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Determination of Critical Influencing Factor on pH Stability of Yuxingcao Injection

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Abstract

The change of drug quality of Yuxingcao injection after formulation is considered as one of the causes for adverse reactions. In this study, orthogonal experimental design was used to investigate the influencing factors on the pH stability of Yuxingcao injection. Two methods of accelerated tests were carried out in the experiments. The results showed that using Tween 80 as the solubilizer could significantly decrease the pH value of Yuxingcao injection after accelerated tests. There was no significant difference in the pH value after the accelerated tests when the other 6 factors varied, including the type of liquid processed, the dosage of activated carbon, heating temperature, reagents for pH adjustment, pH value range after adjustment, and sterilization conditions. In order to improve the quality stability of Yuxingcao injection, the quality of Tween 80 should be strictly controlled to minimize the change of pH value of Yuxingcao injection.

Keywords

Houttuyniae Herba, Chinese Medicine Injection, Quality Stability, Tween 80

1. Introduction

The Chinese medicine herb Yuxingcao is a fresh whole plant or dry above-ground part of *Houttuynia cordata* Thunb. It possesses the effects of clearing heat, detoxification, eliminating carbuncle and pus, and diuresis. Pharmacological researches have shown that Yuxingcao possesses antibacterial, antiviral, anti-inflammatory, immune enhancement, anti-allergic, anti-tumor effects [1] [2] [3] [4]. Yuxingcao

injection is used for viral pneumonia, respiratory tract infection, viral enteritis, and acute urticaria of children. Quick onset and definite curative effects are the advantages of Yuxingcao injection [2].

Due to many adverse reactions, seven different injections made from Yuxingcao were temporarily prohibited of use by the China Food and Drug Administration in 2006. There are many reasons for the adverse reactions caused by Yuxingcao injection [5] [6] [7] [8], including fluctuations in the quality of injections, poor quality of excipients, unreasonable drug compatibility, excessive dosage, excessively rapid injection speed, and individual patient factors. Yuan *et al.* investigated the influences of drug compatibility of Yuxingcao injection and other injections [9]. However, less work is focusing on the pH stability of Yuxingcao injection.

This study investigates the factors that may affect the pH stability of Yuxingcao injection after formulation process. The results of this study will be beneficial to improve the quality control of Yuxingcao injection.

2. Materials and Methods

2.1. Materials and Chemicals

Hydroxypropyl-beta-cyclodextrin was purchased from Xi'an Deli Biochemical Co., Ltd. Hydrochloric acid and sodium hydroxide were purchased from Hengyang Kaixin Chemical Reagent Co., Ltd. Citric acid and sodium citrate were obtained from Hunan Huari Pharmaceutical Co., Ltd. Tween 80 was purchased from Nanjing Well Chemical Co., Ltd. Redistilled Yuxingcao aromatic water and purified water were obtained from Hunan Zhenqing Pharmaceutical Group Co., Ltd.

2.2. Experimental Design

A total of 7 factors were investigated using the pH value after an accelerated test as the evaluation index, including the type of liquid processed, solubilizer type, the dosage of activated carbon, heating temperature, reagents for pH adjustment, pH value range after adjustment, and sterilization conditions. There are 3 levels for each factor in the Taguchi designed experiments, as seen in **Table 1**. The orthogonal table $L_{18}(3^7)$ was selected to arrange the experiments, as seen in **Table 2** and **Table 4**. No repetition experiment was performed. Level 1 of the factor E was also used in Level 3 because there are only two different levels for factor E. The experiments were carried out according to the formulation method of Yuxingcao injection. The samples were prepared according to the combination of factors, potted into 10 ml ampoules, and sterilized.

After sterilization, the samples was divided into two parts and treated with two different accelerated test methods. For Method A, ampoules was placed at 100˚C for 6 hours, cooled to room temperature, refrigerated at 0˚C - 4˚C for 12 hours, placed at 100˚C for 6 hours, and cooled to room temperature. After that, pH value of the samples was measured, and compared with that measured before

Table 1. Experimental factors and their levels.

Factor No.	Factor name	Level		
		1	2	3
Factor A	The type of liquid processed	Purified water	Redistilled aromatic water	Redistilled aromatic water after refrigeration and filtration
Factor B	The type of solubilizer	Tween 80 (0.25%)	Hydroxypropyl β-cyclodextrin (0.4%)	No solubilizer added
Factor C	The dosage of activated carbon	0.05%	no activated carbon added	0.10%
Factor D	Heating temperature	80˚C, 5 min	100˚C, 5 min	No heating process
Factor E	Reagents for pH adjustment	Hydrochloric acid/sodium hydroxide	Citric acid/sodium citrate	Hydrochloric acid/sodium hydroxide
Factor F	pH value range after adjustment	4.5 - 4.8	5.5 - 5.8	7.5 - 7.8
Factor G	Sterilization conditions	100˚C, 30 min	115˚C, 30 min	121˚C, 13 min

Table 2. Results of the change of pH value after accelerated tests with method A.

Test No.	Factors							Results of pH value		
	A	B	C	D	E	F	G	Before sterilization	After the accelerated tests	ΔpH value
1	1	1	1	1	1	1	1	4.85	3.67	−1.18
2	1	2	2	2	2	2	2	5.60	6.44	0.84
3	1	3	3	3	3	3	3	6.31	7.21	0.90
4	2	1	1	2	2	3	3	7.28	7.38	0.10
5	2	2	2	3	3	1	1	5.35	5.79	0.44
6	2	3	3	1	1	2	2	5.91	6.59	0.68
7	3	1	2	1	3	2	3	5.95	4.20	−1.75
8	3	2	3	2	1	3	1	6.39	6.79	0.40
9	3	3	1	3	2	1	2	4.73	4.93	0.20
10	1	1	3	3	2	2	1	5.47	4.20	−1.27
11	1	2	1	1	3	3	2	6.38	7.04	0.66
12	1	3	2	2	1	1	3	5.17	6.28	1.11
13	2	1	2	3	1	3	2	6.86	5.57	−1.29
14	2	2	3	1	2	1	3	4.83	5.34	0.51
15	2	3	1	2	3	2	1	5.79	6.60	0.81
16	3	1	3	2	3	1	2	4.97	3.85	−1.12
17	3	2	1	3	1	2	3	5.96	6.30	0.34
18	3	3	2	1	2	3	1	7.14	7.97	0.83
$\overline{K}_1$	0.177	−1.085	0.155	−0.042	0.010	−0.007	0.005			
$\overline{K}_2$	0.208	0.532	0.030	0.357	0.202	−0.058	−0.005			
$\overline{K}_3$	−0.183	0.755	0.017	−0.113	−0.010	0.267	0.202			
R	0.391	1.840	0.138	0.470	0.212	0.325	0.207			

accelerated tests. For Method B, ampoules were stored at 60˚C for 10 days. After that, pH value of the sample was measured on the 10th day, and calculated the pH value change amount on that of Day 0. All the experiments were carried out during 2009-2010 in the lab of Hunan Zhenqing Pharmaceutical Group Co., Ltd., Huaihua, Hunan province of China.

2.3. Preparation of Yuxingcao Injection

A volume of 500 ml of Yuxingcao redistilled aromatic water was used in the experiments of Table 2 or Table 3 for experiments No. 4 - 9 and No. 13 - 18. According to experimental conditions, some aromatic water was filtered after refrigerating at 0˚C - 4˚C. The solubilizer was then added to the filtrate. If the aromatic water need not to be filtered, the solubilizer was added to it directly. Then the aromatic water was treated with activated carbon or heating. The pH

Table 3. Results of the change of pH value after accelerated tests with method B.

Test No.	Factors							Results of pH value		
	A	B	C	D	E	F	G	Before sterilization	After the accelerated tests	ΔpH value
1	1	1	1	1	1	1	1	4.85	3.82	−1.03
2	1	2	2	2	2	2	2	5.60	6.24	0.64
3	1	3	3	3	3	3	3	6.31	6.78	0.47
4	2	1	1	2	2	3	3	7.28	7.48	0.2
5	2	2	2	3	3	1	1	5.35	5.83	0.48
6	2	3	3	1	1	2	2	5.91	6.37	0.46
7	3	1	2	1	3	2	3	5.95	4.26	−1.69
8	3	2	3	2	1	3	1	6.39	6.63	0.24
9	3	3	1	3	2	1	2	4.73	4.96	0.23
10	1	1	3	3	2	2	1	5.47	4.63	−0.84
11	1	2	1	1	3	3	2	6.38	6.76	0.38
12	1	3	2	2	1	1	3	5.17	6.02	0.85
13	2	1	2	3	1	3	2	6.86	5.49	−1.37
14	2	2	3	1	2	1	3	4.83	5.37	0.54
15	2	3	1	2	3	2	1	5.79	6.13	0.34
16	3	1	3	2	3	1	2	4.97	4.03	−0.94
17	3	2	1	3	1	2	3	5.96	6.21	0.25
18	3	3	2	1	2	3	1	7.14	7.78	0.64
$\overline{K}_1$	0.078	−0.945	0.062	−0.117	−0.100	0.022	−0.028			
$\overline{K}_2$	0.108	0.422	−0.075	0.222	0.235	−0.140	−0.100			
$\overline{K}_3$	−0.212	0.498	−0.012	−0.130	−0.160	0.093	0.103			
R	0.320	1.443	0.137	0.352	0.395	0.233	0.203			

value of the aromatic water was adjusted. After that, the aromatic water was filtered, potted, and sterilized. Purified water was used for Experiments 1 - 3 in Table 2, and 10 - 12 in Table 3. Purified water was added solubilizer, and then it was treated with activated carbon or heating. The pH value of the purified water was adjusted. After that, the purified water was filtered, potted, and sterilized.

3. Results and Discussions

3.1. Results and Analysis of Accelerated Tests with Method A

In the accelerated tests with method A, the temperature was changed repeatedly. After that, the change of pH value was measured. The results are shown in Table 2. It can be seen from the table that the pH value of most samples increased after the accelerated tests. The increase can exceed 1.1. But the pH value of a few samples decreased significantly.

The comprehensive average ($\bar{K}$) and range of change of pH value (R) are shown in Table 2. The most extreme difference of range was caused by factor B, which meant that the choice of solubilizer had the greatest impact on the change of pH value after the accelerated tests. The other ranges were all less than 0.5. The results of the analysis of variance are shown in Table 4. Only factor B has a statistically significant influence, and the p value was less than 0.01. Considering the change of the comprehensive average, it can be seen that using Tween 80 as the solubilizer significantly reduced the pH value after the accelerated tests.

3.2. Results and Analysis of Accelerated Tests with Method B

In the accelerated tests with method B, the samples were placed at 60˚C for a long time. After that, the change of pH value was measured. The results are shown in Table 3. The pH value of most samples increased after the accelerated tests. The increase was less than 0.9. The pH value of a few samples dropped significantly.

Table 4. ANOVA results of accelerated tests with method A*.

Factor	Sum of squared deviations	Degrees of freedom	Mean square	F value	p value	Statistical significance
A	0.568	2	0.284	1.788	>0.1	No
B	12.098	2	6.049	38.075	<0.01	Yes
C	0.070	2	0.035	0.220	>0.1	No
D	0.769	2	0.385	2.421	>0.1	No
E	0.164	2	0.082	0.516	>0.1	No
F	0.366	2	0.183	1.152	>0.1	No
G	0.163	2	0.081	0.513	>0.1	No
Error	0.477	3	0.159			

*$F_{0.01(2.3)} = 30.82$, $F_{0.05(2.3)} = 9.55$, $F_{0.1(2.3)} = 5.46$.

The comprehensive average and range of change of pH value are shown in Table 3. The most extreme difference of range was still caused by factor B. The other ranges were all less than 0.4. The results of the analysis of variance are shown in Table 5. Only factor B has a statistically significant influence, and the P value was less than 0.05. Considering the change of the comprehensive average, it can be concluded that using Tween 80 as the solubilizer significantly reduced the pH value after the accelerated tests.

4. Discussion

Xu compared Tween 80 from different manufacturers, and found that the pH value of the aqueous solution of Tween 80 decreased after heating [10]. It was speculated that it was caused by the hydrolysis of the ester bond of Tween 80 [10]. Tween 80 can cause allergic reactions has been confirmed by different researchers [11] [12]. Yi *et al.* pointed out that the main manufacturers of Yuxingcao Injection had reduced the concentration of Tween 80, and used injection grade Tween 80 [6]. These acts reduced the occurrence of allergic reactions [6]. Honemann *et al.* explained the relationship between hydrolysis of Tween 80 and allergic reactions [13]. Long chain free fatty acids will form in the hydrolysis of Tween 80 [13]. The low solubility of long chain free fatty acids in aqueous solution results in the formation of visible and subvisible particulates, which are considered as a cause for allergic reactions [13]. This study confirms that Tween 80 also has a greater impact on the pH value of the injection. This phenomenon is probably caused by the formation of free fatty acids. Therefore, the quality control of Tween 80 should be more stringent.

Although this study found that Tween 80 has a greater impact on the pH value of the formulation, it is not enough to focus on changes of the pH value in terms of formulation quality control. Zeng and Li carried out a long-term stability experiment of Yuxingcao injection and found that the content of houttuynin and mehtyl-nnonylketone changed little [14]. The types of main volatile components

Table 5. ANOVA results of accelerated tests with method B*.

Factor	Sum of squared deviations	Degrees of freedom	Mean square	F value	p value	Statistical significance
A	0.375	2	0.187	0.818	>0.1	No
B	7.914	2	3.957	17.279	<0.05	Yes
C	0.056	2	0.028	0.123	>0.1	No
D	0.477	2	0.238	1.041	>0.1	No
E	0.544	2	0.272	1.187	>0.1	No
F	0.171	2	0.086	0.374	>0.1	No
G	0.128	2	0.064	0.279	>0.1	No
Error	0.687	3	0.229			

*$F_{0.01(2.3)} = 30.82$, $F_{0.05(2.3)} = 9.55$, $F_{0.1(2.3)} = 5.46$.

and their relative contents neither changed significantly [14]. Hao *et al.* suggested that the quality control of Yuxingcao injection should pay more attention to the quality control of Houttuynia herbal material, including avoiding the use of miscible products, performing standardized planting, selecting good varieties, improving the quality standards of Houttuynia, and strengthening heavy metal detection [15].

5. Conclusion

This study investigated the influence of many formulation conditions, including the type of liquid processed, solubilizer type, the dosage of activated carbon, heating temperature, reagents for pH adjustment, pH value range after adjustment, and sterilization conditions, on the decrease of pH value of Yuxingcao injection. It is found that when Tween 80 was used as the solubilizer, the pH value significantly decreased after accelerated tests. The influence of other factors on pH value is not significant. Considering that Tween 80 is also one of the causes of adverse reactions, high-quality Tween 80 should be used for the production of Yuxingcao injection.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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