Design and content determination of Genhuang dispersible tablet herbal formulation

Rui-hua Xin[1], Ge Liang[2] and Ji-fang Zheng[1]*

[1]Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Key Laboratory of New Animal Drug Project of Gansu Province, Engineering & Technology Research Center of Traditional Chinese Veterinary Medicine of Gansu Province, Key Laboratory of Veterinary Pharmaceutics Development, Ministry of Agriculture, Lanzhou, PR China
[2]Sichuan Animal Science Academy, Chengdu, PR China

Abstract: The aim of the present study was to optimize the shaping technology of the traditional herbal formula Genhuang dispersible tablets, and also establish a method for content determination. The optimal formulation of Genhuang dispersible tablets was determined based on the results of single factor test and orthogonal design test. The disintegration was used as the main study indicator. The proportion of each adjuvant in the optimal formulation consisted of 40% MCC as bulking agent, 15% PVPP and 7% L-HPC as disintegrant, ethanol as adhesive, CSD as lubricant, preparing the dispersible tablets with wet granulation. The content of baicalin in Genhuang dispersible tablets was determined by RP-HPLC method, the C_{18} column (150×4.6 mm, 10μm) was used, the mobile phase was methanol-water-phosphoric acid (47: 53: 0.2) with the flow rate of 1mL/min, the detection wavelength was at 280 nm and the column temperature was 30□. The prepared dispersible tablets could be totally disintegrated within three minutes and in accordance with the standard of the Chinese pharmacopoeia. In conclusion, the formulation was suitable for Genhuang dispersible tablets, and the determination method was simple, sensitive and accurate. Therefore, the Genhuang dispersible tablets can be used for industrial production and effectively controlled.

Keywords: Genhuang dispersible tablet, orthogonal design, disintegration time, content determination.

INTRODUCTION

Infectious Laryngotracheitis (ILT) is an acute upper respiratory infection of chickens (B.W. Calnek., 1999; Yin *et al*., 1997), which occurs worldwide and can cause severe production losses (Huang *et al*., 1997; Gao *et al*., 1995), has been classified as one of the List B animal diseases by the Office International des Epizooties (OIE) (May *et al*., 1923). Although there are different kinds of commercial antibiotics available for use in the prevention of ILT, the vaccines remain insufficient attenuation as immunogenicity of ILTV is usually correlated with its virulence and the strains of ILTV used in vaccines can also produce latent infections (Hughes *et al*., 1987; Hughes *et al*., 1989).Therefore, to treat and prevent the ILT is still important and necessary (Kong *et al*., 2005).

Traditional Chinese medicine (TCM), with has long history and rich resources showed many advantages (Zheng *et al*., 1998; Zhang *et al*., 1995) such as the ability to multi-target, efficiency, low toxicity and low residue, it also can meet the safety requirements of animals' food as well. Several single traditional chinese drugs are proved effective on anti-viruses many years ago (Oh *et al*., 2000; Farnet *et al*., 1998; Yeh *et al*., 1993; Xia *et al*., 1989; Zheng *et al*., 1989), such as *Radix Isatidis*, *Scutellaria* and *Forsythia*, etc (Gao *et al*., 2008; Xu *et al*., 2005; Chen *et al*., 2009). Many researchers used the traditional chinese medicine (Deng *et al*., 2010) to prevent ILT based on the theory of traditional chinese veterinary medicine (TCVM). As a traditional chinese herbal formula, Genhuang Tang consists of 6 crude drugs: *Isatis*, *Scutellaria*, *Vietnamese Sophora Root*, *Belamcanda*, *Platycodon* and *Glycyrrhiza* has been found effective on anti-ILT and be used for decades in China.

However, the formulation used of Genhuang Tang was still decoction, which is inconvenient in transportation. Furthermore, some active ingredients of herbs are unstable in liquid. In treatment of disease, the formulation of the drugs has significant impact on release, absorption and use of active ingredients. So in this study we aimed to improve the formulation of Genhuang Tang.

The dispersible tablets are simpler for caregivers to prepare and administer than crushed bitter tablets and easier to take (Committee for the Pharmacopoeia of P.R. China, 2010). They require only a small amount of water for dispersion and are easy to administer. This formulation is very useful in pediatric clinics because it disperses quickly and forms a uniform suspension (Liu *et al*., 2005). This quality also could make it adaptable for suspension in drinking water of broilers, so we hope it will be used in veterinary medicine. In this paper, the pharmaceutical technology of Genhuang dispersible tablets were investigated for selecting the appropriate pharmaceutical excipients of dispersible tablets, and we also established a method for content determination.

**Corresponding author:* e-mail: xinruihuamys@126.com

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using a 2695 Waters HPLC system (Waters Corporation, USA) consisting of an auto sampler and diode array detector (2995); Single-punch tablet press (Zhongcheng pharmaceutical machinery co., LTD, Hunan); Disintegration tester (Chuangxing electronic Technology Co., Ltd, Tianjin); Hardness tester (Chuangxing electronic Technology Co., Ltd, Tianjin); Chromatographic column GL (Sciences Inertsil ODS-3, 4. 6 mm×150 mm, 5μm).

Reagents

MCC, PVPP, L-HPC, CSD, PVP K30 and CMS-Na were purchased from Shanghai Houcheng fine chemical Co., Ltd. *Isatis*, *Scutellaria*, *Vietnamese Sophora Root*, *Belamcanda*, *Platycodon* and *Glycyrrhiza* were purchased from traditional chinese medicine market. Baicalin standard (Lot 110715-200815) was obtained from National Institute for Food and Drug Control (China). Genhuang dispersible tablets were manufactured by our laboratory (Lot 150401, 150402, 150601, 150602, 150701, 150702, 150703, 150704, 150801 and 150803). Methanol of HPLC grade was purchased from Merck Co., Ltd (USA).

Herbal medicine preparation

Take prescribed amount of medicine, using 10 times the amount of water soaking 0.5h, boiled twice, once for two hours, combined decoction, filtration, concentration, vacuum drying, grinding, sieving through 150μm.The herbal formula of Genhuang Tang was showed in table 1.

Tablets preparation

Raw materials and excipients went through 150μm sieve. Weigh the amount of drugs according to the formula and mixing with excipients. Mass fraction of 70% ethanol solution as the adhesive system of soft material, sifting granulation, then dried it at 60□ for 1 hour, and added q.s. lubricant and mixed uniformly and pressed into tablets.

Disintegration

Took 6 pills of samples into glass tube of the disaggregation meter gondola respectively and put basket in 1000ml beaker. Then adjusted the grit of basket position to fall away from the beaker bottom 25mm, as the beaker filled with a temperature (20 ±1)°C water, regulating water level to rise when the grit in underwater basket 15mm. Started disintegration device, observed and recorded the time of collapse.

Single factor test

Granularity screen

The powder extracted from the drugs of the prescription and excipients would go through 150μm sieve. The 1180, 600, 425μm sieve was used to granulate respectively. The disintegration time and the appearance of the tablets were observed and the results were showed in table 2.

Bulking agents screen

The bulking agents were used to increase the tablet weight and size, and also to shape the tablet. Several bulking agents commonly used were selected such as microcrystalline cellulose (MCC), lactose and calcium sulfate. All of the above were sieved the 150μm respectively. According to the formula, PVPP was added as disintegrating agent, silica powder as lubricant. The quantity of bulking agents was equal. According to the method of "tablets preparation", tablets were made and the tablet hardness was controlled between 22N to 25N. The disintegration time and corpuscular angle of repose was measured. The formula and the determination results were showed on table 3.

Disintegrant screen

The disintegrants were also very important to the disaggregation and dissolution results of the dispersible tablets, so disintegrants was the factor to be first considered. Generally the swelling degree of the disintegrant was $5mL \cdot g^{-1}$ above. The disintegrant commonly contained PVPP, CMS-Na, L-HPC ect. In the experiment, the PVPP CMS-Na and L-HPC were selected as the disintegrant to determine. The adjuvant pass through the 150μm sieve. According to the quantity of the formula, the MCC was selected as bulking agent and the silica powder was selected as lubricant while the quantity of the disintegrant was the same. According to the method of “tablets preparation”, the disaggregation time and the angle of repose of the powder were determined. The formula composition and the results of the determination were showed on table 4.

Adhesive agents screen

There were four kinds of adhesive agents including 40% (v/v) ethanol, 70% (v/v) ethanol, 90% (v/v) ethanol and 5% (m/m) PVPK30- ethanol. Meanwhile, the MCC was selected as a bulking agent and the PVPP was selected as a disintegrant. Also the lubricant was the silica powder. The tablet was made according to the method of “tablets preparation” and the tablet hardness was controlled between 22N to 25N. The disintegration time was also determined. The formula and its determined results were showed in table 5.

Orthogonal test

An orthogonal design was used to optimize the best dosage of MCC, PVPP and L-HPC in the tablet and adopted tablet disintegration time as evaluation. The tablet hardness was controlled between 20N ton 25N while the factor’s level design results were shown in table 6.

Chromatography conditions

The Agilent-C_{18} column (250×4.6 mm, 5μm) was used, the optimal mobile phase was methanol-water-phosphoric acid (47: 53: 0.2) with the flow rate of 1mL/min. The

detection wavelength was at 280nm under a column temperature of 30°, sample volume was 10μL. Recorded chromatograms are shown in fig. 1.

Sample preparation

5 Genhuang dispersible tablet were crushed, 0.2gram powder were accurately weighed and dissolved into 10mL volumetric flask, after adding 6 ml methanol, 100Hz power ultrasonic treatment for 30min. After cooling, diluted with methanol to 10mL, shake well and filter with 0.45μm micro porous membrane. Take the filtrate as the sample solution.

Reference solutions

8.624 milligram of baicalin standard were accurately weighed and dissolved in 50mL volumetric flash with methanol, then gently shaken to form homogenous baicalin solution of 101.8μg/mL.

Blank solution preparation

In view to prepare the blank solution, the prescription does not contain scutellaria was obtained following the same method as in the sample solution. 10μL of sample, reference solution and blank were determined by HPLC respectively and their chromatograms were recorded in fig. 1.

Linear relationship

A series of working reference solutions were prepared by diluting the baicalin reference solution with methanol at concentrations of 4.31, 5.39, 8.624, 10.78, 17.248, 21.56, 34.496 and 43.12μg/mL, respectively. Then, 10μL of each resulting standard solution was determined by the HPLC method. Thereafter, the mean calibration curve and the correlation coefficient were calculated.

Precision

Different operators evaluated the precision of the method by analyzing six samples of baicalin standard solution. The precision was expressed as the relative standard deviation (RSD %).

Repeatability

The repeatability of the assay method was evaluated by analyzing six replicates of baicalin sample solution by under the same conditions by one operator.

Determination

Ten batches of Genhuang dispersible tablet were tested, each batch containing three samples. Sample solutions were prepared as described above. 10μL of each sample was determined by HPLC method and the content of baicalin was determined referring to regression equation.

RESULTS

Single factor testing

When the particle size decreases, the homogeneity of granularity increases the tablet was also good and the disintegration was accelerated. If the 425μm sieve was used, the disintegration time was the shortest, but there would be too much powder after it was dried and the tablet weight would be unstable. So the final selection was the 600μm sieve.

The results of bulking agent screen showed that the different bulking agents had a significant effect on disintegration time. When MCC was selected as the bulking agents, the disintegrating property could be better than the others, it also had the minimum angle of repose and particle flow ability was the best. So MCC was used as the bulking agent.

The results of disintegrant screen showed that the disaggregation time was PVPP < L-HPC < CMS-Na. The appearance of the tablets made by L-HPC was better than the others. The tablets made by PVPP were looser than the others and there were cracks in the appearance of the tablets. We think that viscosity of the powder may not be sufficient. In order to select the suitable appearance and disaggregation time of the tablets, the PVPP and L-HPC were selected as the composite disintegrating.

The results of adhesive agents screen showed that viscosity would be increased and granules couldn't be sieved when the 40% (v/v) ethanol was used to make granules. The disintegration time would be longer when the tablets were made by 5% (w/v) PVP K_{30}-solution of ethanol. The powder would be more when the granules were made by 90% (v/v) ethanol. The disintegration time would be shortest when tablets were made by 70% (v/v) ethanol and the cost was the lowest. So 70% (v/v) ethanol was selected as adhesive agents.

Orthogonal testing

Orthogonal design was shown in table 7 and analysis of variance results were shown in table 8. The range analysis and variance analysis results showed that the factors affect the disintegration time were B>A>C, as the optimum conditions were $A_3B_3C_1$. And factor A and B had a significant difference on the disintegration time (P<0.05). The results determine the best process contained that MCC content in mass fraction of 40%, PVPP content of the mass fraction of 15%, L-HPC content of the mass fraction of 7%. The mixing of two disintegrating agents should be admixed silica powder as lubricate agent and the mass fraction of 70% ethanol solution as the adhesive agents, while 600μm sieve was used for granulation.

Chromatogram of baicalin

Baicalin reference and sample exhibited a characteristic peak appearing approximately at the same time. Furthermore, solvent and excipients had no chromatographic interference with baicalin (fig. 1).

Table 1: Ingredients and Actions of *Genghuang Tang*

English Name	Chinese Pin-Yin	Action	%
Isatis	*Ban Lan Gen*	Clear heat	18.18%
Scutellaria	*Huang Qin*	Clear heat	18.18%
Vietnamese Sophora Root	*Shan Dou Gen*	Clear heat	18.18%
Belamcanda	*She Gan*	Clear heat	18.18%
Platycodon	*Jie Geng*	Transform phlegm and relieve cough and asthma	18.18%
Glycyrrhiza	*Gan Cao*	Tonify deficiency	9.10%

Table 2: Screening test on particle size

d_{mesh}/μm	Appearance	t (disintegrating)/s
1180	rough	310
600	smooth	235
425	Smooth	218

Table 3: Screening test on bulking agents

Prescription		No.		
		1	2	3
Main material	%	30	30	30
Bulking agent	MCC %	32.5		
	Lactose %		32.5	
	$CaSO_4$ %			32.5
Disintegrant	PVPP %	36.5	36.5	36.5
Lubricant	CSD %	1	1	1
Moistening agent	70% ethanol	q.s	q.s	q.s
Disintegration time (s)		68	90	132
Angle of repose(θ)		29.5	35.5	30.0

Table 4: Screening test on addition of disintegrant

prescription		No.		
		1	2	3
Main material	%	30	30	30
Bulking agent	MCC %	40	40	40
Disintegrant	PVPP %	29		
	CMS-Na %		29	
	L-HPC %			29
lubricant	CSD %	1	1	1
Moistening agent	70% ethanol	q.s	q.s	q.s
Disintegration time (s)		88	185	122
Angle of repose(θ)		34.6	37.3	36.9

Table 5: Screening test on adhesive agents

Prescription		No.			
		1	2	3	4
Main material	%	30	30	30	30
Bulking agent	MCC%	40	40	40	40
Disintegrant	PVPP %	29	29	29	29
lubricant	CSD %	1	1	1	1
adhesive agents	40% ethanol	q.s			
	70% ethanol		q.s		
	90% ethanol			q.s	
	5%$PVPK_{30}$- ethanol				q.s
Disintegration time (s)		97	88	76	192

Table 6: Factors and levels

	Factor		
Level	*A*	*B*	*C*
	W (MCC)%	W (PVPP)%	W(L-HPC)%
1	20	9	7
2	30	12	10
3	40	15	13

Table 7: Result of experiments

Formulation	Factor			T(disintegrating)/s
	A	*B*	*C*	
1	1	1	1	169
2	1	2	2	155
3	1	3	3	101
4	2	1	3	120
5	2	2	1	111
6	2	3	2	84
7	3	1	2	123
8	3	2	3	98
9	3	3	1	67
I	425	412	347	
II	315	364	362	
III	288	252	319	
R	137	160	43	

Table 8: Result of variance analysis of disintegrating time

Variance	SS	*f*	*F*	*P*	Remark
A	3509.85	2	79.355	< 0.05	*
B	4493.61	2	101.596	< 0.05	*
C	317.87	2	7.1867		
Error	44.23	2			

Table 9: Baicalin content of three batches Genhuang dispersible tablets

No.	Content (mg/table)			average content (mg/tablet)	RSD/%	Average RSD/%
	1	2	3			
150401	4.0231	4.0313	4.0250	4.0264	0.1074	0.1732
150402	4.0598	4.0611	4.0643	4.0617	0.0569	
150601	4.0567	4.0410	4.0549	4.0508	0.2118	
150602	4.0057	4.0219	4.0151	4.0142	0.2027	
150701	3.9964	4.0126	4.0005	4.0032	0.2103	
150702	4.0118	4.0084	3.9983	4.0061	0.1754	
150703	4.0178	4.0208	4.0001	4.0129	0.2777	
150704	4.0286	4.0185	4.0361	4.0278	0.2196	
150801	3.9615	3.9593	3.9495	3.9568	0.1612	
150802	3.9514	3.9596	3.9533	3.9547	0.1094	

Linearity

The method displayed a good linearity within the ranges of 4.31-43.12μg/mL. The regression equation was A=29204*C*+4678.5 with a correlation coefficient R^2=0.9996 (n=8); where A represents the peak area and C represents the concentration.

Precision

Precision was investigated using prepared baicalin sample solution. The method exhibited a good precision with RSD of 0.14%.

Repeatability

RSD of the content determination was 0.19%. Therefore, the method reproducibility was satisfactory.

Average recovery

The recoveries of Nimesulide in low, medium, and high concentrations were 97.23% (RSD=0.10%), 99.03% (RSD=0.09%) and 98.76% (RSD=0.08%), respectively. The average recovery was 99.00% and the RSD was 0.09%.

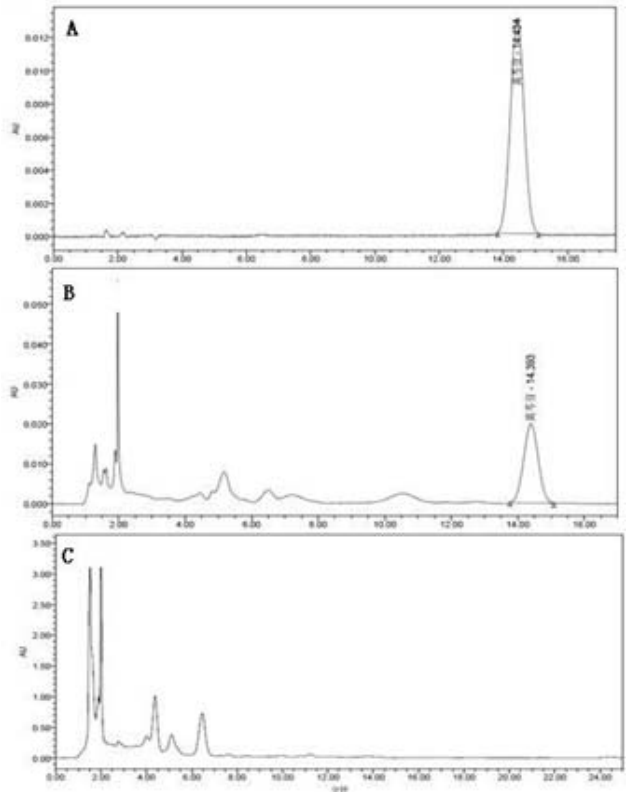

Fig. 1: Chromatogram of Genhuang dispersible tablets content.

Baicalin content

Baicalin content was determined by calculating the peak area with the external standard method. Furthermore, the main self-compare component without calibration factor was used to calculate baicalin related substances content and was found to be 0.1732% (table 8).

DISCUSSION

As the extract of Chinese traditional medicine was very viscous, drug-containing tablets and blanks had large differences in the selection of disintegrant (Wang *et al*., 2008). For example, the disintegrating property of PVPP in the blanks, this had general performance, but had excellent performance in the tablet when extract was added. Therefore, in the preparation of dispersible tablets of Chinese traditional medicine, the extract should be added into a prescription directly to check the performance of a disintegrating property.

The combined disintegrating agents had the feature of good compressibility and good effect of rapid disintegration (Dong *et al*., 2007). Then the MCC and the super-disintegrant PVPP or L-HPC could get satisfactory results. The addition of silica powder in a prescription significantly changed the mobility of the material. As the grain was light and fine; therefore, we should pay more attention to mixing.

In the China Pharmacopoeia 2015 edition, methanol-water is described as the appropriate mobile phase to determine baicalin content in *Scutellaria* crude drug or drug products containing *Scutellaria* (Committee for the Pharmacopoeia of P.R. China, 2010). A small quantity of phosphoric acid was added in order to solve the chromatographic peak tailing problem.

On the basis of single factor experiment and orthogonal test results, our research group developed and optimized Genhuang dispersible tablets. Moreover, we established an effective HPLC method for content determination that showed high resolution, accuracy, precision, high recovery and good reproducibility. This method was applied to all ten prepared batches of Genhuang dispersible tablets, wherein, the measured levels were within a range of 90.0%-110.0%. Hence, the method demonstrated to be suitable for content determination.

CONCLUSION

The developed formulation was appropriate for Genhuang dispersible tablets. Moreover, the HPLC established method was simple, sensitive and accurate, so it could be used in industrial production and quality control work.

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