Optimization of Formulation Technology for Gastric Floating Tablets of total alkaloids from *Chelidonium majus L.*

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Abstract. In this paper, the formulation technology of gastric floating tablets of total alkaloids from *Chelidonium majus*(TAC).was optimized according to its behavior of floating and the release characteristics. Gastric floating tablets were prepared by direct compression with dry powder. The floating time and release performance were used as indexes to evaluate and optimize the formulation. Single factor tests and orthogonal tests were carried out to optimize formulation parameters which impacted properties of TAC gastric floating tablets. The optimal prescription processes were 20% of total alkaloid extracts from *Chelidonium majus L.*, 15 % $HPMCK_{15M}$, 15 % $NaHCO_3$ and 50 % microcrystalline cellulose PH-101 (MCC). The average release of the drug within 12h was 91.23%, the floating time was 3s, and the duration time was over 12h. In conclusion, the TAC gastric floating tablets prepared by the optimized orthogonal test showed a good floating performance within 12 h, and presented a good sustained release property which could extend the retention time of TAC gastric floating tablets in the stomach and enhance its therapeutic effects.

1. Introduction

Chelidonium majus belongs to the Poppy family of plants, which was first recorded in "Herbal for Relief of Famines" [1] . It has a widerange of application due to the rich resources and low costs. *Chelidonium majus* possess the efficacy of relieving spasm and pain, relieving cough and asthma. Modern pharmacological studies have shown that chelidonine, chelerythrine and sanguinarine in *Chelidonium majus* all have anti-tumor activity, which could significantly inhibit gastric cancer, liver cancer, nasopharyngeal cancer, leukemia and so on[2-5]. At present, the main dosage form of *Chelidonium majus* for clinical application is the decoction, and suitable dosage forms of active total alkaloid ingredients have not been developed. As gastric retention agents, stomach floating tablets can extend the residence time of drugs in the gastrointestinal tract[6]. Therefore, the total alkaloids of *Chelidonium majus* (TAC) are prepared into gastric floating tablets to improve the anti gastric cancer effect, which has been clinically proved. The prescription of tablets is determined and the efficacy of *Chelidonium majus* is further improved in treatment of human gastric cancer.

2. Instruments and reagents

BSA 1/10000 electronic balance (Beijing sartorius Scientific Instrument Co.); Agilent 1100 high performance liquid chromatography (Agilent Technologies Inc.); Intelligent dissolution tester (Tianjin

University Radio Factory).Chelerythrine (Shenzhen Mei Ho Biotechnology Co., lot number: 20160708, purity: 98%); TAC(preparated by our team). HPMC $_{E4M}$ (Tianjin Mitsuko Fine Chemical Research Institute), $HPMC_{F4M}$ (Tianjin Fuyu Fine Chemical Co., Ltd.), $HPMC_{K15M}$, MCC, Monoglyceride, HEC, PVP, Stearyl alcohol, Sodium carbonate, Calcium carbonate, Sodium bicarbonate(Tianjin Tianxin Fine Chemical Development Center).

3. Methods and results

3.1. Single factor tests

3.1.1. Screening of hydrophilic gel matrix materials. $HPMC_{E4M}$, $HPMC_{F4M}$ and $HPMC_{K15M}$ were selected as hydrophilic gel skeleton. TAC was dissolved in absolute ethanol, and then well dispersed in MCC which was further evaporated to dry. MCC were mixed with the three kinds of hydrophilic gel matrix respectively, which were then evenly mixed with $NaHCO_3$, PVPP and octadecanol. The mixture was sieved 3 times with a 60-mesh sieve and mixed uniformly for tablet press. The hardness of the tablet was controlled in 6~7Kg. The floating performance and duration time of the tablets were observed and the results were shown in Table 1. $HPMCK_{15M}$ showed better floating performance.

3.1.2. Screening of low density excipients. Monoglycerides, HEC and MCC were selected as low-density excipients. TAC were dissolved in absolute ethanol, which were then evenly dispersed into monoglycerides, HEC and MCC, respectively and were evaporated to dry. Then the 3 low-density excipient samples were evenly mixed with $HPMC_{K15M}$, $NaHCO_3$, PVPP, stearyl alcohol, which was sieved 3 times with 60-mesh siece for tabletting. The tablet hardness was controlled in 6-7 Kg. The floating performance and duration time of the tablets were observed and the results were shown in Table 1. The tablets prepared with MCC showed better floating performance.The tablets prepared with MCC showed better floating performance. The character of MCC was similar to HPMC, which did not dissolve in alcohols. As TAC were easily soluble in water and alcohols, MCC would not interfere the following content determination.

3.1.3. Screening of foaming agents. Na_2CO_3, $CaCO_3$ and $NaHCO_3$ were selected as foaming agent alternative materials. TAC were dissolved in absolute ethanol, and then well dispersed in MCC which was further evaporated to dry. Then the mixture of MCC and $HPMCK_{15M}$ were further mixed with Na_2CO_3, $CaCO_3$ or $NaHCO_3$ respectively. The mixtures were then uniformly mixed with PVPP, octadecanol and other accessories, which were sieved 3 times with 60-mesh sieve for tabletting. The tablets hardness was controlled in 6-7Kg. The floating performance and duration time of the tablets were observed and the results were shown in Table 1. Na_2CO_3, as a foaming agent, showed better floating performance. Therefore, $NaHCO_3$ was chosen as a foaming agent for the preparation of floating tablets.

Table 1. The results of theadjuvantmaterials screening

	Skeleton materials			Low-density accessories			Foaming agents		
	HPMC E4M	HPMC F4M	HPMC K15M	Monoglyceride	HEC	MCC	Na_2CO_3	$CaCO_3$	$NaHCO_3$
Float time (S)	24	18	13	8	15	4	2	78	3
Duration (h)	5	7	>12	2	10	>12	8	3	>12

3.2. Investigate with Orthogonal test

The factors and levels were selected according to the above single factor tests, shown in Table 2. According to the release requirements of sustained-release preparations and the comprehensive score of floating tablets in the Chinese Pharmacopoeia (2015 Edition, Part IV), the TAC cumulative release contents and tablet floating time of 2 time points were investigated emphatically. The release degree of

chelerythrine in the floating tablets was selected as an index, and the standards were as follows: releasing 30% of chelerythrine in 2h, releasing 90% of chelerythrine in 12h, floating time of 3s. The parameters were taken as the index of investigation and calculated as follows：C = | A1-30% | * 100 + | A2-90% | * 100 + | B-3 |，in which A1 and A2 stand for the cumulative release percentages of the prepared gastric floating tablets in 2 and 12 hours respectively, B stands of the floating time of the tablets, C value is the sum of absolute value of deviation of A1, A2 and B to the standard values. The smaller the C value, the better the sustained-release efficacy and floating properties of TAC. The orthogonal test results were shown in Table 3 and the ANOVA analysis results were presented in Table 4. According to the grading standards, the order of influence of each factor was C> A> B, and the optimal combination is $A_2B_2C_3$. $HPMC_{K15M}$ and $NaHCO_3$ mass ratio are all 15%. And MCC mass ratio is 50%. The results also showed that the hydrophilic gel matrix and the low-density excipients were the significant effects on the floating performance of the TAC floating tablets(P<0.05), and the low-density excipients has a greater influence than the hydrophilic gel matrix. While the foaming agent had no significant effect on the floating characteristics of TAC floating tablets(P>0.05).

Table 2. The factor level able of screening accessories dosage

Level	Factor		
	$HPMC_{K15M}$（A）%	$NaHCO_3$（B）%	MCC（C）%
1	10	10	30
2	15	15	40
3	20	20	50

Table 3. Orthogonal test results

Group	A	B	C	D（error）	Grading results		
					Float time (s)	Release performance (2h / 12h) (%)	Totalcore
1	1	1	1	1	5	39.62/95.69	17.31
2	1	2	2	2	2	27.53/85.29	8.18
3	1	3	3	3	1	27.87/91.43	5.56
4	2	1	2	3	4	25.30/87.79	6.91
5	2	2	3	1	3	30.27/92.16	2.43
6	2	3	1	2	4	35.98/95.91	12.89
7	3	1	3	2	3	25.68/90.43	4.75
8	3	2	1	3	1	23.47/95.43	13.96
9	3	3	2	1	4	24.28/91.68	7.40
K1	10.350	9.657	14.720				
K2	7.410	8.190	7.497				
K3	8.703	8.617	4.247				
R	2.940	1.467	10.473				

Table 4. Analysis of variance results

Sources of variance	Square deviation of the sum	Degree of freedom	Mean square	F value	P value
A	13.028	2	6.514	44.776	0.022
B	3.415	2	1.707	11.736	0.079
C	172.430	2	86.215	592.633	0.002

3.3. Confirmatory experiments

Three batches of TAC floating tablets were prepared in accordance with the optimal formula obtained above. The floating time and release performance were observed. The results showed that these three batches of the tablets (Lot No. 2016111901, 2016111902 and 201616111903) were relatively better than the results of any other process conditions in the orthogonal test. The mean floating time was 3 seconds, the average duration was more than 12 hours and the mean total score was 2.86.

3.4. Determination of total alkaloid content

3.4.1. Chromatographic condition. Diamonsil C_{18}（5μm,4.6×200mm）was selected as chromatographic column, solution of acetonitrile-1% trimethylamine(pH was regulated to 3.0 with phosphoric acid)(26:74) was used as mobile phase. The flowing velocity was 1mL/min and the detection wavelength was 290nm. The numbers of theoretical plates were more than 2000, which was calculated by chelerythrine.The chromatographic peak of 3 reference substances showed good separation degree.

3.4.2. Preparation of the test solution. 20 tablets were ground into fine powder, placed in 50mL conical flask. 15mL methanol was added into the powder, which was then treated with ultrasound for 20min, filtered and recycled upper liquid with rotary evaporates. In the end, the residue was dissolved with 1mL methanol and filtered with 0.45μm microporous membrane to prepare the test solution.

3.4.3. Preparation of the control product solution. Appropriate amounts of chelidonine, chelerythrine and coptisine were weighed precisely which were then dissolved and set to the constant volume with methanol to prepare the mixed reference substance solution. The the concentration of chelidonine, chelerythrine and coptisine were 0.1, 0.05, 0.1mg/mL respectively.

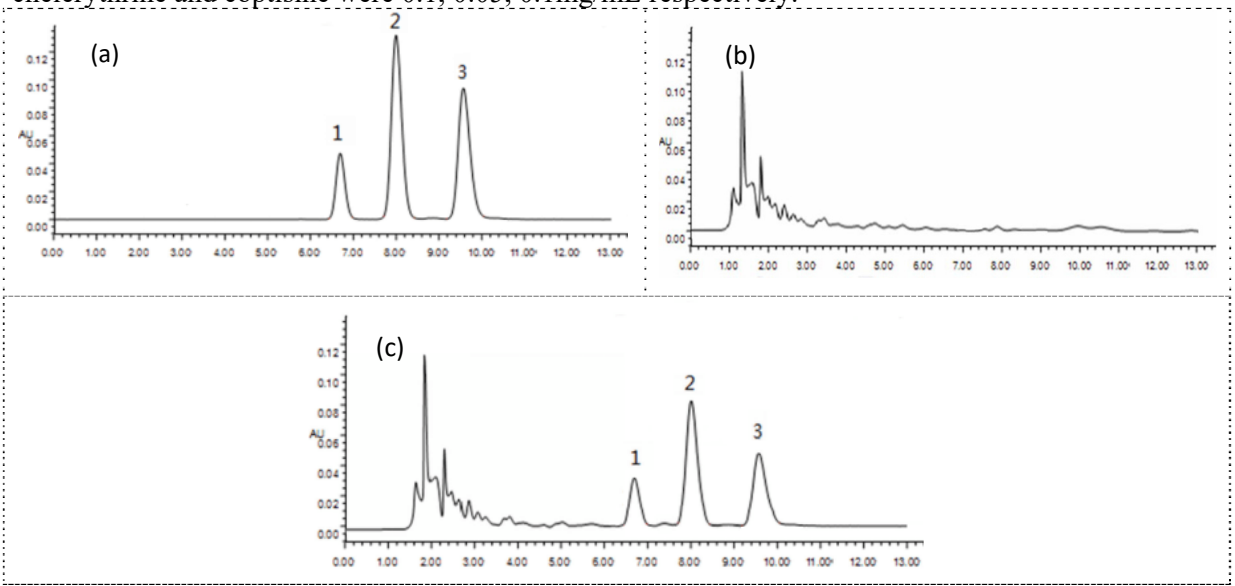

Figure 1.comparison product (a), negative liquid (b) and sample (c) HPLC chromatogram
(1. Chelerythrine 2. Chelerythrine 3. Coptisine)

3.4.4. Preparation of the negative sample solution. The gastric floating tablets without TAC were prepared in accordance with the prescription and preparation of TAC gastric flotation tablets.

3.4.5. Investigation of Linear relationship. 0.25, 0.5, 1.0, 2.0 and 4.0mL of mixed reference solution were accurately measured and transfer into 5mL volumetric flask constant-volumed by methanol. Respectively, 10μL of mixed reference substance solutions of different concentration were accurately

measured by HPLC. Chromatographic peak area integral value was served as the ordinate (Y) and the reference substance mass concentration was served as abscissa (X). Linear regression analysis was performed and a linear regression equation was established. The regression equation of chelidonine was Y = 34087X + 4480 (r=0.9999) with a linear range of 4-60μg/mL. The regression equation for chelerythrine was Y = 27375X-1271 (r=0.9999) with a linear range of 1.5-24 μg/mL. Coptisine regression equation was Y = 22247X-483799 (r = 0.9999), the linear range of 5 -80μg/mL.

3.4.6. Methodological investigation

Mixed reference solution was determined for 6 timesaccording to the chromatographic conditions of "3.4.1"part. The RSD of the peak area of chelidonine, chelerythrine and coptisine were 0.39%, 0.56% and 0.47%, indicating a good precision of the instrument.The test solutions were accurately detected at 0, 2, 4, 6, 8 and 10 h. The RSD of the peak area of chelidonine, chelerythrine and coptisine were 0.33%, 0.32% and 0.60% respectively which showed a good stability of the test solution within 10 hours. 6 solutions of TAC Gastric floating tablets were prepared in parallel following the "3.4.2"method. The RSD of the content of chelidonine, chelerythrine and coptisine were0.45%, 0.95% and 0.56%,showing a good repeatability of the experiment. 6 portions of powder of TAC Gastric floating tablets(the content of chelidonine, chelerythrine and coptisine were 1.13mg/g, 0.58mg/g and 3.2mg/g) were weighed about 100mg for each portion. The reference substance of chelidonine, chelerythrine and coptisine were added into the powder in the weigh ratio of 1:1. The samples solutions were prepared and the peaks area were measured. The recovery rates of chelidonine, chelerythrine and coptisine were calculated as 98.58%, 99.33% and 99.50%. The RSD were 0.31%, 0.29% and 0.24%, respectively. The above results certified that the method was reliable and accurate.

3.5 Determination of release degree of TAC gastric flotation tablets

According to the third method of dissolution rate and releasing detection assay recorded in the forth part of "Chinese Pharmacopoeia"(2015 edition)[7], 6 TAC gastric floating tablets were accurately weighed and respectively placed in 900 mL of artificial gastric juice, which were then incubated at the speed of 100r/min and temperature of 37 ℃. 1mL sample solutions were precisely measured at 1, 2, 4, 6, 8, 10, 12, 24h time points, respectively. Meanwhile, the artificial gastric juice of the same volume, temperature and pH were complemented. Then samples were filtered with 0.45μm microporous membrane. The initial filtrate was discarded, after which 10 μL subsequent filtrate were used for the HPLC analysis. The TCA contents were measured (Based on the sum content of chelidonine, chelerythrine and coptisine) and the dissolution rates were calculated.

3.6 Determination of floatation performance of gastric flotation tablets

6TAC gastric floating tablets were put in the spin basket and placed in 900mL of artificial gastric juice under the simulating gastric motility condition (at the rotate speed of75r/min and temperature of 37℃). The floating time and holding time of the tablets were recorded.

3.7 The quality evaluation of TAC floating tablets

3.7.1 Floating performance measurement. The results of the floating performance detection in vitro showed that the TAC gastric-floating tablets floated within 3 seconds, and the duration time was more than 12h, indicating a good floating performance of the tablets.

3.7.2. Detection of dissolution rates. According to the operation sequence in the "3.2" part, the dissolution rates of TAC gastric floating tablets were detected and the results were shown in Table 5.

Table 5. The results of dissolution rates Detection of TAC gastric floating tablets

Time (h)	2	4	6	8	10	12	24
Dissolution	29.76%	49.32%	60.74%	71.89%	82.07%	91.23%	99.83%

3.7.3. Weight difference detection of TAC gastric floating tablets. The total weights of the 3 batches of TAC gastric floating tablets (20 tablets for each batch) were accurately weighed for the calculation of the average tablet weight. Then, the weight of single tablet in each batch was weighed accurately, which was compared with the calculated average tablet weight of each batch.

The weight difference of the 3 batches of tablets meet the requirement of weight difference limit of ± 7.5% recorded in "Chinese Pharmacopoeia"(2015 edition). As shown in Table 6.

Table 6. Results of the weight difference of TAC gastric floating tablets

Batch	Average weight (g)	Detection limit	Minimum	Maximum	Result
20161017	0.29959	±7.5%	0.2970	0.3014	qualified
20161021	0.29817	±7.5%	0.2963	0.3003	qualified
20161026	0.29997	±7.5%	0.2981	0.3007	qualified

4. Discussion

Gastric cancer is one of the most common malignancies worldwide, with approximately 95.1 million new cases and 72.3 million deaths in 2012, which ranks fifth in cancer incidence and third in mortality rate[8]. The number of cases and deaths of gastric cancer in China account for 42.6% and 45.0% of the world's. The development of efficient and low toxicity anti-cancer drugs is extremely urgent. Gastric floating tablet (GFT) is a slow-release agent, made of high polymer material. As it has the advantages of slow releasing of drugs, prolonging treatment time, reducing the number of taking times and directly applying to the lesion site, GFT can play a targeted role in the stomach. In this paper, TAC stomach floating tablet was made into gastric targeting agent, which could inhibit the proliferation of gastric cancer cells more effectively and enhance the clinical treatment effect of TAC.

The single factor method was used to screen the hydrophilic gel scaffold, the low density auxiliary and the foaming agent for preparing TAC gastric floating tablets with the indexes of the appearance and floating performance of the tablets. The dosage of these 3 main factors was optimized by orthogonal test. Finally, $HPMC_{K15M}$ was confirmed to be the hydrophilic gel scaffolds, MCC was selected as low-density excipients, and sodium bicarbonate acted as foaming agent. The optimal technological condition was $A_2B_2C_3$, namely, $HPMC_{K15M}$ mass ratio of 15%, $NaHCO_3$ mass ratio of 15%, the MCC mass ratio of 50%. The average release of TAC gastric floating tablets within 2h and 12h were 28.76% and 91.23%.The floating time was within 3s. The duration time of the tablets was more than 12h. The TAC gastric floating tablets showed favorable controlled-release characteristics.

Reference

[1] Nanjing University of Chinese Medicine2006*Traditional Chinese medicine Dictionary(2nd edition)*(Shanghai: Shanghai Science and Technology Press) P1000.
[2] Qu Z Y, Zou X, Zhang X J, Sheng J J, Wang Y M, Wang J Q, Wang C and Ji Y B 2016*Mol. Med. Rep*.**13** 1336-44
[3] Zou X, Wang YM, Wang JQ, Long LL, Wang C, Liu Y, Sheng J J, and Qu ZY 2014*Drugs Clinic.***29** 1326-30.
[4] Paul A, Bishayee K, Ghosh S, Mukherjee A, Sikdar S, Chakraborty D, Boujedaini N and Khuda-Bukhsh AR.*Zhong Xi Yi Jie He Xue Bao.***10**1025-38
[5] Kemény-Beke A, Aradi J, Damjanovich J, Beck Z, Facskó A, Berta A and Bodnár A2006*Cancer Lett*. **237** 67-75
[6] Xu X H, Zhang TJ, Song XB, Gong S X, and Liu K Y 2007 Chin. Tradit. Herb. Drug. 38 779-82.
[7] Chinese Pharmacopoeia Commission 2015 *Pharmacopoeia of the People's Republic of China(part four)*(Beijing: China Medical Science Press)P 121
[8] Zuo TT, Zheng RS, Ceng M, Zhang S W and Chen W Q 2017 *Chin. J.Clin.Oncol*.**44** 52-8.