View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

ELSEVIER

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 18 (2011) 86 – 94

Procedia Engineering

www.elsevier.com/locate/procedia

The Second SREE Conference on Chemical Engineering

Determination of anthraquinone content in lac dye through combined spectrophotometry and HPCE

Zhang Hong[a,b], Fang Guigan[a,*], Zheng Hua[b], Li Kun[b]

[a] *Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, Nanjing 210042, China*
[b] *Research Institute of Resources Insects, Chinese Academy of Forestry, Kunming, Yunnan 650224, China*

Abstract

This study is aim to establish the method for determination of anthraquinone content in lac dye with the combined spectrophotometry and HPCE, With the 0.5%$Mg(Ac)_2$-CH_3OH solution as the colour-developing agent, the carminic acid concentration within the concentration of 5~50μg/mL presents a good linear relationship with the absorbance under the wavelength of 540nm (R=0.9994). Through the test, the results showed that the total anthraquinone content in lac dye was 82.13%, average recovery rate 97.80%, RSD 1.31%. Under 291nm detection wavelength and 25 ˚C column temperature, with pH8.035 60mmol/LNa_2HPO_4-$Na_2B_4O_7 \cdot 10H_2O$ mixed buffer as background electrolyte and 60cm × 75μm uncoated capillary column as separation lanes, separation voltage 20kV, pressure 0.5psi × 10s for sample injection, the relative contents of five components laccaic acids A, B, C, D, E were measured by peak area normalization method; combined with the spectrophotometry, the contents of five anthraquinone components - laccaic acids A, B, C, D, E were determined, respectively 40.42%, 17.66%, 2.54%, 1.51%, 20.00%.

Keywords: spectrophotometer; high performance capillary electrophoresis (HPCE); lac dye; laccaic acid; anthraquinones

Laccaic acid is a derived component of lac red, a type of multi-hydroxy anthraquinone substances extracted from the lac secreted from lac insects. The pigment itself is in red or pink, water-soluble. The aqueous solution is in pink to purple [1-3]. The physical and chemical properties of lac dye is very stable, which is not only a common food coloring[4-7], but also can be used as the natural dyes[8-16] of silk and cotton, etc; Moreover, it has better stability and stronger resistance to oxidation [17]. It is an excellent colorant that is extensively used.

The known laccaic acid is composed of five anthraquinone carboxylic acids A, B, C, D, E with similar structure [1], seen Fig.1. It can be seen that only R group is slightly difference for A, B, C, E in

* Corresponding author. Tel.: +86-871-386-1466, +86-25-8548-2548; fax: +86-871-386-0021, +86-25-8548-2536.
E-mail address: hua-zheng@sohu.com, fangguigan@yahoo.com.cn.

1877-7058 © 2011 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
doi:10.1016/j.proeng.2011.11.014

structure. The structural similarity causes it difficult for separation and detection of different anthraquinone component from laccaic acid[18], moreover, it is difficult to calibrate the laccaic acid contents in the lac dye. The product quality is expressed by the absorbance [19]. To detect the laccaic acid contents in the lac dye rapidly, conveniently and accurately, in this test, the carmine acid, a multi-hydroxy anthraquinone, is used as a control sample, to determine the total anthraquinone content in lac dye with the spectrophotometer[20-24], that is the content of laccaic acid; and then separate the lac dye by the high performance capillary electrophoresis (HPCE), and measure the relative content of five anthraquinones (laccaic acid) in the lac dye by the peak area normalization method [25-28], to provide a reference for the detection and quality control of lac, lac products and lac dye in research and practices.

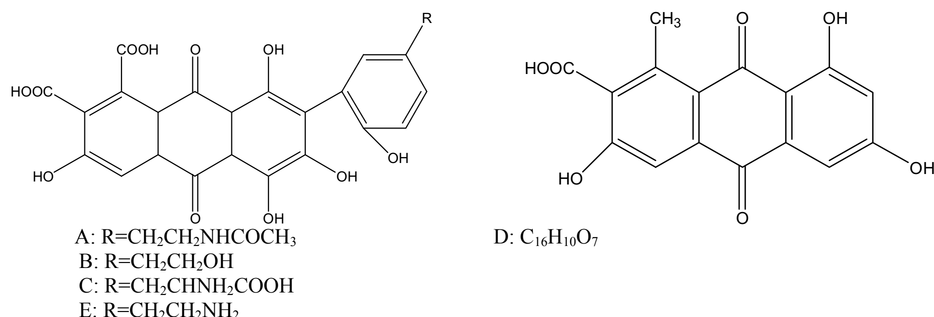

Fig. 1 The structural formula of five anthraquinone components in lac dye

1. Materials and Method

1.1. Materials and reagents

Lac dye (Yunnan Mojiang Lac Plant); Magnesium acetate (AR) (Silian Chemical Plant); methanol (AR) (Shanghai Chemical Reagent Co., Ltd); disodium hydrogen phosphate, sodium dihydrogen phosphate (AR) (Shanghai Xinhua Chemical Plant); carminic acid standard sample (purity ≥ 96%) (Japan F.F.I. Food Industry Co., Ltd.); borax (AR) (Guangdong Shantou Xinning Chemicals Plant); boric acid (AR) (Chongqing Chuanjiang Chemical Reagent Factory); anhydrous ethanol (AR) (Tianjin Fengchuan Chemical Reagent Co., Ltd); phosphoric acid (AR) (Tianjin Damao Chemical Reagent Factory); sodium hydroxide (AR) (Tianjin Fengchuan Chemical Reagent Co., Ltd); sodium carbonate (AR) (Tianjin Fengchuan Chemical Reagent Co., Ltd); sodium bicarbonate (AR) (Chengdu Jinshan Chemical Reagent Factory).

1.2. Instruments and equipments

DU 800 UV - visible spectrophotometer (U.S. Beckman Coulter); Beckman P/ACE™ system MDQ HPCE system (U.S. Beckman Coulter); fused silica capillary (60cm × 75μm) (Hebei Yongnian optical fiber factory); PHS-3C precision pH meter (Shanghai Precision Scientific Instrument Co., Ltd.); AB204-S precision electronic balance (Mettler Toledo (China) Co., Ltd.); Purelab Ultra ELGA ultrapure water system (U.K. ELGA).

1.3. Sample pretreatment

Precisely weigh 10mg carminic acid standard product and dissolve them in 100mL volumetric flask. Prepare 0.1mg/mL standard stock solution at constant volume with 0.5%$Mg(Ac)_2$-CH_3OH solution;

respectively pipette 0.5 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mL standard stock solution to 10mL volumetric flask to prepare the standard solutions of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50μg/mL (mass concentration) for use. Measure the absorbance of different concentrations of standard solution of carmine acid with a spectrophotometer, three times for each sample and calculate the average value of the three times of measurement results. Plot a standard curve with absorbance as the ordinate and the concentration of the standard sample as the abscissa, and calculate the regression equation.

Accurately weigh 10mg of lac dye sample and dissolve them in a 100mL volumetric flask, and then respectively pipette 1, 2, 3, 4, 5, 6, 7 mL of the sample into 10mL volumetric flask, and add 0.5%Mg $(Ac)_2$-CH_3OH developing agent to prepare lac dye solution of mass concentration of 10, 20, 30, 40, 50, 60, 70 μg / mL for future use.

Precisely weigh 50mg of lac dye sample and dissolve it in a 100 mL volumetric flask, add ultra-pure water to constant volume. Prepare the lac dye sample solution with mass concentration of 0.5mg/mL for use. Filter all solutions before sample injection with 0.45μm cellulose membranes.

1.4. Conditions for Measurement

Measure the absorbance of lac red sample solution with the developing agent 0.5%$Mg(Ac)_2$-CH_3OH under the 540nm wavelength by spectrophotometry, three times for each sample and calculate the average value of the three times of results; then calculate the total anthraquinone in the lac red sample according to the regression equation. Conduct the repeatability, stability and standard recovery rate tests respectively according to the above test results.

The uncoated quartz capillary 60cm × 75μm (effective length 50cm), separation voltage 20kV, separation time 25min, detection wavelength 291nm, column temperature 25 °C were adopted. Sample injection at the pressure 0.5psi × 10s, with 60mmol/L disodium hydrogen phosphate - borax mixed buffer at pH 8.035. Before the capillary was used, flush them with methanol, water, 0.1mol/L hydrochloric acid, 1mol/L sodium hydroxide and buffer solution successively. Flush with water, 0.1mol/L sodium hydroxide, water, electrophoresis buffer successively for 5 min, 3min, 3 min and 5 min at the interval of two samples running.

2. Results and analysis

2.1. Determination of total anthraquinone content in lac dye

2.1.1 Selection of the wavelength

Scan the 0.1mg/mL carmine acid standard product and lac red sample prepared in 1.3 in the visible light zone. The results were shown in Fig.2. It was discovered the maximum absorption of carmine acid at 540nm and greater absorption of laccaic acid at 540nm, therefore, 540nm was selected as the detection wavelength.

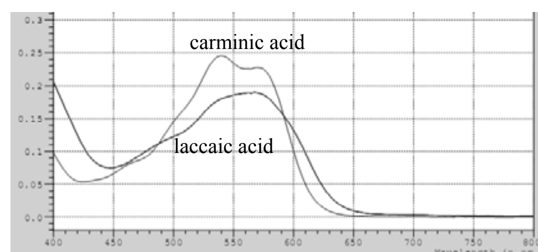

Fig. 2 Scanning peaks of carminic acid and lac red

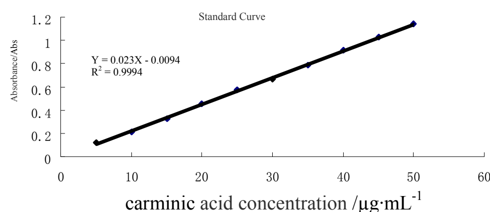

Fig.3 Standard curve carminic acid sample

2.1.2 Plotting of standard curve

Measure the absorbance with standard carminic acid with mass concentrations 5, 10, 15, 20, 25, 30, 35, 40, 45, 50μg/mL prepared in 1.3 with UV spectrophotometer according to the method in 1.4. With the absorbance as the ordinate and the carminic acid standard sample concentration as the abscissa, obtain the standard curve equation Y=0.023X-0.0094 (Fig. 3).

2.1.3 Determination of total anthraquinone in laccaic acid samples

Measure the total anthraquinone of the lac dye samples with mass concentrations of 10, 20, 30, 40, 50, 60, 70 μg / mL prepared in 1.3 according to the method of 1.4. The results were shown in Table 1.

Table 1 Determination of the total anthraquinone in lac dye samples

Sample number	1	2	3	4	5	6	7
Absorbance/Abs	0.1755	0.3762	0.5528	0.7528	0.9481	1.1078	1.3125
Total mass of anthraquinone determined in lac dye sample/μg	80.3910	167.6520	244.4350	331.3910	416.3040	485.7390	574.7390
Weight of lac dye/μg	100	200	300	400	500	600	700
Content of total anthraquinone in lac dye sample/%	80.39	83.83	81.48	82.85	83.26	80.96	82.11
Average/%				82.13			

2.1.4 Determination of repeatability

Measure the lac dye samples of 50μg/mL prepared in 1.3 for 9 successive times according to the method in 1.4 and obtain the absorbance. RSD is 0.14%.

Table 2 Determination results of repeatability of lac dye

Sample number	1	2	3	4	5	6	7	8	9
Absorbance/Abs	0.9375	0.9354	0.9344	0.9351	0.9342	0.9342	0.933	0.9351	0.9336
Average/Abs					0.9347				
RSD/%					0.14				

2.1.5 Determination of stability

Measure the 50μg/mL lac red sample prepared in 1.3 once every 2h according to the method in 1.4. The results see Table 3. The RSD is 0.34%, suggesting that the sample has good stability within 8h.

Table 3 Determination results of stability of lac dye

Test time/h	0	2	4	6	8
Absorbance/Abs	0.9397	0.9368	0.9348	0.9322	0.9310
RSD			0.34		

2.1.6 Determination of standard recovery rate

Fetch 1, 2, 3, 4, 5, 6, 7 mL of 0.1mg/mL lac red newly-prepared in 1.3 into 10 mL volumetric flask, and then add 1mL 0.1mg/mL carmine acid standard product, and then add 0.5%Mg $(Ac)_2$-CH_3OH solution to constant volume, to determine the absorbance, as shown in Table 4.

Table 4 Recovery rate of total anthraquinone in different concentrations of samples

Sample number	Absorbance/Abs	Actual concentration of total anthraquinone in the sample/μg/mL	Theoretical concentration of total anthraquinone in the sample/μg/mL	Recovery rate/%	Average/%	RSD/%
1	0.3903	17.3783	18.2130	95.42		
2	0.5935	26.2130	26.4260	99.19		
3	0.7633	33.5957	34.6390	96.99		
4	0.9625	42.2565	42.8520	98.61	97.80	1.31
5	1.1432	50.1130	51.0650	98.14		
6	1.3204	57.8174	59.2780	97.54		
7	1.5226	66.6087	67.4910	98.69		

2.2. Determination of relative content of five anthraquinone components in lac dye

2.2.1 Optimization of electrophoresis conditions

2.2.1.1 Composition and concentration of electrophoresis buffer

The rationale of capillary electrophoresis is that different mass charge ratios of charged ions can be separated with the variance of migrant rate under a certain electric field. The buffer composition and concentration will certainly affect the separation, migration and electroosmotic flow of the sample during the electrophoresis process, therefore, selection of appropriate buffer has decisive role for the separation of samples. In this test, we investigated the impact of different concentrations of phosphate, borax, carbonate, and mixed buffer system on the separation of lac dye. As shown in Fig.4, when the 60mmol/L Na_2HPO_4-$Na_2B_4O_7 \cdot 10H_2O$ mixed buffer was used as the basic electrolyte, the retention time of lac dye was relatively short with better separation effect. In addition, after the background electrolyte was selected, we explored the impact of SDS, β-cyclodextrin, ethylene glycol, acetonitrile and other additives on the separation effect; however, due to multiple components of lac red with similar structure, the separation peaks canoe be significantly improved, therefore, we finally selected 60mmol / L disodium hydrogen phosphate – borax solution as the buffer system.

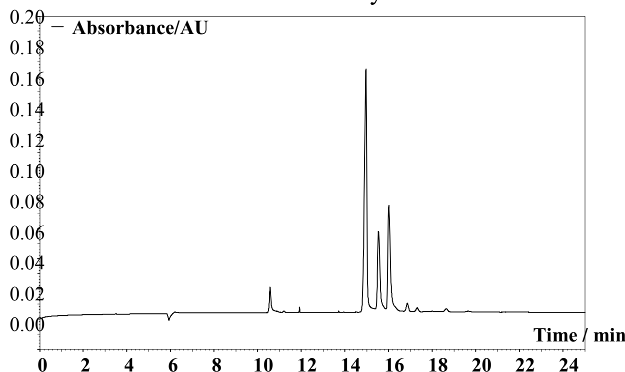

Fig.4 The electrophoresis spectra with 60mmol / L disodium hydrogen phosphate - borax solution as the buffer system

2.2.1.2 Selection of pH values of buffer solution

As seen from Fig.1, the laccaic acids A, B, C, E only differ from one R group and their molecular weight difference is small. We selected Na_2HPO_4-$Na_2B_4O_7 \cdot 10H_2O$ system as the buffer solution in 2.2.1.1, so, pH values will affect the separation and migration of components, and change the inherent binding effect of borate buffer system on the separating components, therefore, it is obvious that the pH value will improve the separation effect. In this test, we investigated that effect of the buffer solutions with pH 7.356, 7.945, 8.324, 8.524, 8.632, 8.709, 8.820, 9.143, 9.433, 9.656, 9.886 on the separation. The results showed that when the pH value was about 8.0, the separation effect was better.

2.2.1.3 Selection of separation voltage

Under the conditions of the selected background electrolyte composition, concentration and pH, we investigated the effect of separation voltages 10, 15, 18, 20, 23, 25 kV on the separation. The results are as shown in Fig.5. When the separation voltage was 10, 15, 18kv, the retention time was significantly extended and the peak tailing was obvious; and when the separation voltages were 23, 25kv, the retention time was not reduced significantly and the separation effect was exactly the same as that of 20kv. Considering the above factors, we selected 20kV as the separation voltage of the buffer solution.

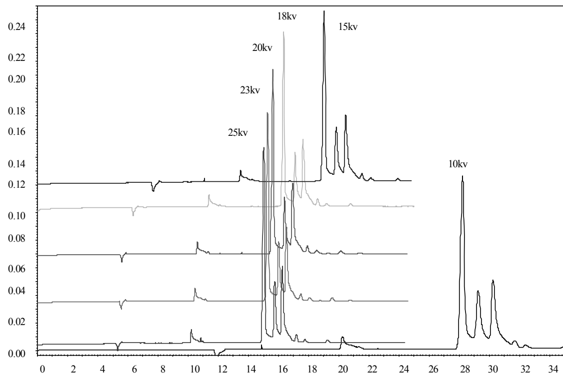

Fig.5 HPCE of lac dye under different separation voltages

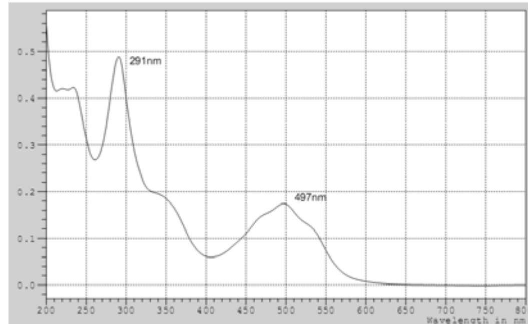

Fig. 6 Scanning peaks of lac dye with reference of buffer solution

2.2.1.4 Selection of detection wavelength

With the reference of disodium hydrogen phosphate - borax mixed buffer system with concentration of 60mmol/L and pH8.035, full-wave band scanning on the UV and visible light regions of the 2.0×10^{-3}mg/mL lac dye solution containing 50% ethanol, as shown in Fig.6, the maximum absorption peak appeared at 291nm and 497nm; but as seen from the electrophoresis spectrum, we discovered that, when the detection wavelength was 291nm, the baseline was stable, the peak shape was better, and the lac dye has stronger absorption, so the 291nm was selected as the detection wavelength.

2.2.2 Determination of five anthraquinone components in laccaic acid

2.2.2.1 Determination of peak appearance of five anthraquinone components

According to the basic separation mode of capillary zone electrophoresis (CZE), the separation of substances by the capillary electrophoresis is based on the ion mobility of different components, and its ion mobility is proportional to the effective charge[29]. The formula of electrophoretic mobility [30] is as follows:

$$\mu_{app} = \frac{v_{app}}{E} = \frac{v_{eof} \pm v_{eff}}{E} = \mu_{eof} \pm \mu_{eff} \qquad (1)$$

(1) Where, μ_{eo} is electroosmosis mobility, which can be expressed as $\mu_{eof} = \frac{v_{eof}}{E} = \frac{\varepsilon\zeta}{4\pi\eta}$ (2)

(2) Where, ξ is double layer potential, related to the capillary material, the charging conditions of the inner wall and composition and concentration of electrolyte solution; ε is the dielectric constant of electrolyte solution and η is the viscosity of electrophoretic medium.

(1) Where, in formula (1), μ_{eff} is the effective electrophoretic mobility, which can be expressed as

$$\mu_{eff} = \frac{v_{eff}}{E} = \frac{q}{6\pi\eta r} \qquad (3)$$

(3) Where, in formula (3), q and r are the quantity of electric charge and radium respectively, and η is the viscosity of the electrophoretic medium.

As seen from formula (2), since the five components of lac red can realize separation and detection under the same conditions, so their double layer potential ξ, dielectric constant of electrolyte solution ε and viscosity of electrophoretic medium η are the same, that is, the electro-osmotic mobility of five component μ_{eof} is the same. Therefore, the separation sequence of five anthraquinone components

depends on the effective electrophoretic mobility μ_{eff}. As seen from formula (3), $6J\eta$ is a constant, so the ion mobility μapp depends on q / r, that is, the charge density. As seen from Fig.1, laccaic acid D is lack of a carboxyl and two hydroxyl groups compared with other four components, so its charge density is lowest. A, B, C, E differ from each other in R group and only C has one carboxyl group, so the charge density of C is highest. A, B, E have no ionization groups, so its charge density is dependent on the ionization ability of phenolic hydroxyl on the para-position caused by electrophilic induction effect of R group. Since the electrophilic ability $CH_2CH_2NHCOCH_3$>CH_2CH_2OH>$CH_2CH_2NH_2$, so the order of charge density is A> B> E. To sum up, the peak appearance of five anthraquinone components of lac red is laccaic acid C> A> B> E> D.

2.2.2.2 Determination of relative contents of five anthraquinone components

0.5mg/mL lac red sample solutions prepared in 1.3 were fetched for continuous sample injection for 9 times according to the condition in 1.4, and then the relative contents of five anthraquinone components of laccaic acids A, B, C, D , E in lac red were measured by peak area normalization method. The results are shown in Table 5.

Table 5 Determination of relative contents of five anthraquinone components in lac red by peak area normalization method

Sample number	Peak area of laccaic acid A	Peak area of laccaic acid B	Peak area of laccaic acid C	Peak area of laccaic acid D	Peak area of laccaic acid E	Total peak area	Percentage of laccaic acid A /%	Percentage of laccaic acid B /%	Percentage of laccaic acid C /%	Percentage of laccaic acid D /%	Percentage of laccaic acid E /%
1	950219	412862	59145	34792	489554	1946572	48.81	21.21	3.04	1.79	25.15
2	94194	424876	65072	37461	502967	1971570	47.74	21.55	3.30	1.90	25.51
3	1060470	456718	57922	37106	526494	2138710	49.58	21.35	2.71	1.74	24.62
4	1060715	444783	60789	37248	510487	2114022	50.18	21.04	2.88	1.75	24.15
5	1086741	479700	81034	43921	533949	2225345	48.83	21.56	3.64	1.98	23.99
6	1140738	499267	75765	45395	545166	2306331	49.46	21.65	3.29	1.96	23.64
7	1077351	492584	64233	40309	553629	2228106	48.35	22.11	2.88	1.81	24.85
8	1222267	523849	74750	43817	539102	2403785	50.85	21.79	3.11	1.82	22.43
9	1197518	517311	72088	44888	608348	2440153	49.08	21.20	2.95	1.84	24.93
Average	-	-	-	-	-	-	49.21	21.50	3.09	1.84	24.36

2.3. Contents of five anthraquinone components in lac red

According to the content of total anthraquinone (laccaic acid) in lac red measured by spectrophotometer, the separation of lac red by HPCE and the relative contents of five anthraquinone components in lac red measured by peak area normalization method, we calculated the relative content of five anthraquinone components- laccaic acid A, B, C, D, E in lac red. The results were shown in Table 6. The results showed that, laccaic acid A is the major component in lac red, up to 40.42%; followed by laccaic acid E and laccaic acid B, respectively 20.00% and 17.66% in lac red; the contents of laccaic acid C and laccaic acid D is relatively lower, only 2.54% and 1.51% in lac red.

Table 6 Calculation of five anthraquinone components in lac red

Sample	laccaic acid A	laccaic acid B	laccaic acid C	laccaic acid D	laccaic acid E
Relative content of laccaic acid/%	49.21	21.50	3.09	1.84	24.36
Content of total anthraquinone in lac dye sample/%	82.13				
Content of laccaic acid in lac dye sample/%	40.42	17.66	2.54	1.51	20.00

3. Conclusion

3.1. With the carmine acid as the standard sample, 0.5%Mg $(Ac)_2$-CH_3OH solution as developing agent, the content of total anthraquinone (laccaic acid) in lac red are determined by a spectrophotometer at 540nm wavelength. The sample processing is simple, measurement results are accurate, the linearity, precision and stability are good and the standard recovery rate as high as 97.80%.
3.2. Establish a method of separation of lac red –HPCE and optimize the electrophoresis conditions: under the 291nm wavelength, 25°C column temperature and 0.5psi × 10s sample injection conditions, with 60mmol/L Na_2HPO_4-$Na_2B_4O_7 \cdot 10H_2O$ mixed buffer (pH8.035) as the background electrolyte, 60cm × 75μm uncoated capillary column as the separation lanes, five types of laccaic acids are effectively separated; the relative contents of five component of laccaic acids A, B, C, D, E were measured by the peak area normalization method.
3.3. Determination of the total anthraquinone content (laccaic acid) of lac red samples by the combined spectrophotometer and capillary electrophoresis. The total anthraquinone content (laccaic acid) is 82.13%, of which, the laccaic acid A accounts for about 49.21%, which is 40.42%, the major component in lac red; the laccaic acid B accounts for about 21.50%, which is17.66%; the laccaic acid C accounts for about 3.09%, which is 2.54%, the laccaic acid D accounts for about 1.84%, which is 1.51% and the laccaic acid E accounts for about 24.36%, which is 20.00%.

Acknowledgements

This investigation was supported by Fund Program in Conversion of Agricultural Science and Technology Achievement, Ministry of Science and Technology of the People's Republic of China (2010GB24320619) and State Forestry Project on Promotion of Scientific and technical achievement, SFA, China ([2010]11). We gratefully thank the contributions of everyone who joined in these research projects mentioned above.

References

[1] Chen X M, Chen Y Q, Zhang H, Shi L. *Lac insect Cultivation and Lac processing*. Beijing: China Forestry Press, 2008: 170-184.
[2] Hirokado M, Kimura K, Suzuki K, Sadamasu Y, Katsuki Y, Yasuda K. Detection Method of Madder Color, Cochineal Extract, Lac Color, Carthamus Yellow and Carthamus Red in Processed Foods by TLC. *Journal of the Food Hygienic Society of Japan*, 1999, 40(6): 488-493.
[3] Katsuhiro W, Takatoshi K, Hiromitsu A. Analytical and preparative separation of Kaoliang and Lac Colors by pH-Zone-Refining CCC. *Journal of Liquid Chromatography & Related Technologies*, 2005, 28(12): 2097-2106.
[4] Ling G T. *Food Additive Manual*. 3rd ed. Beijing: Chemical Industry Press, 2003:988.
[5] GB 2760-2011 The national food safety standards *Use of food additives standards*. Beijing: Health Ministry of China.
[6] Lu Y M, Zhou M C, Zheng H, Zhang H. Characteristics and Research Progress of Quinone Pigments. *Chemistry and Industry of Forest Products*, 2007, 27(Supp.):147-152.
[7] Zheng H, Zhang H, Zhang Z H. Characteristicss and Extraction Technology of Natural Pigments from Animals and Plant. *Forest Research*, 2003, 16(5): 628-635.
[8] Fu J J. *The UV-protection of Lac dye and its Application to Silk*. Suzhou: Suzhou University, 2008.

[9] Hou X N, Wang X R, Ding L, Chen W L. Study on the Dyeing Behavior of Lac Dye on Silk. *Textile Auxiliaries*, 2009, 26(2):16-19.
[10] Paisan K, Aroonsiri S, Nontalee C. Thermodynamics of adsorption of laccaic acid on silk. *Dyes and Pigments*. 2002, 53 :179-185.
[11] Montra C. Thermodynamics Study of Lac Dyeing of Silk Yarn Coated with Chitosan. *Walailak J Sci & Tech*, 2009, 6(1): 93-107.
[12] Montra C, Udomsak D, Sanae R. Thermodynamics of Adsorption of Laccaic Acid onto Chitosan and Associated Dye Toxicity Studies. *Fibers and Polymers*, 2010, 11(2): 205-212.
[13] Montra C, Saowanee R, John B. B, Vichitr R. Adsorption kinetic study of lac dyeing on cotton. *Dyes and Pigments*, 2008, 76: 435-439.
[14] Saowanee R, Montra C, John B. B, Vichitr R. An adsorption and thermodynamic study of lac dyeing on cotton pretreated with chitosan. *Dyes and Pigments*, 2007, 72: 88~96.
[15] Sorapong J, Ruangsri W, Surasak W, Peter G, Orn-Anong A, Wimol N. Comparative study of lac dye adsorption on cotton fiber surface modified by synthetic and natural polymers. *Dyes and Pigments*, 2006, 71:188-193.
[16] Kamel M. M., El-Shishtawy R M., Yussef B. M., Mashaly H.. Ultrasonic assisted dyeing III. Dyeing of wool with lac as a natural dye. *Dyes and Pigments*, 2005, 65: 103-110.
[17] Zhang H, Fang G G, Zheng H, Chen J, Tang LY, Gan J. Study on the stability of Lac dye. *Food Science*, 2009, 3(23): 65-69.
[18] Eizo T, Timothy H.M, Toshiro W, Shiro N, Hikoya H, Tomoe N. Preventive effects of anthraquinone food pigments on the DNA damage induced by carcinogens in Drosophila. *Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesisvv*, 2001, 480-481: 139-145.
[19] GB 4571-1996 *Food additive-Lac dye*. Beijing: General Administration of Quality Supervision,Inspection and Quarantine of P.R.C..
[20] Wei Y H, Wu X A, Chen L, Zhang C Z. Study on determination methods of anthraquinones component of rhubarb. *Journal of Lanzhou University (Medical Sciences)* , 2005, 31(1): 13-15.
[21] Man R L, Ni W D, Jia H T, Lin J, Li Z M, Li B, et al. Simultaneous determination of stilbene and anthraquinones compound in polygonum cuspidatum sieb.et znce by the spectrophotometric method. *Chinese Journal of Analytical Chemistry*, 2005, 33(12): 1808-1809.
[22] Li H B, Fang K Y, Li X E. Study on Determination Methods of Anthraquinones Component of Cassia tora L. *Food Science*, 2007, 28(7): 427-429.
[23] Ke Y C, Wu W K. Content determination of total anthraquinones in Gushangkangfu Wash-out Granules by UV spectrophotometric. *China Medical Herald*, 2010, 7(18): 61-63.
[24] Wu Q S, Bai X H, Tian J. Determination of anthraquinones in Rhizome Polygoni Cuspidati by magnesium ion color UV spectropotometry. *Shanxi Med Univ*, 2008, 39(12): 1099-1102.
[25] Zhang H Y, Ge L J, Chen H, Jing C, Shi Z H. Improvement of reproducibility in capillary electrophoretic characterization of rhubarb by normalization of migration time. *Chinese Journal of Chromatography*, 2009, 27(4): 489-493.
[26] Zhang J Y, LiI Q F, Pan Z W, Chen X G, Hu Z D. Determination of Anthraquinones in Chinese Rhubarb and Compound Preparations by Micellar Electrokinetic Capillary Chromatography. *Journal of Lanzhou University (Natural Sciences)* , 2003, 39(3): 101-102.
[27] Yan L S, Wang Z H, Luo G A, Wang Y M. Simultaneous Separation of Five Anthraquinone Compounds in Chinese Herb Rhubarb Extracts by Pressurized Gradient Capillary Electrochromatography. *Chemical Journal of Chinese Uniersities*, 2004, 25(5): 827- 830.
[28] Chen A J, Chen X F, Li J R, Li X, Li Q S, Hu Z D. Separation and determination of anthraquinones in Paedicalyx attopevensis Pierre ex Pitard by microemuision electrokinetic chromatography. *Journal of Lanzhou University (Natural Sciences)*, 2008, 44(6): 72-77.
[29] Zhou S N, Tan H R, Li H, Lu N. Simultaneous Separation of Polyphenols by High Performance Capillary Electrophoresis. *Journal of the Chinese Cereals and Oils Association*, 2010, 25(6): 114-118,122.
[30] Chen Y. *Capillary electrophoresis technology and application*. sec ed. Beijing: Chemical Industry Press, 2006.