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Research Article

Development and Validation of a HPLC-UV Method with Pre-column Derivatization for Determination of Cinnabar in Jufang Zhibao Pills

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ABSTRACT

In this work, a reliable and accurate high-performance liquid chromatography method with pre-column derivatization was established and validated for determination of cinnabar in Jufang Zhibao pills. Scanning electron microscope (SEM) image was used to identify the types of cinnabar crude drug in Jufang Zhibao pills. The chromatography separation was performed on a Welch XB-C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile phase consists of water spiked with 0.022 mmol/L sodium diethyldithiocarbamate (A, pH adjusted to 8–9 by ammonia water) and methanol (B, 80:20, *v/v)* at flow rate of 1.0 ml/min with the detected wavelength was 272 nm. The oven temperature was set at 35°C. The calibration for cinnabar content has good linearity (R^2 =0.9999) over the range of 2.43–300 μg/ml and the average recovery was less then 1.90%. The limits of detection and quantification were 0.1127 μg and 0.2065 μg/ml. The results indicated that the proposed method has advantages of high accuracy, good repeatability and stability and can be successfully used for determination of cinnabar in Jufang Zhibao pills. It provides a basis for drug manufacture quality control and proves the feasibility of the pre-column derivatization method during the determination of cinnabar in Jufang Zhibao pills.

Introduction

Ju Fang Zhi Bao (labeled JFZB) pills[1] is a classic Chinese herbal prescription drug, consisting of powdered buffalo horn extract, bovis calculus, hawksbill, lamber, artificial musk, benzoin, cinnabar, realgar, borneol, which was original appeared in "Lingyuanfang" (compiled by Shenkuo, a distinguished scientist in North Song dynasty) and later was recorded in "Taipinghuiminhejijufang" in Song dynasty. JFZB pills is the representative of Chinese medicine during in emergency, which has an obviously effect for coma symptoms, spasm, irritability and pediatric shock convulsion[2].JFZB pills contain 8.7% Cinnabar (a Chinese mineral medicine) that is Cinabre, dansha, the major chemical component is mercuric sulfide (HgS), and which still containing a little free mercury and soluble mercury salt. The shocked world of Minamata disease was caused by mercury poisoning in Japan in 1956. It is universally acknowledge that mercury and its compounds exhibit highly chronic toxicity [3] , which has threaten to our kidneys, the central nervous system and the reproductive system[4-10]. Hence, mercury was strictly limited applied to medicine in international society, which not only affect the medicine export but also damage to the reputation of traditional Chinese medicine. It may result in renal dysfunction if one following long-term use of cinnabar containing in traditional Chinese medicines. To the best of our knowledge there are 46 traditional Chinese medicine containing cinnabar and the content of mercuric sulfide in cinnabar crude drug should not less than 96% in pharmacopoeia of the People's Republic of China (2015) according to the records. Therefore, it can be known that applied widely in traditional medicines in China. The daily dose for cinnabar is 0.1–0.5 g according to CH.P. The amount of mercury plays an important role in traditional Chinese medicines for evaluating the safety and effectiveness of drug. However, the current national standards still has not been

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determination of cinnabar in JFZB pills. The quality of cinnabar crude drug is controlled by simple microscopic identification items. Due to the price of crude cinnabar drug is expensive and apt to appear fake and substitute matters which could not only have the effect on quality of drug but also even threaten to our health. It is therefore urgently to qualitative and quantitative determination of cinnabar in traditional Chinese medicines. Up to date, several analytical procedures have been available for quantitative measurement of cinnabar content, including titration[11,12], atomic absorption spectroscopy(AAS)[13,14], atomic fluorescence spectroscopy (AFS)[15], inductively coupled plasma mass spectrometry (ICP-MS)[16,17] and high performance liquid chromatography (HPLC)[18,19] et al. However, there are several shortcomings due to titration method having strong interferences during the detection of the traditional Chinese medicines. AAS and AFS exhibit major errors and also greater interference due to the existing of realgar in JFZB pills prescription. ICP-MS was limited by high advanced equipment expenditure and complex sample preparation process. HPLC was an ordinary analytical instrument and has been used widely in our routine laboratory analysis. Thus, in order to enhance the safety and efficiently control quality of Chinese herb medicine, it is necessary to obtain the content of cinnabar in JFZB pills. Recently, our research put forward a novel, sensitive and reliable method for determination of cinnabar content in JFZB pills by HPLC-UV method with pre-column derivatization. Sodium diethyldithiocarbamate[20,21]（abbreviation of NaDDC) is an excellent precolumn derivatizing reagent, which can form the colored chelate with Hg(II) ions that has absorption values in UV wavelength range. The formed chelate is stable in weak alkaline medium, thus can be separated by C_{18} column with HPLC. By means of above principle, a simple, sensitive, reliable method was developed to determination of cinnabar in JFZB pills based on the HPLC separation of $Hg(DDC)_2$ chelates.

Materials and Methods

Chemicals and reagents

The commercial products of JFZB pills were purchased from Tongrentang pharmaceutical factory (Being, China). Batch numbers were 3010201, 3010203, 3010204, 3010206, 3010207. Reference compound of mercuric sulfide was obtained from Fluka company (purity≥99.0%). Standard solution of mercury was purchase from National Institute of Metrology (Beijing, China). Methanol and 65% nitric acid of HPLC grade was purchased from Fisher Scientific company (Fisher Scientific, USA). Sulfuric acid, sodium hydroxide, sodium dihydrogen phosphate, dibasic sodium phosphate, sodium diethyldithiocarbamate (abbreviation of NaDDC) and potassium nitrate of analytical grade were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Purity water was obtained from Wahaha Co., Ltd. (Hangzhou, China). The analysis of cinnabar content in JFZB pills was performed on an Agilent high performance liquid chromatography system (Agilent, USA). Chromatographic separation was performed on an HPLC Welch-XB C_{18} column (250 mm × 4.6 mm,5 μm） (Welch Corporation, China). Cinnabar crude drug in JFZB pills was identified via Leica dm 3000 microscope (Leica, Germany).The maximum absorbance wavelength measurement was performed with a Shimadzu UV–2700 spectrophotometer (Shimadzu, Japan). The morphology of the cinnabar crude drug powder was observed by a scanning electron microscopy (Hitachi S-4800, Japan). Samples solutions were centrifuged by HC-2518 high speed centrifuge (Anhui USTC Zonkia Scientific Instruments Co., Ltd, China). The mobile phase was consisted of methanol and water (containing 0.022 mmol/L NaDDC, pH 8–9).The optimal wavelength of proposed method was 272 nm which obtained from their UV spectrum. The column temperature was maintained at 35°C, with the flow rate was 1.0 ml/min, and the injection volume was 10 μl. Original date was acquired from Agilent Chemstation and processed with origin and office software. The results were calculated by external standard method.

Preparation of standard solutions

Measure accurately 1.0 ml of the mercury reference solution (1000 μg/ml) into 10 ml volumetric flask with 2% nitric acid to volume and mix well as reference stock solution. The stock solution was diluted serially step by step with 2% nitric acid to make up five different concentrations of 300, 90, 27, 8.1, and 2.43μg/ml as working solutions. Measure accurately 5.0 ml of the working solutions in 10 ml Teflon centrifuge tube, respectively. Accurately weighted 30.0, 30.0, 10.0, 10.0, and 10.0 mg NaDDC in Teflon centrifuge tube respectively and shaked for several minutes for purpose of mixing well. Then the mixed centrifuge for 10 minutes(6000 r/min).The supernatant solution was discarded, then dissolved the precipitation with methanol into 10 ml volumetric flask and filtered through 0.45 μm nylon filter membrance prior to injection into system. All solutions were stored at refrigerator at 4°C before beginning to analysis.

Preparation of sample solutions

JFZB pills and equal quality of diatomite were triturated and blended. Accurately weighted the mixed fine powder 0.5 g placed into a 100 ml triangular bottle dissolved with 8 ml sulfuric acid and 1.2 g potassium nitrate. Digestion of JFZB pills on the electric furnace and optimized digestion when the solution turned transparent without any without reddish brown smoke. The sample stock solution was dissolved and quantitatively transferred into a 25 ml volumetric flask with dilute nitric acid. Measure accurately 1.0 ml of sample stock solution in 10 ml Teflon centrifuge tube. 2 ml 10 mol/L sodium hydroxide, 2 ml phosphate buffer solution and two drops of Phenolphthalein test solution were added to Teflon centrifuge tube until the solution turned pink (if not, 5 mol/L sodium hydroxide can be used to adjust it to alkaline). Accurately weighted 8.0 mg NaDDC in Teflon centrifuge tube and shaked for several minutes in order to mix well. Then the mixed centrifuged for 10 minutes (6000 r/min). The supernatant solution was discarded, then dissolved the precipitation in 10 ml volumetric flask with methanol and filtered through 0.45 μm nylon filter membrane prior to injection into HPLC system.

Method validation

The method of established in our paper was validated by checking its specificity, linearity, sensitivity, precision, repeatability, stability and recovery according to international guidelines. The relative standard deviation (RSD) is usually to evaluate the precision, repeatability, stability and recovery of the proposed method.

Specificity

Specificity was determined by comparing the samples and negative samples. The negative samples were prepared by absence of cinnabar crude drug according to the samples extraction process. Chromatographic conditions were the same as described above method of samples. No co-eluted peak of the same retention time appeared of the same chromatographic conditions which indicated the established method has good specificity.

Linearity

Five different concentrations of reference solutions were prepared by diluted serially gradually. The linear equations was described by the form of Y= aX+b, where Y and X were the peak areas versus their corresponding concentration, respectively. Table 1 is the results of their linear equation and related parameters.

Table 1 : Regression equation, coefficient, linearity, LOD and LOQ

Component	Linear equation	Linearity(**ng**)	Coefficient($\mathbf{R^2}$)	LOD **μg/mL**	LOQ **μg/mL**
cinnabar	Y=8.8599x-38.802	4.86-600.0	0.9999	0.1127	0.2065

Sensitivity: The limits of detection (LOD) and quantification (LOQ) values were expressed by the known content sample solutions which were measured at signal-noise (S/N) ration of 3:1 and 10:1 respectively. Table 1 showed the results of the LOD and LOQ in our established method.

Precision: Intra-day precision analysis test was measured by determination of reference solutions with three different concentrations on the same day, while the inter-day analysis test was examined in duplicates for consecutive three days. RSD was used to analyze the variations of the proposed method.

Repeatability: Six replicates of the sample were prepared as the method described above and analyzed twice to determine the mean. The chromatography condition was adopted as the previous mentioned above. RSD was usually evaluated the repeatability of the established method.

Stability: The stability of method proposed was evaluated by analyzing the sample solution at 0, 1, 2, 4, 8, 12, 16, 20, 24 h. Sample solution was prepared by the method described as above. RSD is a significant indicator of assaying the stability of method.

Recovery: Accuracy was measured by the recovery of sample solution. The recovery tests of JFZB pills were investigated by spiking with three different concentration (low, middle, high concentration) authentic content mercuric sulfide reference substances to the samples. Six replicates were accurately weighed and prepared by the method described as previously and analyzed by HPLC. The recoveries were showed in Table 2.

Table 2: Recoveries of Cinnabar Content in JFZB Pills (n=9)

Component amount(mg)	Original amount (μg)	Spiked amount (μg)	Determined amount(μg)	Recovery (%)	RSD (%)
52.02	3617.3	1870.1	5552.0	103.45	
50.06	3481.0	1880.3	5465.5	105.54	1.25
51.48	3579.7	1954.3	5648.5	105.86	
50.30	3497.7	3721.8	7454.9	106.32	
49.96	3474.1	3805.9	7292.6	100.33	2.90
51.48	3579.7	3750.4	7467.9	103.67	
50.55	3515.1	5615.5	9388.0	104.58	
51.09	3552.6	5559.9	9205.8	101.68	1.42
52.32	3638.2	5559.9	9390.3	103.46	

Cinnabar morphological characterization

The traditional Chinese medicine containing the artificial cinnabar is prohibited in Ch. P due to the toxicity of soluble mercury salt is more than nature cinnabar. Therefore, SEM image was carried out in order to distinguish the types of cinnabar in JFZB pills. Firstly of all, accurately weighed the JFZB sample 2.0 g then 30 ml chloroform was added and ultrasonic treatment for 10 min then filtered The precipitation was rinsed with methanol for several times until the cinnabar crude drug powder was appeared. It is obtained the cinnabar crude drug powder based on the principle of cinnabar can not insoluble in chloroform and methanol. Eventually, SEM experiment was carried out for the purposing of distinguishing the types in traditional Chinese medicine. The image of nature cinnabar, artificial cinnabar and the cinnabar in JFZB pills was displayed in Fig. 8.

Application

The developed method was applied to the determination of cinnabar content in JFZB pills with five different batch numbers from Tongrentang pharmaceutical factory. The content of cinnabar in JFZB pills were presented in Table 3.These date indicated the method seem to be a promising for the quantitative analysis of cinnabar content in JFZB pills.

Table 3: Content of Cinnabar in JFZB Pills (n=5)

Batch number	Cinnabar contents($mg \cdot g^{-1}$)	RSD（%）
3010201	68.86	1.22
3010203	62.92	0.43
3010204	65.92	1.12
3010206	66.21	0.97
3010207	64.89	1.39

Results and Discussion

Principle of reaction

The NaDDC is an excellent metal chelating agent, which can be existed stably in alkaline medium and its anions have three resonance structure (Fig. 1). Hg(II) ions can be dissociated from the cinnabar during the processing of JFZB pills was digested. NaDDC and Hg(II) ions can form a stable and have the strongest UV absorption chelates in alkaline medium (Fig. 2). We can concluded from Fig. 3 there are two absorption peak from 200–400 nm UV range at least that is B1 band and B2 band which produced by N=C and S=C π-π* transition respectively. From the figure 2 it is concluded that the wavelength emerged blue shift which may be caused by the chelates formation when Hg(II) ions and NaDDC reagents. Sodium oxide was used to adjust the pH of chelation reaction solution. To ensure the reaction completely, excess NaDDC need to be added. It can be separated from the aqueous phase because of $Hg(DDC)_2$ chelates show poor solubility in water (pK_{sp}=43.5, 20°C), but have good solubility in methanol. The chelates can be separated by HPLC and analysis by means of this reaction principle. During the processing of our experiment, we also found the chromatographic column efficiency decreased and the resolution of between two adjacent peaks increased in the number of experiments. It maybe caused by the mercury ions and chelates ligand exchanged each other, which could be solved by the rinsing the chromatographic column for 10 min with the 0.1 mol/l nitric acid.

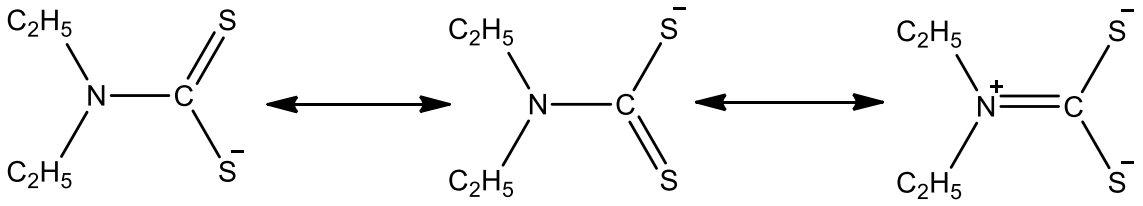


Fig. 1: Three resonance chemical structures of NaDDC

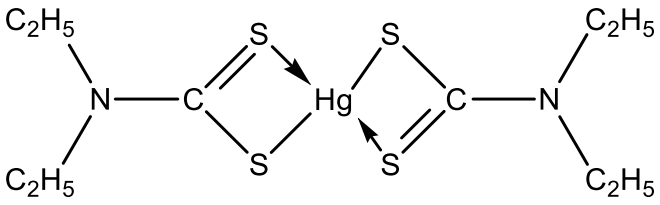


Fig. 2: The chemical structure of $Hg(DDC)_2$ chelates

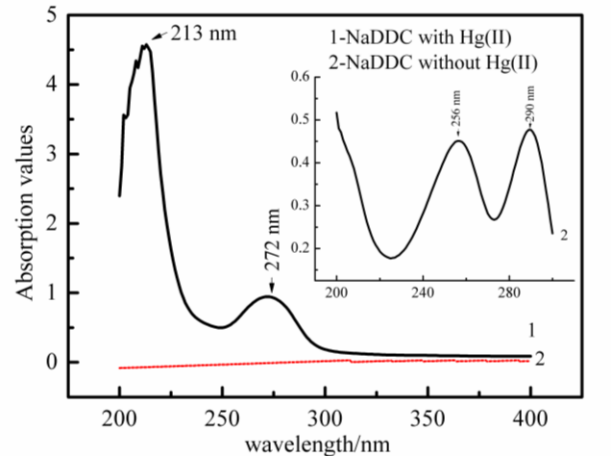
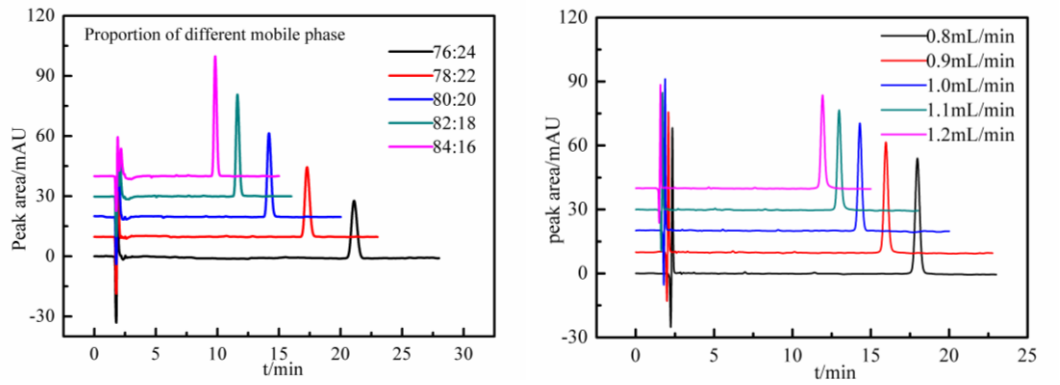


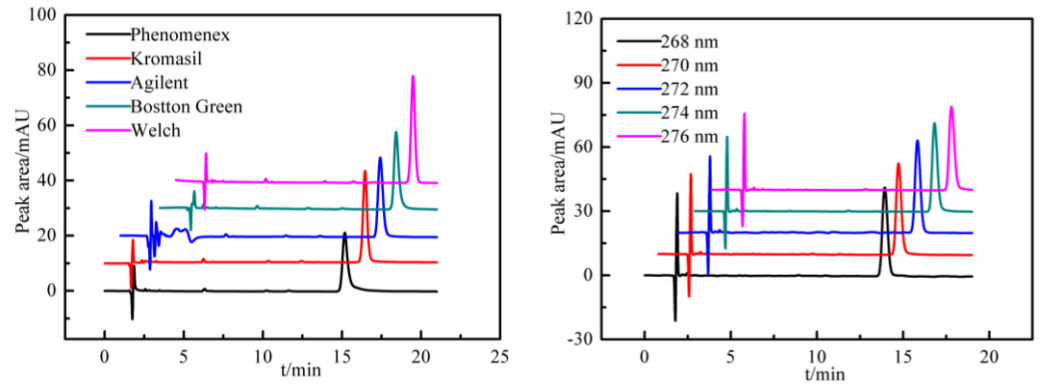
Fig. 3: UV spectrum of $Hg(DDC)_2$ chelates

Optimization of Chromatographic conditions

The detection of wavelength was screened by the UV spectrum of derived solution of reference 2 through scanning from 200–400 nm range. The results show that there are two maximum absorption peaks at 213 nm and 272 nm, respectively. Eventually, 272 nm is decided to the optimization of detection wavelength for the purpose of eliminating interference. We also optimized the conditions of chromatograms by the requirement for the better resolution of peaks, including the types of column such as Phenomenex Luna-C_{18} (250 mm × 4.6 mm, 5 μm), Welch XB-C_{18} (250 mm × 4.6 mm, 5 μm), Agilent XDB-C_{18} (250 mm × 4.6 mm, 5 μm), Boston Green C_{18} (250 mm × 4.6 mm, 5 μm), Kromasil 100-5 C_{18} (250 mm × 4.6 mm, 5 μm), series of oven temperature(25–45°C), detection wavelength(268 nm–276 nm), various proportions of mobile phase system(methanol: water (containing 0.022 mmol·L^{-1} NaDDC, pH 8–9) and flow rate(0.8–1.2 ml/min) (Fig. 4). We also found that the baseline noise will decrease if the mobile water phase containing of little amount NaDDC. All influence factors were considered the optimization of chromatograms. Implementation of system robustness experiment and study the factor of resolution, tailing factor and the number of theoretical plates. Eventually, it is concluded that welchXB-C_{18} (250 mm × 4.6 mm, 5 μm) column, oven temperature of 35°C, detection wavelength of 272 nm, the ratio of mobile phase system of 80:20 and flow rate of 1.0 ml/min was adopted. During the above chromatogram conditions the peaks have the good shape, resolution and tailing factor meets the requirements without interference. The Chromatograms figures of reference substances solution, sample solution and negative solution were shown in Fig. 5.



A B



C D

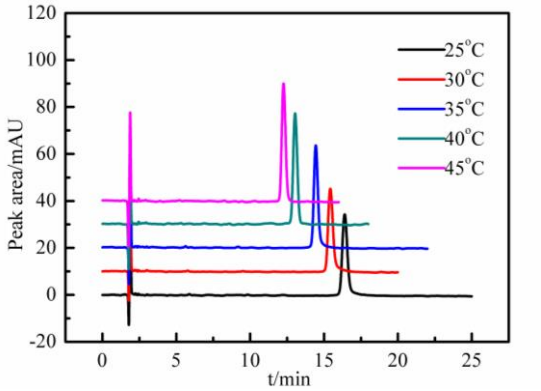


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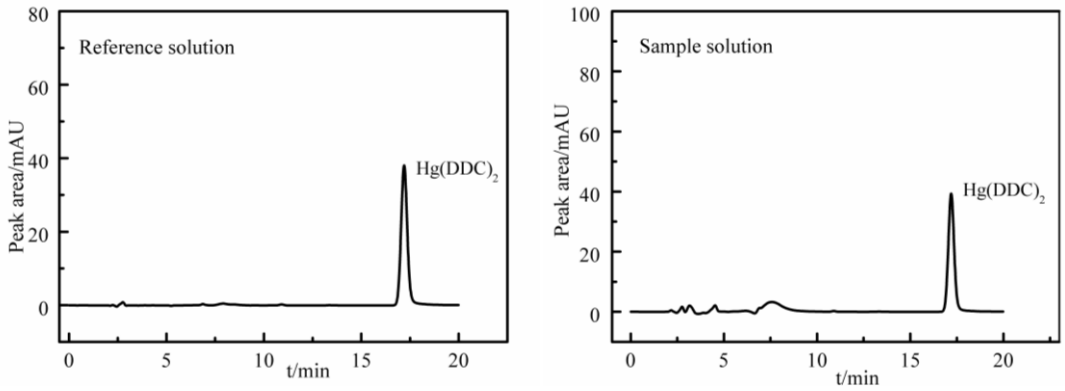
Fig 4: Chromatograms of robustness: (A) proportion of different mobile phase; (B) various flow rates; (C) different columns; (D) different wavelengths ;(E) different column temperatures



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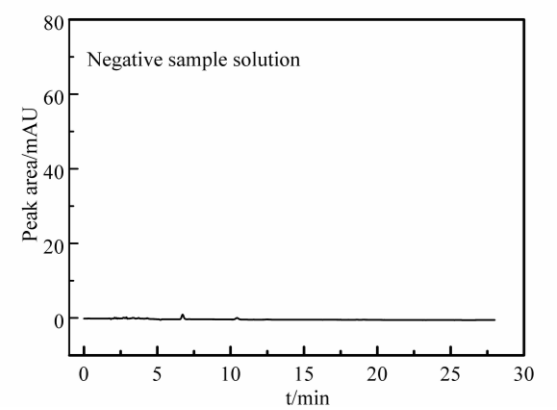

C

Fig 5: Chromatograms of reference substances solution and sample solution: (A) reference substances solution; (B) sample solution; (C) negative sample solution

Optimization of sample preparation

The volume of sulfuric acid, amount of potassium nitrate and sodium diethyldithiocarbamate were screened with orthogonal test. The results show that there was a significant distinction with the amount of potassium nitrate and the volume of sulfuric acid during the analysis. On the contrary, the amount of sodium diethyldithiocarbamate had no obvious distinction on the cinnabar content, 8 mg of sodium diethyldithiocarbamate was the best choice and excess sodium diethyldithiocarbamate would give rise to the noise increased. Eventually, the results demonstrated that the developed method (8 ml sulfuric acid, 1.2 g potassium nitrate and 8 mg sodium diethyldithiocarbamate) was adequate and suitable for the sample analysis.

Method validation

The linearity of the proposed method was determined by the method described as above. The linearity range from 2.43 to 300 μg/ml has broad linear range and more suitable for the trace mercury ion than the previous method [22]. Mercury in water solution was adopted as the reference solution is accurate than the analytical grade mercury nitrate. The correlation coefficients R^2 values was 0.9999 which means the method has a good linear relationship during the investigated ranges of sample concentration and corresponding peak areas (Fig. 6). The LOD and LOQ of the method were 0.1127 μg and 0.2065 μg/ml, respectively. The RSDs of intra-day and inter-day variations were 1.08% and 1.63%, respectively. The results showed that the RSD of repeatability and stability were 1.53% and 1.27% which indicated the established method has good repeatability and relatively stable and efficient. The recoveries ranged from 100.33% to 106.33% with the RSD less than 3.0% which also suggested that the method has good accuracy and can be used to determinate of cinnabar content in JFZB pills.

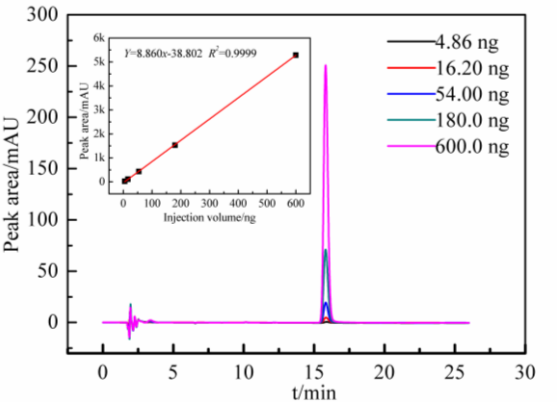

Fig 6: Plot of peak area versus concentration of reference solutions

Cinnabar identification

In this work, the cinnabar crude drug in JFZB pills could be identified via microscopic identification measure. The photomicrograph of cinnabar in JFZB pills under 400 times microscopic was presented in the Fig. 7. It was found that there are dark brown red irregular small particles with the sparking and dark in edge of the power which was in accordance with the national standards of cinnabar microscopic features. In fact, we also identified the nature cinnabar and artificial cinnabar of JFZB pills by SEM (scanning electron microscope) measure. SEM images further can be used to obtain the information of its micro-morphology of cinnabar. From the Figure 8, it is easily concluded that the image of cinnabar (ca. 200–800 nm) in JFZB pill size was smaller than the artificial cinnabar (ca. 0.8–6 um), which was similar to the nature cinnabar (ca. 200–1000 nm).Based on above the discussion the method of determination of cinnabar content in JFZB pills was developed. The study of distinguishing the nature and artificial cinnabar for purposing the safety and quality of traditional drugs can be realized.

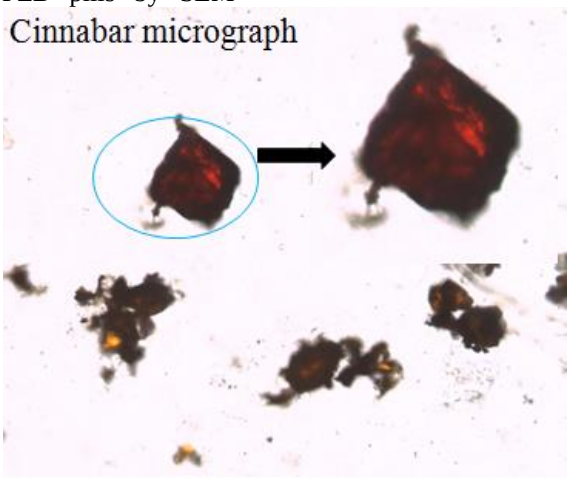

Fig. 7: Micrograph of cinnabar crude drug in JFZB pills

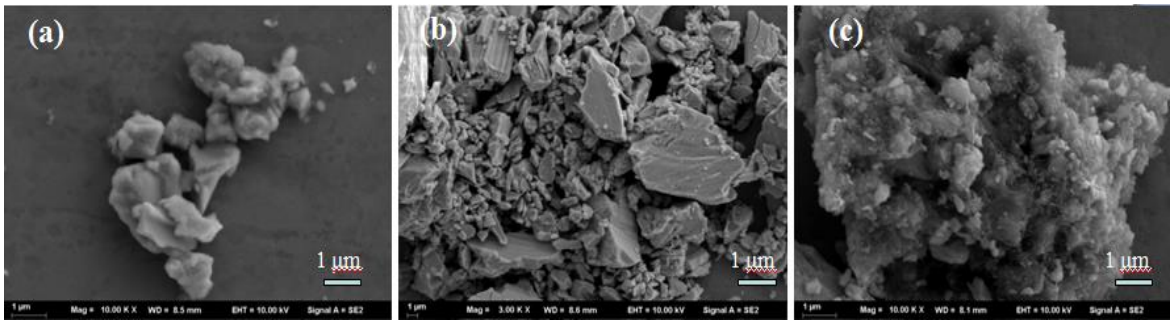

Fig 8: SEM image of cinnabar crude drug powder: (a) nature cinnabar crude drug; (b) artificial cinnabar crude drug; (c) cinnabar crude drug in JFZB pills

Conclusion

In our study, a validated, sensitive and reliable method was successfully applied for determination of cinnabar content in JFZB pills by HPLC-UV with pre-column derivatization method. The results demonstrated the proposed method was sensitive and selective which can be suitable to routine laboratory analysis and revised the standards of drug quality. It will be a promising method for quality assessment in traditional Chinese medicines containing cinnabar in the future.

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References

1. Chinese Pharmacopoeia Commission, “National Standard for Chinese Patent Drugs of the People's Republic of China”, China Medical Science Press, Beijing, 1996; 12:95.

2. Chinese Pharmacopoeia Commission, "Pharmacopoeia of the People's Republic of China", China Medical Science Press, Beijing, 2015;1:1008-1009.

3. Liu J, Shi JZ, Yu LM, Goyer RA and Waalkes. MP. Mercury in traditional medicines: is cinnabar toxicologically similar to common mercurials? Exp. Boil. Med., 2008; 233(7): 810-817.

4. Lu YF, Wu Q, Liang SX, Miao JW, Shi JS and Liu J. Evaluation of hepatotoxicity potential of cinnabar-containing An-Gong-Niu-Huang Wan, a patent traditional Chinese medicine. Regul. Toxicol. Pharm., 2011; 60(2): 206-211.

5. Young YH, Chuu JJ, Liu SH, and Lin-Shiau SY. Neurotoxic mechanism of cinnabar and mercuric sulfide on the vestibulo-ocular reflex system of guinea pigs. Toxicol. Sci., 2002; 67(2): 256-263.

6. Chuu JJ, Liu SH, Linshiau SY. Differential neurotoxic effects of methylmercury and mercuric sulfide in rats. Toxicol. Lett., 2007; 169(2):109-120.

7. Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharm. Sci., 2002; 23(3):136-9.

8. Bartosiewicz MJ, Jenkins D, Penn S, Emery J, and Buckpitt A. Unique Gene Expression Patterns in Liver and Kidney Associated with Exposure to Chemical Toxicants. J. Pharmacol. Exp. Ther., 2001; 297(297):895-905

9. Liang A, Li C, Xun B, Wang J, Zhao Y, Liu T, Cao C, Yi Y, and Hao R.Study of mercury cumulation in Cinnabar-treated rats. China. J. Chin. Mate. Med., 2009; 34(23):3068-72.

10. Lu YF, Wu Q, Yan JW, Shi JW, Liu J, and Shi JS. Realgar, cinnabar and An-Gong-Niu-Huang Wan are much less chronically nephrotoxic than common arsenicals and mercurial. Exp. Biol. Med., 2011; 236(2):233-9.

11. Huang X, Huang C, Xie X, Lv Y. Titrimetric determination of cinnabar(HgS) content in baoying powders. Chin. Med. Pharm., 2015; 5(20):201-203

12. Wang L, Lai P. Determination of Cinnabar in Zhike Taohua Powders. Chin. Pharm., 2013; 22(20):55-56

13. Tian N, Yang G. The Research on the Content of Dissoluble Mercury Saitsin Processed Cinnabar Determined by Atomic Absorption Spectrometry. Chin. J. Pharm. Anal., 1995; 15(1):27-30

14. Huang RJ, Zhuang ZX, Tai Y, Huang RF, Wang XR, and Lee FS. Direct analysis of mercury in Traditional Chinese Medicines using thermolysis coupled with on-line atomic absorption spectrometry. Talanta, 2006; 68(3):728-734.

15. Lin LT, Huang HM, Zhuang BY. Determination of cinnabar in Saimeian vaginal suppositories by AFS. Strait. Pharm. J., 2012; 24(3):70-71

16. Chen Q, Cheng Y, Meng Z, Zhang Q, Liu Y, and Zhang X. Determination of leachable mercury in Chinese traditional patent medicine containing cinnabar by bionic extraction-ICP-MS. Chin. J. Pharm. Anal., 2012; 32(6):1036-1039.

17. Santos J S D, Guárdia M D L, Pastor A, Santos M LPD. Determination of organic and inorganic mercury species in water and sediment samples by HPLC on-line coupled with ICP-MS. Talanta, 2009; 80(1):207-211.

18. Zhang J, Feng X. Determination of Cinnabar in Xiao'er Jindan Tablets by HPLC with Pre-column Derivatization. China Pharm., 2010; 13(8):1120-1122

19. Ding J H, Wang N, Jiang H, Chang B, and Sun GF. Determination of the Content of Soluble Mercury in Cinnabar by High Performance Liquid Chromatography. Chem. Res., 2010; 21(2): 80-82

20. Atanassova D, Stefanova V, Russeva E. Co-precipitative pre-concentration with sodium diethyldithiocarbamate and ICP-AES determination of Se, Cu, Pb, Zn, Fe, Co, Ni, Mn, Cr and Cd in water. Talanta, 1998; 47(5): 1237-1243.

21. King JN, Fritz JS. Determination of cobalt, copper, mercury, and nickel as bis (2-hydroxyethyl) dithiocarbamate complexes by high-performance liquid chromatography. Anal. Chem., 1987; 59(5): 703-708.

22. He YM, Ren HZ, Liu XQ. Determination of Mercury in Cinnabar by HPLC with Pre-column Derivatization. Chin. J. Pharm., 2008; 39(11):849-852.

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