

Comparison of hydroxy naphthoquinone from North Qinglongyi with different storage times

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Abstract. Objective: To determine the appropriate solvent for the extraction of hydroxy naphthoquinone, and to establish a method for the determination of the content of hydroxy naphthoquinone in the North Qinglongyi, and compare the changes of the content of hydroxy naphthoquinone in North Qinglongyi with different storage times. Methods: According to the nature of hydroxy naphthoquinone in alkaline solution will be discolored, so this experiment for Juglone as the standard reagent, 5% KOH solution as a developer, and the absorbance was measured by UV-spectrophotometry at the wavelength of 515 nm. The content of hydroxy naphthoquinone in North Qinglongyi was determined by colorimetric method, and the contents of hydroxy naphthoquinone in North Qinglongyi of different storage times were compared. Results: The optimum extraction solvent was ethyl acetate. The recoveries were 97.73%±1.11% and the RSD was 1.14% (n = 6). The contents of hydroxy naphthoquinone in the North Qinglongyi were 0.0141%, 0.0104% and 0.0073%, respectively, for one year, two years and three years. The content of hydroxy naphthoquinone decreased with the storage time prolonged. Conclusion This experimental method was stability, high recovery rate, simple and reliable. According to the results of this experiment, we can see that the storage time of North Qinglongyi should not be too long. Should try to choose this year's North Qinglongyi for experimental research.

1. Introduction

North Qinglongyi, the immature frugivorous dry pericarp of Manchurian walnut of platymiscium of walnut of juglandaceae(*Juglands mandshurica Maxim.*), is produced chiefly in northeast china, with the potential resources most abundant in Hei Longjiang Province. Qinglongyi originally was recoded named as walnut Qinglong in the *Chinese material medica*, named as green walnut in *First aid medicine*, but it was usually used since it was called “Qinglongyi” in the *Chinese herbal medicine handbook of Shandong Province*^[1]. Hydroxy naphthoquinone compound is the effective component to play the role of anti-tumor effect in the North Qinglongyi^[2]. Thus, it is of great significance to carry out the content determination of the hydroxynaphthoquinone in the North Qinglongyi on the evaluation of the medicinal quality. The experiment, based on the Borntrager reaction that hydroxy quinones will have color changes in an alkaline solution, takes the juglone as the reference substance^[3], with 5% potassium hydroxide as the chromogenic reagent, and use UV-visible spectrophotometer to testify the content of the hydroxynaphthoquinone in the North Qinglongyi.



2. Preparation of the experimental equipment

2.1. Herbs

North Qinglongyi herbs, acquired from Bin county, Harbin City, was identified as the immature frugivorous of Manchurian walnut of platymiscium of walnut of juglandaceae(*Juglands mandshurica Maxim.*), While it is fresh, strip the green peel to make it dry and natural and the medical materials are stored in the basic laboratory medicine of the Life Sciences and Environmental Sciences Center in Harbin University of Commerce. According to the storage time, it is classified as the medical materials that can be stored for one, two or three years.

2.2. Reagent、reference substance

Ethyl alcohol, chloroform, ethyl acetate, methyl alcohol is analytically pure, double distilled water, 5%KOH liquor. Juglone reference substance(Purchase of TianJinYiFang Limited Company, purity is 98%).

2.3. Apparatus

LIV-2102C Ultraviolet-Visible spectrophotometer(UNICO Limited Company), KQ5200DB Ultrasonic instrument(Kunshan Ultrasonic instrument Limited Company), N-1000 Rotary evaporators(EYE-LA Shanghai's Ailang Limited Company), BS110S Electronic analytical balance(Beijing Sartorius Limited Company).

3. Determination of the content of the hydroxynaphthoquinone in the North Qinglongyi herbs

3.1. Preparation of the reference solution and the test solution

3.1.1. Preparation of the reference solution

Weigh 2.5 mg of juglone as the reference substance which is put into the volumetric flask of 25b mL, dissolved in methanol and set the volume to obtain a concentration of 0.01 mg/mL of the reference solution.

3.1.2. Preparation of the test solution

Smash the North Qinglongyi herbs with the storage life of one year, two years and three years into 30 mesh powder, and accurately weigh five shares of three kinds of medicinal powder respectively, each for 5g, which are then put into a beaker. Add 50 mL of the ethyl acetate soaked for 24 h, with ultrasonic extraction three times (power 40 KHz, temperature 25 °C) ^[4-5], each time for 30 min, and combine the filtrate that has been filtrated three times, rotary evaporators are used for solvent recovery. Get the concrete until it is dried to constant weight. Unguents of the storage life for one, two and three years respectively weight 131 mg, 126 mg and 127 mg; the yield of the concrete was respectively 2.62%, 2.52% and 2.54%. Weigh 5 mg respectively and put them into 10 mL volumetric flask, dissolved in methanol and set the volume.

3.2. Selection of the wavelength for detection

Take the reference solution of 10 mL, added into the 1 mL 5% KOH for color and scanned within the wavelength range from 200 to 800 nm. The scanning results show the juglone has an absorption maximum at 515 nm of the visible wavelengths sector. Take the North Qinglongyi herbs of the storage period for one year and the test solution for 10 mL, added into 1 mL 5% KOH for color scanned under the same conditions, with the results also indicating that in 515 nm of the wavelength of maximum absorption, it is determined that the detection wavelength was 515 nm. The results are shown in figure 1 and 2.

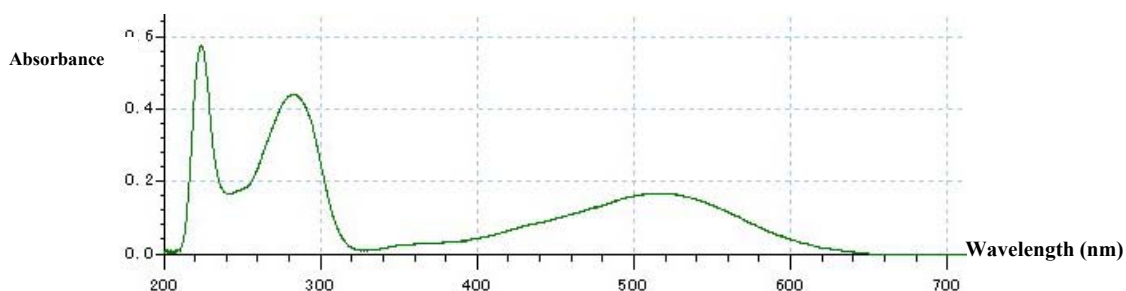


Figure 1. Juglone reference substance full wavelength spectrogram

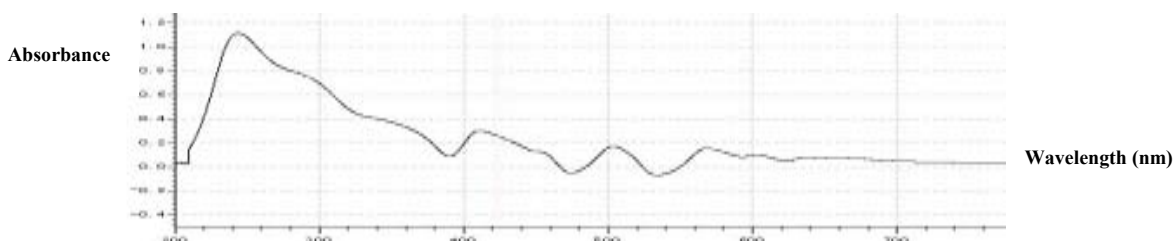


Figure 2. Storage for one year North Qinglongyi's extractive full wavelength spectrogram

3.3. Study on the linear relationship

Take the precise amount of the reference solution for 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL and place them in the 10 mL volumetric flask, respectively added to 1 mL 5% KOH for color and methanol is added to set the volume. Take the precise amount of 1 mL 5% KOH into 10 mL volumetric flask, add methanol to volume as the blank solution. Absorbance values are measured at 515 nm wavelength. Take A of the absorbance value for the vertical axis, the juglone concentration C ($\mu\text{g/mL}$) as the abscissa, linear regression is carried out resulting in the regression equation $y = 0.1089x + 0.1410$, $r = 0.9999$. The results showed that juglone in 2~12 $\mu\text{g/mL}$ range has a good linear relationship.

3.4. Precision tests

Fetch exact 0.1 mg/mL juglone and 0.2 mL reference solution to 10 mL volumetric flask, 1 mL 5% KOH solution for color, with methanol to set volume, repeat measurements of the absorbance values six times at 515 nm to calculate the relative standard deviation. As a result, the RSD was 0.25% ($n = 6$). This experiment enjoys a good precision.

3.5. Repeatability test

Take 6 shares of the North Qinglongyi powder of the storage life for one year, with ethyl acetate as the extraction solvent, according to 3.1.2, "Preparation of the test solution" Method is used for the Preparation of the test solution. Take respectively the test solution for each 10 mL, and then add 1 mL 5% KOH aqueous solution the color. The absorbance value was measured at 515 nm wavelength. As a result, the RSD = 0.58% ($n = 6$), showed a good reproducibility.

3.6. Stability test

Take one share of the North Qinglongyi powder of the storage life for one year, with the ethyl acetate as the extraction solvent, according to 3.1.2 entitled "Preparation of the sample solution for" method which can be used to prepare for the test solution, take the test solution 10 mL, added 1 mL 5% KOH water solution for color, with methanol to set volume, and measure the absorbance value in 0, 10, 20, 30, 40, 50 min. The RSD was for 0.21% ($n = 6$) which showed that the test solution of the North Qinglongyi herbs is basically stable within 60 min.

3.7. Sample recovery rate experiment

Take exact amount of 6 shares of the North Qinglongyi powder (hydroxynaphthoquinone content of 0.0141 percent) of the storage life for a year, and respectively add 1 mL juglone reference substance to 6 shares of the test sample solution, according to 3.1.2 entitled "prepared for the test solution" methods for the preparation, take the test solution each for 10mL, and then added with 1 mL 5% KOH aqueous solution for color and calculate the recovery rate. As a result, the recovery was 97.73%±1.11% and the RSD was 1.14% (n = 6), indicating that the assay results are accurate. See table 1.

Table 1. Sample recovery rate result

	1	2	3	4	5	6	RSD
Weight (mg)	769.5	777.3	769.5	785.1	777.3	781.6	
Test article (mg)	0.1085	0.1096	0.1085	0.1107	0.1096	0.1102	
Reference substance (mg)	0.1	0.1	0.1	0.1	0.1	0.1	1.14%
Result (mg)	0.2074	0.2058	0.2067	0.2088	0.2081	0.2067	
Recovery rat (%)	98.9	96.2	98.2	98.1	98.5	96.5	

3.8. Hydroxynaphthoquinone Determination in the North Qinglongyi of different storage years

Take the test solution of the North Qinglongyi of different storage life for each 10 mL, and add respectively 1 mL 5% KOH aqueous solution for the color, and the absorbance value was measured at 515 nm and the results are as in table 2.

Table 2. The absorbance of North Qinglongyi at different storage times

Times	Absorbance			Weight (μg/mL)			Average weight (μg/mL)	Original medicinal
1 years	0.435	0.433	0.434	2.7000	2.6814	2.6905	2.6906	0.0141%
2 years	0.366	0.365	0.363	2.0661	2.0569	2.0386	2.0539	0.0104%
3 years	0.296	0.298	0.298	1.4233	1.4417	1.4417	1.4356	0.0073%

4. Conclusion and discussion

Naphthoquinone ingredients are a sort of secondary metabolites in plants with a wide range of physiological activity. The naphthoquinone ingredient in the North Qinglongyi is one of the active ingredients of anti-cancer. Studies conducted have shown that the crude naphthoquinone can significantly inhibit a variety of transplanted tumors, and have stronger impact on the vitro killer cells and less toxic^[2]. The hydroxynaphthoquinone compound of the North Qinglongyi mainly includes the juglone, 2-methoxy-8-hydroxy-1,4-naphthoquinone, 5,8-dihydroxy -1, 4-naphthoquinone, hydrogenated juglone its glycosides etc, in which the anti-tumor effect of juglone and the study of the mechanism are more thorough^[6-8]. The research group has established HPLC method for the determination of the content of the North Qinglongyi juglone^[4], based on which establishment of the determination method for measuring the contents of the hydroxynaphthoquinone plays a great significance in the comprehensive evaluation of the quality of this kind of medicines. Determination method of the total pigment content of the hydroxynaphthoquinone in the lithospermum established in the *Chinese Pharmacopoeia* takes the Shikonin as reference, using the UV-visible spectrophotometry with its maximum at a wavelength of 516 nm visible light to measure^[9]. In this paper, UV-visible spectrophotometry is adopted, but the difference is that the nature of the Borntrager reaction of hydroxy quinone is the basis to carry out the development of color on the content of the hydroxynaphthoquinone, avoiding interference with other components and thus improving the sensitivity. This method is good in stability, high in recovery rate, simple and reliable, which thus is suitable for testing the content of the Chinese medicine and its preparation containing hydroxynaphthoquinone, and also provides a scientific basis for formulating the standards of the North Qinglongyi quality.

With juglone as the reference substance, the author investigated in this paper appropriate extraction solvents for hydroxy naphthoquinone in North Qinglongyi. For this purpose, three solvents (i.e. chloroform, ethyl alcohol and ethyl acetate) were screened. Upon measurement, the content of hydroxy naphthoquinone extracted is 0.0055%, 0.0095% and 0.0141% respectively. Therefore, ethyl acetate was

determined as the most appropriate extraction solvent. On this basis, the author also compared the content of hydroxy naphthoquinone in North Qinglongyi in different years of storage. According to the comparison result, there is obvious difference in the content of hydroxy naphthoquinone in North Qinglongyi in different years of storage. With the increase in time, the content of hydroxy naphthoquinone in North Qinglongyi gradually decreases. It is of instable components for naphthoquinone. It is speculated that such instability is caused by decomposition with the increased time of storage^[10-11]. Therefore, it is inappropriate to store North Qinglongyi medicinal materials for a long time. Instead, medicinal materials in the current years should be used for clinical application or antineoplastic experiment research as much as possible.

Acknowledgments

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