ISSN: 2688-8645

# Study on the Purification Technology of Isolavonoids of Radix Puerariaes

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## Abstract

**The study compared adsorption properties of five macroporous resins, including S-8, NKA-9, AB-8, x-5, D4006, AB-8 resin was determined as the best sorbent. The optimum purification condition obtained was: the dried resin as reference, the flow rate on absorption is 0.5 mL/min, 100mL80%ethanol, the flow rate on desorpfion is 2.0mL/min. Under these conditions, the purity of pueraria isoflavones had much greatly improved from 21.65% to 87.55%, and The content of puerarin increases from 9.79%to 92.80%.**

## Keywords

**Radix puerariaes, Isolavonoids, Extraction, Purification.**

## 1. Introduction

At present, there are many reports of the extraction and purification of isoflavones of Radix Puerariae. Lead acetate precipitation method was put forward in Japan. Junei K refined isoflavones of Radix Puerariaes with water and n-butyl alcohol solution [1]. Jian-ping Guo with silica gel column chromatography could get higher recovery rate of flavonoids with water saturation n-butyl alcohol solution, Wei-jie Pan got puerarin, daidzein and refined flavonoid by using acid hydrolysis of ethyl acetate extraction and purification [2]. In recent years, macroporous adsorption resin chromatography separation dynamics has been researched and reported, using macroporous resin purification flavonoids has obtained the good effect [3].

Macroporous resin adsorption separation process to avoid the recovery difficult, big loss, high cost, flammable and explosive, the environmental pollution, caused by using organic solvent purification [4-6]. In this paper, using macroporous resin adsorption separation process for purification of crude flavonoids (Purity is only 21.65%), in order to provide theoretical reference for industrial production of pueraria isoflavones purification.

## 2. Materials and Equipment

### 2.1. Materials

Pueraria isoflavones extract (homemade), ethanol (95%), macroporous adsorption resin, hydrogen chloride, sodium hydroxide (A.R).

### 2.2. Experimental Apparatus

Winnowing chinese medicine grinder (WKX-160); stainless steel electrothermal blowing display (101-1); one over ten thousand electronic balance (Sartorius); constant temperature water-bath water (HH-S); vacuum pump (SHB-III); rotary evaporation instrument (RE-52AA); high-speed centrifuge (Avanti J 25); spectrophotometer (CARY 100).

### 2.3. Experimental Method

2.3.1 Pretreatment of the resin

ISSN: 2688-8645

After pretreatment, the new resin before use, to remove oligomer, organic matter and harmful ions.

2.3.2 Preparation and determination of Radix Puerariaes samples

Accurately measured 30.0g dry puerarin, Extraction isoflavones of Radix Puerariaes, according to the literature [7]. Accurately weighed rough flavonoid, with 95% ethanol solvent, constant volume, got the sample solution.

2.3.3 Analysis method purification of isoflavones

Calculated the content of isoflavones, according to the formula (1), Calculated the purity of isoflavones, according to the formula (3), according to the literature [7].

2.3.4 Static adsorption and analytical experiment

(1) Static saturated adsorption experiments

Accurately weighed five kinds of macroporous adsorption resin respectively 1.0g for S-8, NKA-9, AB-8, X-5, D4006, put in conical flask, join the sample liquid 20 mL, every 5 min vibration wave l0s, last 2 h, shading stand for 24 h, saturated adsorption. Accurately measured absorb a certain amount, diluted the determination of mass concentration of isoflavones, according to the literature [7]. By the formula (1) and formula (2), macroporous adsorption resin static adsorption capacity and adsorption rate were calculated respectively.

$Q_a = (C_o\text{-}C_l)\times V_a / W$ (1)

$E_a = (C_o\text{-}C_l)/ Co\times 100\%$ (2)

$P_f = (W_f/ W_l) \times 100\%$ (3)

In above formula: Qa-adsorption quantity(mg/g); Co-mass concentration of isoflavones before adsorption samples (mg/mL); Cl-mass concentration of isoflavones after adsorption samples (mg/mL); Va-adsorption volume(mL); W-macroporous adsorption resin dry weight (g); Ea-adsorption rate (%); Tf-extraction yield of isoflavones(%);Wf-total isoflavones content in the sample solution(g); W0- Radix Puerariae dry weight(g).

(2) Static analytical test

Accurately weighed five kinds of macroporous adsorption resin respectively 1.0g for S-8, NKA-9, AB-8, X-5, D4006, static saturated adsorption isoflavones, coarse filter befor put in plug taper bottle, add 70% ethanol, every 5 min vibration wave 10 s, for 2 h. Accurately measured analytical solution a certain amount, diluted the determination of mass concentration of isoflavones, according to the literature [7]. With the formula (4) and formula (5), the resolution of macroporous adsorption resin was calculated.

$Q_e = (C_e\times V_e)/W$ (4)

$E_e = (Q_e/Q_a) \times 100\%$ (5)

In above formula: Qe- amount of parsing (mg/g); Ce-mass concentration of isoflavones of analytic solution (mg/mL); W- macroporous adsorption resin dry weight (g);

2.3.5 Determination of eluant concentration

Static saturated adsorpted saturation AB-8 resin, coarse filtration, put in plug taper bottle, corresponding to join concentration were 20%, 30%, 50%, 30%, 80%, 95% ethanol 20 mL, every 5 min vibration wave 10 s, 2 h duration, fully desorption. Accurately measured analytical solution a certain amount, diluted the determination of mass concentration of isoflavones, according to the literature [7]. With the formula (5), the resolution of macroporous adsorption resin was calculated.

2.3.6 Dynamic adsorptionand analytical experiment of AB-8 resin

Accurately measured 10.0g AB-8 resin, wet packing column and acid-alkali treatment, the sampling rate was 0.5 mL/min.

(1) Selecting the dosage of eluent

ISSN: 2688-8645

Accurately measured a certain volume sample solution, to join in the resin column at 0.5 mL/min flow velocity, after adsorption saturation, elution with distilled water at 2.0 mL/min flow rate, untill eluent was very light in color collecting water wash, constant volume and enrichment befor determination of mass concentration of isoflavones. Measured the minimum level of consumption was the highest water elution amount of resin, then using 80% ethanol elution with 2.0 mL/min flow rate, once every 10 mL collection eluent, determination of mass concentration of isoflavones, content was almost equal to zero to determine the dosage of eluent.

(2) Selecting the velocity of eluent

Accurately measured a certain volume sample solution, to join in the resin column at 0.5 mL/min flow velocity, after adsorption saturation, elution with distilled water, untill eluent was very light in color, then using 80% ethanol elution, respectively, with 3.0 mL/min, 2.0 mL/min, 1.0 mL/min flow rate, once every 10 mL collection eluent, determination of mass concentration of isoflavones and the appropriate elution velocity.

2.3.7 Determination of refined flavonoid content and purity

Purification experiment was carried out to join in the resin column at 0.5 mL/min flow rate, selecting the determined types of resin, the concentration of ethanol elution agent, dosage of eluent and elution velocity. Collect eluent, purified by reduced pressure concentration, dissolved with 70% ethanol, vacuum drying to extract refined flavonoid.

## 3. Results and Discussion

### 3.1. Screening Macroporous Adsorption Resin

Static adsorption and analytical test were carried out to determine the best adsorbent. The results are shown in table 1, table 2 and table 3.

**Table 1.** Physical properties of five kinds of macroporous adsorption resins

| Resin | Polarity | Particle size Range (mm) | Specific surface area ($m^2/g$) | Average pore diameter (nm) | Porosity (%) |
|---|---|---|---|---|---|
| S-8 | polarity | 0.3-1.25 | 100-120 | 28.0-30.0 | 0.78-0.82 |
| NKA-9 | polarity | 0.3-1.25 | 250-290 | 15.5-16.5 | 46-50 |
| AB-8 | weak polarity | 0.3-1.25 | 480-520 | 13.0-14.0 | 42-46 |
| X-5 | nonpolar | 0.3-1.25 | 500-600 | 29.0-30.0 | 50-60 |
| D4006 | nonpolar | 0.3-1.0 | 400-440 | 6.5-7.5 | 43-48 |

**Table 2.** Results of static saturated adsorption tests

| Resin type | S-8 | NKA-9 | AB-8 | X-5 | D4006 |
|---|---|---|---|---|---|
| Adsorption quantity (mg/g) | 36.28 | 17.20 | 29.74 | 23.54 | 9.86 |
| Adsorption rate (%) | 89.17 | 66.25 | 84.96 | 76.66 | 32.28 |

**Table 3.** Results of static desorption tests

| Resin type | S-8 | NKA-9 | AB-8 | X-5 | D4006 |
|---|---|---|---|---|---|
| Analytical rate (%) | 53.41 | 76.32 | 88.94 | 78.36 | 80.44 |

The table 1 shows that five kinds of macroporous adsorption resin has significant differences for adsorption and resolution of isoflavones, due to the difference of physical properties. From table 2 shows that S-8 polarity resin and AB-8 weak polarity resin on the saturated adsorption

ISSN: 2688-8645

amount of isoflavones are high, D4006 nonpolar resin adsorption quantity is minimum. Aperture smaller D4006 not only slow adsorption speed, adsorption volume was small, and impurity separation effect was poorer, suggesting that the aperture size is an important factor of affect the adsorption effect of isoflavones. It show that the material is adsorpted through the aperture of the resin to adsorb spread to inner surface, only when the aperture is large enough, specific surface area can give full play to the role. At the same time, the adsorption capacity of macroporous resin is also associated with the molecular weight of pueraria isoflavones and configuration, so that the resin purification for material has certain selectivity. From table 3 shows the resolution for isoflavones of AB-8 resin was higher than other resins. Considering the cost of macroporous resin, physical properties and adsorption-resolution effect, with high specific surface area, appropriate pore diameter and large adsorption capacity for isoflavones, higher analytical rate of AB-8 resin as the best adsorbent.

## 3.2. Determination of Eluant Concentration

According to the above method to determine the optimum eluant concentration. The results shown in figure 1.

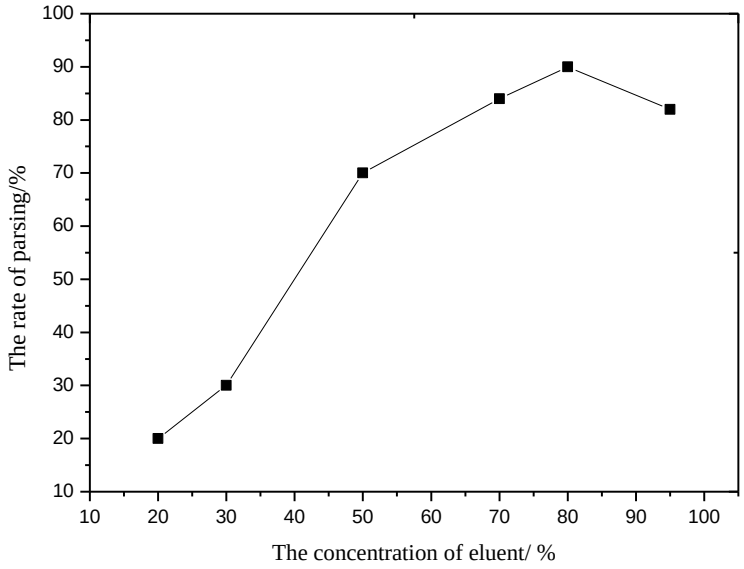


**Figure 1.** The effect of the concentration of ethanol on purification

From figure 1 shows that using 70% ethanol elution, analytic rate greatly enhanced with the increase of the concentration, when 70% slow growth, fell slightly after rose to 80%. The reason is that different isoflavone solubility in the different concentrations of ethanol. Considering resolution of isoflavones and the production cost, to determine 80% ethanol solution as the best eluent.

## 3.3. Determination of Eluent Dosage

According to the above method to determine the optimum eluent dosage. The results shown in figure 2.

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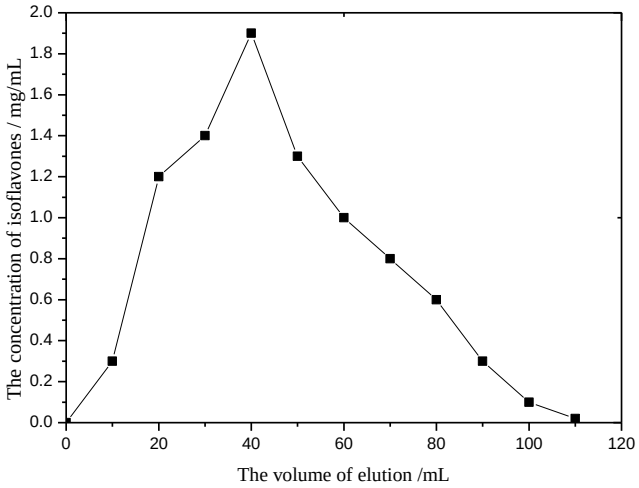


**Figure 2.** The effect of the dosage of ethanol on purification

The figure 2 shows that with the increase of number of elution, isoflavone content in the eluent gradually increase, isoflavone content of part 5 eluent was the highest. The reason was that the small molecule organic acids, coumarins, steroids and other organic compounds were cleared off first, flavonoids was cleared again. Along with the increase in number of elution, isoflavones content gradually reduce in eluent, part 10 almost no isoflavones composition. So the best ethanol elution volume was 100 mL for 10.0 g macroporous adsorption resin.

## 3.4. Determination of Eluant Flow Velocity

According to the above method to determine the optimum eluant flow velocity. The results shown in figure 3.

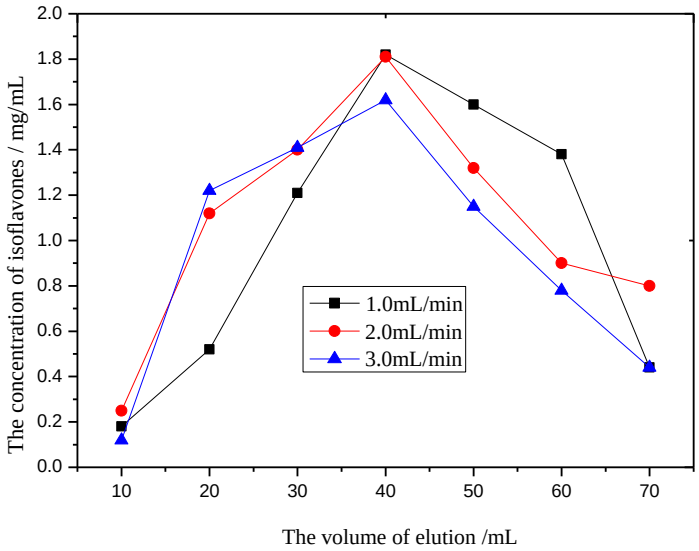


**Figure 3.** The effect of the flow velocity on purification

The figure 3 shows that the elution rate of 1.0 mL/min and 2.0 mL/min, have higher resolution of isoflavones. Comprehensively consider the resolution of isoflavones and purification efficiency, the best elution rate was 2.0 mL/min.

## 3.5. Determination Results of Refined Flavonoids Content and Purity

According to the above method, 2.6104 g rough flavonoid was and 0.0681g refined flavonoid were made. With 95% ethanol dissolved, sample solution were got, Calculated by the formula

ISSN: 2688-8645

(3), the purity of no purification and purification of isoflavones were 21.65% and 87.55%, respectively, purity increased about 4 times.

## 4. Physical Conclusion

(1) The best adsorbent was determined. Compared S-8, NKA-9, AB-8, X-5, D4006 five kinds of macroporous resin adsorption separation characteristics for isoflavones, determine the AB-8 macroporous resin was the best adsorbent.

(2) The optimal purification conditions was determined. With AB-8 resin as adsorbent, ethanol as the eluent, the eluant concentration, dosage of eluent and elution rate were examined on effect for refined isoflavones. The optimal purification conditions as follows: 10.0 g dry resin, 0.5 mL/min flow injection, 80% ethanol dosage was 100 mL, the elution flow rate 2.0 mL/min. Under this condition, isoflavones purity increased from 21.65% to 87.55%, puerarin content increased from 9.79% to 92.8%. It shows that macroporous resin adsorption purification method is a feasible and effective method for refined isoflavones and pueraria.

## Acknowledgements

The authors wish to thank the helpful comments and suggestions from my leaders and colleagues in college of chemistry and chemical engineering, zhoukou normal university. This work are supported by the Scientific Research Innovation Foundation of college of chemistry and chemical engineering, zhoukou normal university (Grant No. ZSHYCX0207).

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