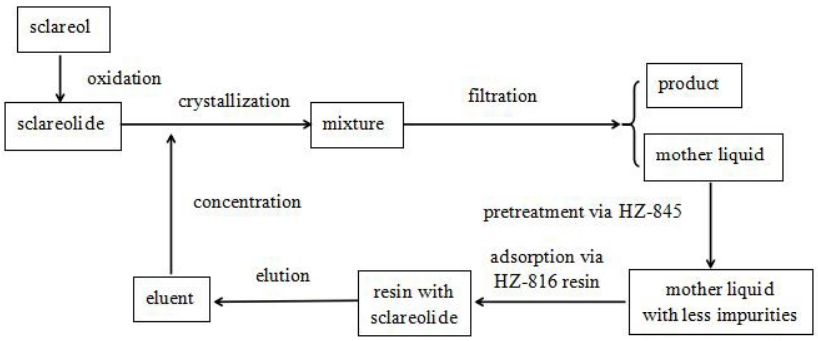

Fig. 1 The flowchart of process

II. Material and Method

A. Experimental Materials

Materials used in the experiment include standard sample of sclareolide (wt97.3%), crude product of sclareolide (wt80.1%), sclareolide crystallization mother liquor (wt14%), provided by Guangchang Mei Yue Technology Co., Ltd.

The macroporous adsorption resin used in the study was HZ-816 and HZ-845 provided by Shanghai Huazhen Corporation of East China University of Science and Technology. HZ-816 resin is non-polar and HZ-845 resin is medium polar.

Ethanol, dibutyl phthalate, cyclohexane of analytically pure, were provided by First Chemical Factory of Shanghai Zhenxing. Methanol, while butyl acetate, ethyl acetate, ethylene dichloride, isopropyl alcohol of analytically pure, were purchase from Sinopharm Chemical Reagent Co., Ltd.

B. Analytical Method

In the study sclareolide content was analyzed by gas chromatography. Relevant instruments were 9790FID gas chromatography (Zhe Jiang Fuli Ltd.), SGE-D3 Capillary Column (Nanjing Xin Hang Scientific Instrument Co., Ltd.), N2000 Chromatography data workstations (Zhejiang University Zhida Information Engineering Co., Ltd.).

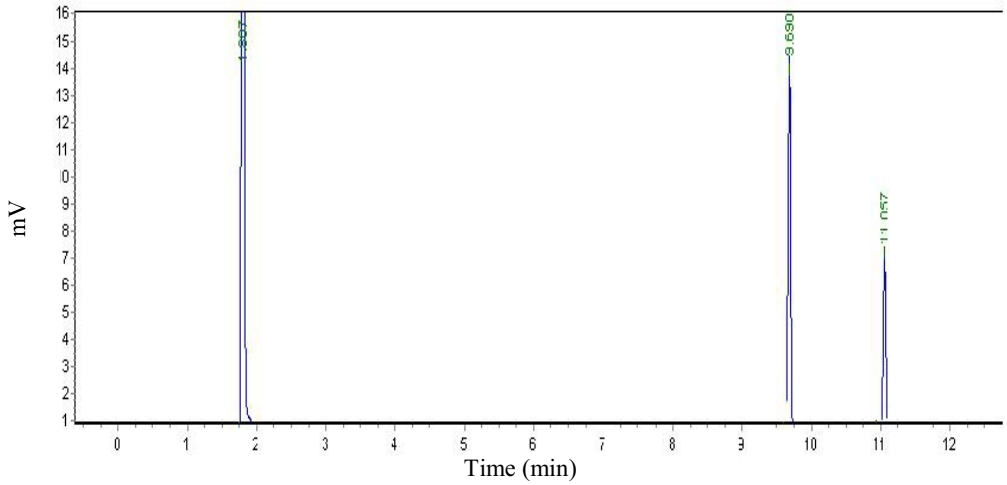

Fig. 2 The gas chromatogram of sclareolide, ethanol and internal standard material

Dibutyl phthalate was chosen as an internal standard with ethanol being used as solvent [12]. Typical chromatographic analysis chart is shown in Fig. 2. The peaks with retention time of 1.832, 9.690 and 11.05 min correspond to ethanol, dibutyl phthalate and sclareolide respectively. After many experiments by internal standard method, the standard curve of sclareolide can be calibrated with A_2/A_1 as abscissa and m_2/m_1 as ordinate, where A_2 and A_1 refer to peak area of sclareolide and dibutyl phthalate, m_2 and m_1 represent mass of sclareolide and dibutyl phthalate in the injection sample. When sclareolide concentration is 6.8~34.1g/L, the standard equation of the curve is y=1.0711x-0.0332. The correlation coefficient is 0.9955 and the fitting can be regarded as satisfactory.

III. Results and Discussion

A. Pretreatment of the Mother Liquid

Considering the complexity of mother liquor components, some pretreatment, before adsorption chromatography, is required to reduce part of the impurities and decrease the difficulty of subsequent separation. According to the theory of "similarity and attraction", moderate polar resin could adsorb moderate polar impurity in the mother liquid. So HZ-845 resin was chosen to adsorb part of impurities in the mother liquid.

40ml methanol was mixed with 20ml mother liquor thoroughly and 10g HZ-845 macroporous resin was added into the mixture. Static adsorption last for 8 hours at normal temperature and then adsorption capacity of HZ-845 resin and the effect of impurity removal were inspected. Before and after the solution was pretreated by HZ-845 resin, the sclareolide concentration was analyzed by gas chromatographic respectively. Chromatograms are shown as Figs. 3 and 4.

Fig. 3 represents the chromatogram after the mother liquid being pretreated by the resin. Compared with Fig. 4, the mother liquid's chromatogram, the peaks of impurities after 18 minutes have disappeared. Namely, these impurities were adsorbed by HZ-845 resin. What's more, the sclareolide content increased from the initial 14%wt to 20%wt.

The adsorption capacity of sclareolide with HZ-845 resin is calculated by (1):

$$Q = (C_0 - C_1)\ V / m_1 \quad (1)$$

where C_0 is the initial sclareolide concentration before adsorption and C_1 is the equilibrium sclareolide concentration after adsorption, V is the volume of the solution and m_1 is the dry weight of the resin, Q is adsorption capacity of sclareolide with HZ-845 resin.

It has been experimentally found that the adsorption capacity of HZ-845 resin for sclareolide is 17.78mg/g. The adsorption capacity is low and only 3.10% loss of sclareolide is found, which will not cause obvious loss of sclareolide yield. So it is feasible to use HZ-845 for pretreatment of the mother liquid.

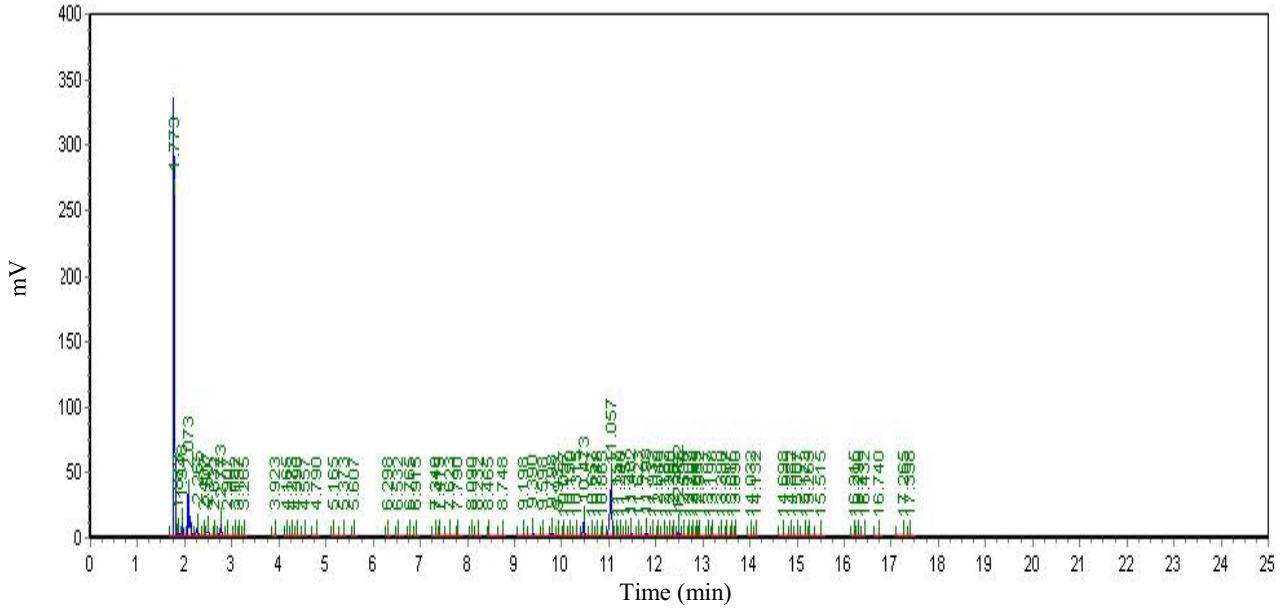

Fig. 3 The gas chromatogram of mother liquid pretreated by HZ-845 resin

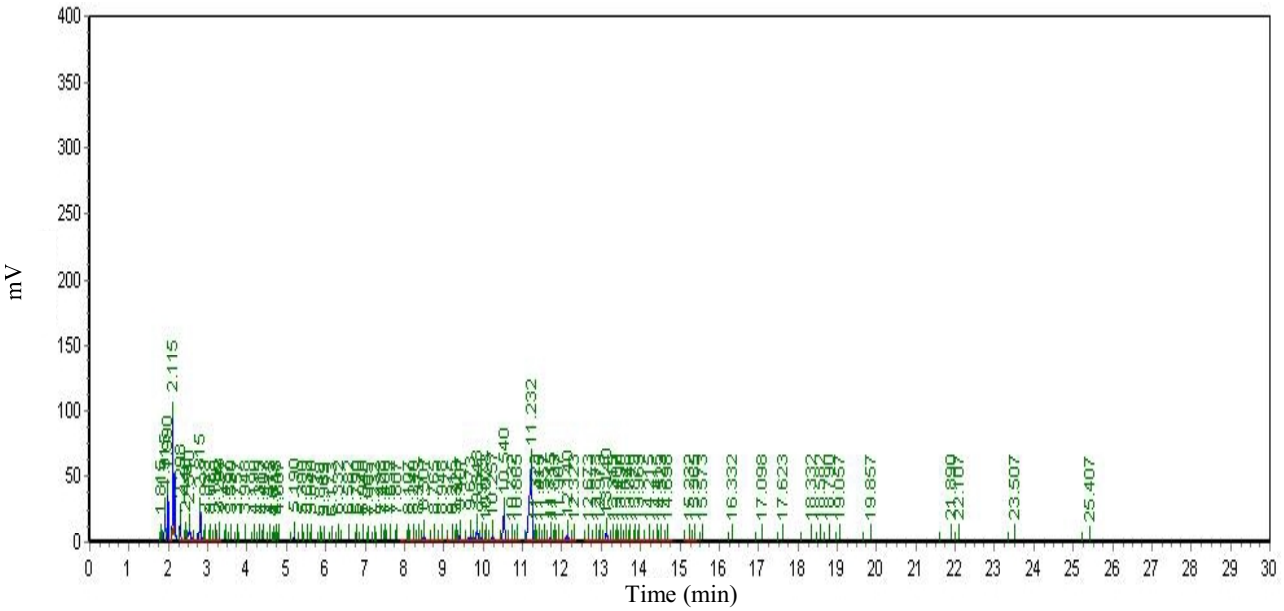

Fig. 4 The gas chromatogram of mother liquid

B. Selection of Adsorption Solvent

Certain mass of sclareolide crude product, certain mass of pretreated HZ-816 resin and 50ml solvent were mixed and the mixture was stirred for 8 hours at room temperature. After sufficient adsorption, the solution was filtered and sclareolide content in the filtrate was analyzed by gas chromatography. The adsorption capacity of sclareolide with HZ-816 resin was calculated according to (1). Six kinds of solvents including ethanol, ethylene dichloride, isopropyl alcohol, petroleum ether, ethyl acetate and methanol were considered for adsorption solvent. The result is shown as Table I.

TABLE I
THE ADSORPTION CAPACITY OF SCLAROLIDE WITH HZ-816 RESIN IN DIFFERENT SOLVENT

solvent	Resin (g)	Sclareolide (g)	C_0 (g/L)	C (g/L)	Adsorption capacity (mg/g)
ethanol	20.71	2.05	40.90	13.10	67.12
ethylene dichloride	20.70	2.31	46.18	27.19	33.31
isopropyl alcohol	33.31	2.03	20.30	8.69	58.07
petroleum ether	19.99	2.03	20.30	8.69	58.07
ethyl acetate	20.69	2.04	46.18	22.31	46.23
methanol	20.72	2.06	37.61	14.23	61.33

As can be seen from Table I, adsorption capacity of resin for sclareolide in the ethanol and methanol is greater than in other solvents. The polarity of methanol is stronger than ethanol and methanol was cheaper. So methanol is chosen as solvent adsorption.

C. Effects of Flow Rate and Sclareolide Feed Concentration on Sclareolide Adsorption Ratio in the Fixed Bed

Sclareolide Adsorption Ratio in the fixed bed was conducted at different flow rate and different sclareolide feed concentration. The flowchart of fixed bed is shown as Fig. 5.

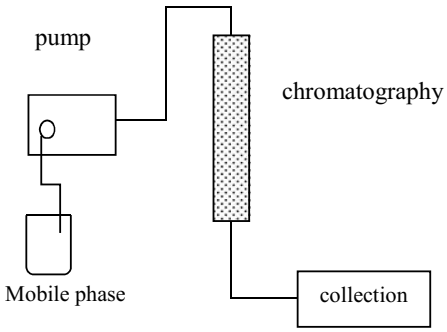

Fig. 5 The flowchart of fixed bed

Experimental procedure was as follows. 5g HZ-816 resin was loaded into the column (1cm I.D.). Then 2.5BV solution, consisting of sclareolide crude product and methanol, was fed into the column by the pump. The effluent was collected by a collector and sclareolide concentration in the effluent was analyzed by gas chromatography. The adsorption ratio of HZ-816 resin for sclareolide could be calculated by (2):

$$R = (C_0 - C_2)/C_0 \quad (2)$$

where C_0 is initial sclareolide concentration before adsorption and C_2 is sclareolide concentration in the collector after adsorption is finished, R is adsorption ratio.

Three sets of flow rate, 1BV/h, 2BV/h and 3BV/h,were researched and initial sclareolide concentration was 0.105g/ml. Then three sets of sclareolide feed concentration, 0.04g/ml, 0.08g/ml and 0.12 g/ml, were reaearched and flow rate was 2BV/h.

TABLE II
ADSORPTION RATIO OF SCLAREOLIDE IN THE FIXED BED AT DIFFERENT FLOW RATE AND DIFFERENT SCLAREOLIDE FEED CONCENTRATION

Flow rate (BV/h)	Sclareolide feed concentration (g/ml)	Adsorption ratio (%)
1		84.26
2	0.105	94.80
3		65.04
	0.04	75.59
2	0.08	84.54
	0.12	75.67

Table II shows effects of flow rate and sclareolide feed concentration on sclareolide adsorption ratio in the fixed bed. As can be seen from it, adsorption ratio increases with increment of flow rate changed from 1BV/h to 2BV/h. However, adsorption ratio of 3BV/h is much lower than that of 1BV/h and 2BV/h. If flow rate is too high, sclarolide would penetrate the column quickly. So 2BV/h is chosen as proper flow rate. Adsorption ratio of 0.08g/ml is higher than that of 0.04g/ml and 0.12g/ml when flow rate is 2BV/h. So 0.08g/ml is chosen as sclareolide feed concentration.

D.Selection of Elution Solvent

9.5754g sclarolide was dissolved in 200ml ethanol and then 98g HZ-816 resin was mixed with the solution. After stirred for 8h sufficiently, the mixture was filtered and resin, whose adsorption capacity of sclarolide was 147.99mg/g, was prepared to do elution experiments, whose flowchart was shown Fig. 5. The elution ratio of sclarolide could be calculated by (3):

$$R_2 = C_3 V / m_2 \quad (3)$$

where C_3 is sclarolide concentration in the eluent, V is eluent volume and m_2 is sclarolide mass in the resin.

The pending elution solvent was methanol, ethanol, butyl acetate, ethyl acetate, isopropyl alcohol and cyclohexane. Via a series of static elution experiments, it could be found that elution ratio of butyl acetate and cyclohexane are better, reaching 52.82% and 49.49%. Since good elution effect cannot be achieved with single-component elution solvent, cyclohexane is chosen to be co-solvent with butyl acetate and methanol according to experience.

The volume ratios of cyclohexane, butyl acetate and methanol in the co-solvent of 1:2:7, 3:2:5 and 4:2:4 were considered. Then dynamic elution experiments were conducted with well-prepared resin and co-solvent. Elution rate was 0.5 BV/h, namely 0.59ml/10min. The elution ratio was calculated by the sclareolide concentration, analyzed by gas chromatography. The result is shown as Fig. 6.

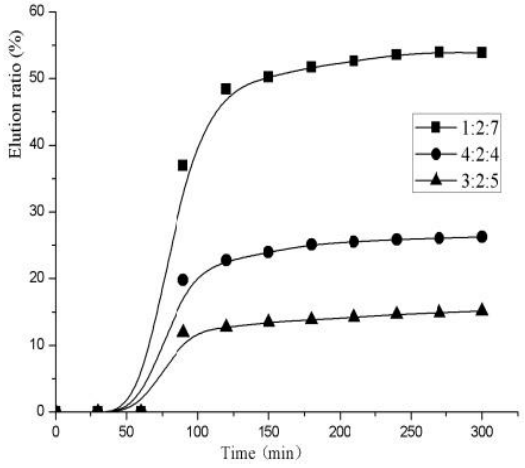

Fig. 6 The elution curve of sclarolide by cyclohexane, butyl acetate and methanol

As can be seen from Fig. 6, elution ratio could reach 55.18% when the volume ratio of cyclohexane, butyl acetate and methanol in the co-solvent is 1:2:7, which is better than co-solvent of cyclohexane and butyl acetate. According to these elution experiments, it could be found that elution ratio had hardly increased when elution last for 100min. Same resin were used in the elution experiments. Therefore, these elution experiments can be compared with each other.

E. Selection of Elution Rate

A certain quality of sclareolide mother liquor was dissolved in methanol. Part of impurities was removed from the solution via HZ-845 resin and then sclareolide in the solution was adsorbed sufficiently by HZ-816 resin. 4.5g HZ-816 resin was loaded into the column and cyclohexane: butyl acetate: methanol in the co-solvent was 1:2:7 (volume ratio). Dynamic elution was conducted. The elution rates were respectively 0.5BV/h, 2BV/h, 6BV/h, 8BV/h, 10BV/h. The result is shown as Fig. 7.

As can be seen from Fig. 7, elution ratio could reach maximum 74% when elution rate is 8BV/h.

F. Crystallization Research of Chromatography Eluent

11.2g sclareolide crude product with 80.1%wt purity was crystallized and the crystallization mother liquid still contained some sclareolide. After solvent recovery, the sclareolide was separated from the crystallization mother liquid via HZ-845 resin and HZ-816 resin. The eluent with sclareolide was concentrated and used as feed for crystallization.

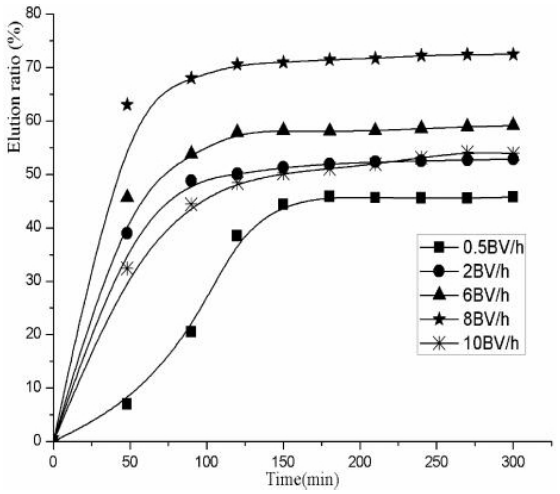

Fig. 7 The elution curve of sclarolide by different speed

The elution experiments were conducted as follows. After 13.14g mother liquid was pretreated by HZ-845, the sclareolide concentration in the mixture of mother liquid was respectively 0.04g/ml, 0.08g/ml, 0.12g/ml with the addition of methanol. The flow rate of solution through HZ-816 resin was 2BV/h. Then the resin was eluted by the co-solvent at the rate of 8BV/h. Cyclohexane: butyl acetate: methanol in the co-solvent was 1:2:7(volume ratio). The data of elution experiments are shown as Table III.

TABLE III
THE DATA OF ELUTION EXPERIMENTS

Concentration（g/ml）	0.04	0.08	0.12
Absorption ratio（%）	78.23	84.55	80.00
Sclareolide purity in the eluent（wt%）	0.3880	0.8610	1.2200
Elution ratio（%）	78	80	79.98

The eluent was merged into the crystallization step after concentrated. The data was shown as Table IV. The first group data were obtained with no eluent merged into the crystallization step. The second group data were obtained in experiment with eluent merged into the crystallization feed after concentration.

TABLE IV
THE COMPARISON OF MIXING THE ELUENT BEFORE AND AFTER

Method	Crystal purity (%)	Crystal mass(g)	Pure sclareolide mass (g)	Recovery rate of sclareolide (%)
I	96.5	8	7.72	86
II	96.7	10.06	9.73	90

As can be seen from Table IV, when crystallization mother liquid purified by the resin adsorption and the elution is merged into the crystallization step, the recovery rate of sclareolide could reach 90%, which higher than 86% the recovery of sclareolide in the first method. What's more, the product purity can be maintained with the second method.

IV. CONCLUDING REMARKS

In this paper, crystallization mother liquor was purified by adsorption and chromgraphy for recycling of sclareolide. Crystallization mother liquor is firstly pretreated by HZ-845 resin to remove part of impurities and the sclareolide content increases from 14% to 20%. Then HZ-816 resin and methanol have been respectively chosen as adsorbent and adsorption solvent. The optimal adsorption condition of fixed bed is that the sclareolide concentration in the feed is 0.08g/ml and the flow rate is 2BV/h. The co-solvent made up of cyclohexane, butyl acetate and methanol has been chosen as elution solvent. It is found that elution ratio can reach 74%wt when volume ratio of cyclohexane, butyl acetate and methanol is 1:2:7 and flow rate is 8BV/h. Finally, the eluent with less impurities is concentrated and merged into crystallization step. The total yield of sclareolide increases from 86% to 90% and the stability of production's purity can also be maintained.

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