

Determination of the content of hypericin in Huanghua Ningshen granules based on HPLC¹

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Abstract. In this study, the HPLC method was used to determine the content of hypericin in huanghua ningshen granules. The Fortisil-C18 chromatographic column (4.6 mm×250 mm, 5 μm) was used with mobile phase of dipotassium hydrogen phosphate solution: acetonitrile (88:12), 1 mL/min⁻¹ flow rate, detection wavelength of 360 nm. The results showed that the content of hypericin was linear in the range of 6.6–67.0 μg mL⁻¹ ($r = 0.9991$). The average recovery was 99.43 %, and RSD was 0.94 %. This method can be used to determine the content of hypericin in Huanghua Ningshen granules.

Key words. Huanghua Ningshen granule, hypericin, HPLC method.

1. Introduction

In today's society, more and more people suffer from varying degrees of depression [1]. This may be due to faster and faster pace of life, more and more intense social competition, and work and life and other aspects of the pressure is also increasing. Depression is the neuropsychiatric disorder caused by a variety of factors and with depression as the main symptoms. Depression is a disease with high incidence, high disability rate and high recurrence rate [2]. The main manifestations of depression include slow thinking, difficulty falling asleep, serious self-mutilation, suicide and other acts. WHO statistics show that depression ranked in the top fourth in the

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ten major diseases. By 2020, it may become the second largest disease after cardiovascular and cerebrovascular diseases, becoming the highest burden of disease in developed countries [3]. Prevention and treatment of depression has attracted the medical profession and the community's attention [4].

Depression is not caused by a single cause, and the causes are very complex. The influence of various factors, such as genetic factors, biochemistry, immunity, psychology, health, society and environment, may lead to different degrees of depression. It is a complex disease caused by multiple etiologies [5]. At present, its pathogenesis is not clear, which is still in the stage of hypothesis. It is thought that the occurrence of depression is closely related to the abnormal metabolism of biogenic amines [6]. There are many hypotheses about its pathogenesis. At present, there are 5 kinds of hypotheses which are recognized by the medical profession, that is, the 5-HT hypothesis, the norepinephrine hypothesis, the dopamine hypothesis, the amino acid neurotransmitter system hypothesis and the acetylcholine hypothesis [7].

At present, the main treatment of depression is drug therapy with Western medicine, SSRI classic antidepressants as the main medicine. However, the drug cost is high, the effect is slow, and there are some side effects. There are 70 % patients with residual effect, and the effect is not ideal [8]. Therefore, the key is to reduce the side effects of drugs and shorten the onset time of drugs. Therefore, a large number of studies began to focus on natural medicines, including traditional Chinese medicine. Chinese medicine has great potential in the treatment of depression, and it also shows a very bright future. Traditional Chinese medicine multi-target, comprehensive treatment has low adverse reaction, which is the absolute superiority superior to Western medicine. Especially for patients with longer course of disease, its curative effect is better than Western medicine [9].

Huanghua Ningshen granule is compound preparation by 12 kinds of herbs including yellow, rehmannia, Acacia flowers, vinegar Cyperus, turmeric, licorice, floating wheat, wheat, tuckahoe, lily, calamus, and jujube according to a certain proportion. Its main function is the curative effect of restoring consciousness and inducing liver and relieving depression, which has better clinical effect on treating depression. Based on the reports on the determination of the content of hypericin in the Huang Ning ning granules, in this paper, HPLC method was used to determine the content, which provided a new basis for quality analysis, evaluation and control.

2. State of the art

2.1. Clinical study of Chinese medicine in the treatment of depression

Traditional Chinese medicine (TCM) is an important part of Chinese traditional culture. Traditional Chinese medicine a very influential and valuable subject at home and abroad and it has been extensively studied [10]. Chinese medicine is a kind of medicine which is produced under the guidance of the basic theory of traditional medicine with a wide application, and it has played an important role to cure various diseases and human health in the whole world, and it has made

outstanding contributions [11]. Because of its unique curative effect, mechanism of action and therapeutic effect, more and more attention has been paid to the study of medicine at home and abroad.

At present, the main treatment of depression is drug therapy with Western medicine, SSRI classic antidepressants as the main medicine. However, the drug cost is high, the effect is slow, and there are some side effects. Some people are not suitable for the treatment of Western medicine. In this case, traditional Chinese medicine or acupuncture can be used to treat the disease and to reverse the development of the disease.

For patients with self-mutilation or even suicidal tendencies with major depression, taking a slower onset of Western medicine can increase the risk and it may delay the treatment of the last period. High medical expenses also let many patients give up treatment. Therefore, the key to the development of new drugs is the ability to solve the problem of timeliness and rapid onset.

In clinical application, it is found that the yellow flower has sedative and hypnotic effect, so it has a series of clinical research. The results show that the yellow flower has a better effect of sleep calming, and it may improve the quality of sleep. And there are no greater adverse reactions with fewer side effects, which have a good guiding role in clinical medication. The flower of silk tree *albizzia* belongs to the leguminous plant, and the taste is good. It can be found that the flower can play a role in relieving depression. Flowers can be used for the treatment of depression, insomnia and other symptoms. The extract of the flower of silk tree *albizzia* was found to have the function of resisting depression and hypnosis. Glycyrrhizin has obvious antidepressant effect, and its mechanism is to reduce the probability of BV2 cell death induced by glutamate injury. In the study on the pharmaceutical composition of *liquiritia glycyrrhiza*, it is found that most of the antidepressant drugs in *Glycyrrhiza* are flavonoids. Therefore, the antidepressant effect of flavonoids is one of the important references for current drug screening. In the clinical treatment, the patient has a certain improvement in the short term after taking *Acorus calamus*. With continuous administration of the drug, the patient's mental state will be significantly improved as well as better quality of sleep. *Rhizoma cyperi* belongs to *Cyperus* sedges, and it has a bitter taste. It has the effect of soothing the liver and relieving depression. In the analysis of TCM syndrome of depression, liver qi stagnation is an important cause of depression. Therefore, *Rhizoma cyperi* can treat on liver qi stagnation in clinical. After the drug composition analysis of *Rhizoma Cyperi*, it is found that the main component is the same kind of brass. In addition, it has includes the carrier of saponins. This not only can play a neuro-protective role in anti-oxidation, but also has important pharmacological effects of anti-allergy and reducing blood sugar, with mechanism of increasing the content of DA and *Cyperus* 5-HT. *Shengdi* belongs to *Scrophulariaceae* *Rehmannia* injection, and its water extract has a protective effect on organisms. Its function is to protect the brain tissue of rats, and can promote the repair of nerve function. *Turmeric* belongs to *Zingiberaceae* plants, and it can improve the sport level of depressive rats effectively, including vertical movement and horizontal movement level, and reduce consumption of sugar, which has verified the antidepressant effects of turmeric.

2.2. Study on Chinese medicine preparation

Chinese medicine has made good progress in the treatment of depression, and it also has played a positive role in guiding clinical medication. After a number of repeated rigorous clinical studies, it has confirmed the support of compound drugs in the treatment of depression has a better clinical effect: multiple targets, rapid onset, less side effects, especially in the regulation of neural plasticity and inflammation. Therefore, it is of great significance to explore the active ingredients of traditional Chinese medicine and the prescription of Chinese herbal medicines in the comprehensive understanding of the role of Chinese medicine in the treatment of depression.

Most of the traditional Chinese medicine is based on decoction. The decoction is in accordance with a certain proportion of all kinds of water and medicine in the prescription were mixed, and then after a certain time of boiling. It is not easy to carry, not easy to store, less suitable for the crowd, which limits the widespread use of Chinese herbal medicine decoction.

Huanghua Ningshen granule is a kind of dry extract granule of Chinese traditional medicine extract, which is developed by extraction, concentration, drying and so on. Compared with the decoction, the preparation process is simple, easy to carry and store, providing convenience for most patients. This solves a series of problems caused by the limitation of the conditions, which is more applicable to the crowd and greatly promotes the popularization.

3. Methodology

3.1. Instrument

The main experimental instruments used in this study are as follows: Ultimate 3000 high performance liquid chromatography system, purchased from the United States Diane; Electronic balance, bought from METTLER & TOLEDO instruments Shanghai Co.; The constant temperature water bath box and chromatography analysis platform of Tianjin Test Instruments Company; Ultrasonic cleaning machine of Kunshan, Shanghai.

3.2. Selection of extraction methods

In this study, three methods of extracting hypericin were selected, and the extraction efficiency was compared in the same time. Hot ethanol reflux extraction: To accurately weigh the amount of the Huanghua Ningshen granules. After crushing and screening, 1g was added to the flask of 50 ml. 25 ml of 70 % ethanol was refluxed for 1 h and the loss of 70 % ethanol was made up for fully shaking. After filtration, the former 2 ml filtrate was discarded. Then following 2 ml filtrate was added to the 10 ml flask. Finally, 70 % ethanol was added to the scale and shade well to complete the extraction process.

Ultrasonic concussion: To accurately weigh the amount of the Huanghua Ning-

shen particles. After crushing and screening, 1 g was added to the flask of 50 ml. 25 ml of 70 % ethanol was weighed for 20 min of ultrasonic treatment. After the completion of the ultrasound, it was put with natural cooling to room temperature. Then the loss of 70 % ethanol was made up for fully shaking. After filtration, the former 2 ml filtrate was discarded. Then following 2 ml filtrate was added to the 10 ml flask. Finally, 70 % ethanol was added to the scale and shade well to complete the extraction process.

Soxhlet extraction method: To accurately weigh the amount of the Huanghua Ningshen particles. After crushing and screening, 1 g was added to a Soxhlet extractor. 25 ml of 70 % ethanol was refluxed for 1 h and the loss of 70 % ethanol was made up for fully shaking. After filtration, the former 2 ml filtrate was discarded. Then following 2 ml filtrate was added to the 10 ml flask. Finally, 70 % ethanol was added to the scale and shade well to complete the extraction process.

3.3. Chromatographic condition

Fortisil-C18 chromatographic column (4.6 mm×250 mm, 5 μm), 40 °C as the column temperature, flow rate of 1 ml/min, the sample volume of 10 μL. The mobile phase of acetonitrile (A) - 0.2 % phosphoric acid (B) was used for gradient elution. The detection wavelength was 360 nm.

The hypericin comparative sample in Huanghua Ningshen granules was accurately weighed. The sample obtained was dissolved by methanol, and the solution of 0.5 mg/ml was obtained. The 1 g sample powder was precisely weighed and added to the flask with 25 ml of 70 % ethanol solution after No.3 sieve filtration. Then the total weight was recorded. It was carried out with water bath heating reflux for 45 min, and put at the room temperature for 1 h. Weigh again, and use 70 % ethanol to make up for the loss of weight. After fully mixed and filtration by 0.45 μm membrane, the filtrate was the prepared sample solution. The reference solutions respectively 0.1, 0.2, 0.5, 1, 2, 4, 8 ml was put into 7 flasks of 10 ml. They were diluted with 70 % ethanol to the scale, shade well, and added to high performance liquid chromatography to determine its peak area. The peak area of the sample was the ordinate, the sample volume was the abscissa, and the linear regression was used to obtain the linear concentration range and correlation coefficient r .

3.4. Precision test and stability test

With precision weigh of Huanghua Ningshen God granules for crushing and screening, the same sample was tested for 6 times under the same chromatographic conditions. According to the measured peak area of hypericin, we could determine whether it met the requirements. Similarly, with the same sample, different time points were measured. This study selected 0, 2, 4, 8, 16 h for test, according to the measured peak area of hypericin to determine whether to meet the requirements.

Take about 0.5 g granules with the known concentration, taking 6 pieces. The sample solution was prepared by adding the reference solution of 0.4 ml, and added to the liquid chromatograph. The peak area was determined, and the recovery rate

was calculated according to the established conditions. The results showed that the average recovery rate was 99.43% and RSD was about 0.94%, which was in line with the requirements. In this paper, the sample solution using 0.2 g granules was prepared according to the method described in this paper. 20 μl was added to the liquid chromatography column, and the content of hypericin was determined according to the optimized chromatographic conditions.

4. Result analysis and discussion

4.1. The established of the HPLC method to analyze the results

The results showed that the extraction efficiency of hot ethanol reflux had the highest in the same extraction time. In this paper, the hot ethanol reflux and the HPLC method was used for the determination of the content of hypericin in the granules, and the main instrument was Ultimate 3000 high performance liquid chromatography (Fig. 1). The chromatographic conditions could be used to measure the separation of the peaks of the hypericin and other components in the granules (Fig. 2), which showed a good linear relationship in the range of 2.5–50.0 $\mu\text{g ml}^{-1}$ (Table 1). The recovery was 99.43% and for RSD see (Table 2). Therefore, the method established in this paper could be used to determine the content of hypericin.



Fig. 1. Diode ultimate high performance liquid chromatography system

Table 1. The linear analysis of the hypericin content in the granules

Component	Regression equation	Correlation coefficient	Linear range (mg/ml)
Hypericin	$y = 0.5134x - 0.4612$	0.9991	0.66–67.00

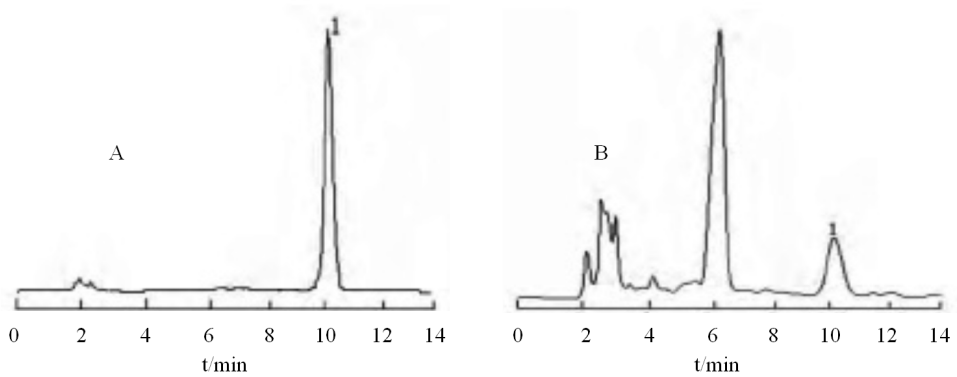


Fig. 2. HPLC chromatogram (A: hypericin reference product; B: sample; 1: hypericin)

Table 2. The results of the recovery of Hypericum in the granules

Component	Sample weight (mg)	Sample content (mg)	Addition amount (mg)	Measured quantity (mg)	Rate of recovery (%)	Average recovery (%)	RSD (%)
Hypericin	0.5021	0.3364	0.3350	0.6681	99.58	9.43	0.94
	0.5053	0.3367	0.3350	0.6781	99.49		
	0.5019	0.3379	0.3350	0.6693	99.14		
	0.5037	0.3372	0.3350	0.6721	99.34		
	0.5042	0.3352	0.3350	0.6737	99.94		
	0.5007	0.3382	0.3350	0.6601	99.05		

4.2. Determination of the content of Hypericum in Huanghua Ningshen granules

According to the established chromatographic conditions, the solution of the sample was analyzed, and the content of hypericin was determined.

Table 3. Results of the determination of the content of hypericin

No.	Content of hypericin (%)	Average content	RSD (%)
1	0.35	0.35	0.97
2	0.33		
3	0.35		
4	0.36		
5	0.35		

5. Conclusion

At present, the treatment of depression is mainly based on drug treatment of Western medicine with SSRI classic antidepressant drugs. But the drug is expensive, slow onset and there are large side effects. Chinese medicine has made good progress in the treatment of depression, and has played a positive role in guiding clinical medication. Repeated rigorous clinical studies have confirmed the good clinical effect of compound medicine to support in anti-depression, multi targets, fast acting, less side effects, especially played an important role in the process of clinical neural plasticity and neural regulation of inflammation (Chaki 2003) [15]. Therefore, closely combining the basic theoretical knowledge of Chinese medicine, rich clinical experience, advanced technology and the latest application of neural science and biology to carry out the systematic research to multiple templates between multiple disciplines will promote the whole society can more in-depth understand the clinical value and scientific connotation of traditional Chinese medicine compound preparation in the treatment of depression.

In this study, the sample was extracted with 70 % ethanol and then determined by Ultimate high performance liquid chromatography (HPLC) system. The operation was very simple and reliable with short operation time. With acetonitrile (A) - 0.2 % phosphoric acid (B) as mobile phase for gradient elution, the peak separation effect was good, and the hypericin could be separated from other miscellaneous peaks obviously. In the process of detection wavelength, the maximum absorption peak could be detected by the 360 nm wavelength, and the lowest degree of interference of the magazine could be guaranteed. There was a good linear relationship in the range of 2.5–50.0 $\mu\text{g ml}^{-1}$. The recovery was 99.43 %, and RSD was 0.94 %. After repeated experiments, it was found that the method used in this study was accurate and reliable, which could provide a new basis for quality analysis, evaluation and control.

References

- [1] V. L. FEIGIN, G. A. ROTH, M. NAGHAVI, P. PARMAR, R. KRISHNAMURTHI, S. CHUGH, G. A. MENSAH, B. NORRVING, I. SHIUE, M. NG, K. ESTEP, K. CERCY, C. J. L. MURRAY, M. H. FOROUZANFAR *Global burden of stroke and risk factors in 188 countries, during 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013*. *The Lancet Neurology* 52 (2016), No. 9, 913–924.
- [2] S. Z. LANGER: α_2 -Adrenoceptors in the treatment of major neuropsychiatric disorders. *Trends in pharmacological sciences* 36 (2015), No. 4, 196–202.
- [3] S. BHAVSAR, A. SARKAR, R. RAM, P. PITHADIA, J. P. MEHTA, D. PARMAR: *Lifestyle practices related to health promotion among adult males in urban areas of Jamnagar city, Gujarat*. *Journal of Research in Medical and Dental Science* 1 (2013), No. 2, 36–39.
- [4] H. I. KUO, W. PAULUS, G. BATSIKADZE, A. JAMIL, M. F. KUO, M. A. NITSCHKE: *Acute and chronic effects of noradrenergic enhancement on transcranial direct current stimulation-induced neuroplasticity in humans*. *The Journal of physiology* 595 (2017), No. 4, 1305–1314.
- [5] T. GUO, Y. T. XIANG, L. XIAO, C. Q. HU, H. F. CHIU, G. S. UNGVARI,

- C. U. CORRELL, K. Y. LAI, L. FENG, Y. GENG, Y. FENG, G. WANG: *Measurement-based care versus standard care for major depression: a randomized controlled trial with blind raters*. *American Journal of Psychiatry* 172 (2015), No. 10, 1004–1013.
- [6] T. H. CHAO, P. K. FU, C. H. CHANG, S. N. CHANG, F. C. MAO, C. H. LIN: *Prescription patterns of Chinese herbal products for post-surgery colon cancer patients in Taiwan*. *Journal of Ethnopharmacology* 155 (2014), No. 1, 702–708.
- [7] Y. GUO, J. ZHU, X. SU, J. YANG, Y. LI, Q. WANG, W. WEI: *Efficacy of Chinese herbal medicine in functional dyspepsia: A meta-analysis of randomized, double-blind, placebo-controlled trials*. *Journal of Traditional Chinese Medical Sciences* 3 (2016), No. 3, 147–156.
- [8] S. S. QU, Y. HUANG, Z. J. ZHANG, J. Q. CHEN, R. Y. LIN, C. Q. WANG, G. L. LI, H. K. WONG, C. H. ZHAO, J. Y. PAN, S. C. GUO, Y. C. ZHANG: *A 6-week randomized controlled trial with 4-week follow-up of acupuncture combined with paroxetine in patients with major depressive disorder*. *Journal of Psychiatric Research* 47 (2013), No. 6, 726–732.
- [9] S. C. MAN, B. H. HUANG, R. M. NG, X. C. YU, H. CHEUNG, M. P. M. FUNG, L. S. W. LI, KEI P. LEUNG, KWOK P. LEUNG, K. W. Y. TSANG, E. ZIEA, V. T. WONG, Z. J. ZHANG: *A pilot controlled trial of a combination of dense cranial electroacupuncture stimulation and body acupuncture for post-stroke depression*. *BMC Complementary and Alternative Medicine* 19 (2014), No. 14, paper 255.
- [10] R. Y. WU, D. D. ZHU, Y. C. XIA, H. S. WANG, W. W. TAO, W. D. XUE, B. M. XIA, L. REN, X. ZHOU, G. C. LI, G. CHEN: *A role of Yueju in fast-onset antidepressant action on major depressive disorder and serum BDNF expression: a randomly double-blind, fluoxetine-adjunct, placebo-controlled, pilot clinical study*. *Neuropsychiatric Disease and Treatment* 11 (2015), 2013–2021.
- [11] M. J. RAMAKER, S. C. DULAWA: *Identifying fast-onset antidepressants using rodent models*. *Molecular Psychiatry* 22 (2017), No. 5, 656–665.

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