Optimization of high pressure machine decocting process for Dachengqi Tang using HPLC fingerprints combined with the Box–Behnken experimental design

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Abstract Using Dachengqi Tang (DCQT) as a model, high performance liquid chromatography (HPLC) fingerprints were applied to optimize machine extracting process with the Box–Behnken experimental design. HPLC fingerprints were carried out to investigate the chemical ingredients of DCQT; synthetic weighing method based on analytic hierarchy process (AHP) and criteria importance through intercriteria correlation (CRITIC) was performed to calculate synthetic scores of fingerprints; using the mark ingredients contents and synthetic scores as indicators, the Box–Behnken design was carried out to optimize the process parameters of machine decocting process under high pressure for DCQT. Results of optimal process showed that the herb materials were soaked for 45 min and extracted with 9 folds volume of water in the decocting machine under the temperature of 140 °C till the pressure arrived at 0.25 MPa; then hot decoction was excreted to soak Dahuang and Mangxiao for 5 min. Finally, obtained solutions were mixed, filtrated and packed. It concluded that HPLC fingerprints combined with the Box–Behnken experimental design could be used to optimize extracting process of traditional Chinese medicine (TCM).
1. Introduction

For these years, machine decocting process has become an acceptable option in Asia, especially China and Korea. The process is particularly designed for an individual. In detail, a patient's herbs prescribed by doctors according to the TCM theory are added into water; the active components in materials are extracted under the condition of high temperature and pressure in a closed stainless steel cooking pot; the obtained liquids are quantitatively packed by an automatic pack machine in a vacuum which is sterile and convenient to be stored and orally administered. So it is generally adopted in clinic.

Dachengqi Tang (DCQT) is a well-known purgative formula consisting of Radix et rhizoma rhei (Dahuang), Cortex magnoliae officinalis (Houpu), Fructus aurantii immaturus (Zhishi) and Natrii sulfas (Mangxiao). It has been widely used in China to treat diseases such as acute intestinal obstruction without complications, acute cholecystitis and appendicitis for thousands years [1,2]. Plenty of research papers have been published to discuss this formula from the perspectives of pharmaceutics, pharmacology, and chemical ingredients [3–6]. However, few papers have focused on the decocting method, especially machine decocting process.

Therefore, in this paper, using DCQT as a model, high pressure machine decocting process was optimized with HPLC fingerprints combined with the Box–Behnken experimental design.

2. Experimental

2.1. Instrumentation

Analysis was carried out on an Agilent 1100 series HPLC system (Agilent Corporation, Germany) consisting of a G1315B Diode Array Detector (DAD), a G1311A low-pressure quatpump, a G1379A online degasser, a G1316A thermostat column compartment and a G1313A automatic sample injector. The YF-20 Donghua automatic decocting machine, YBS250E liquid packing machine and YBS liquid packing machine were all obtained from Beijing Donghuayuan Medical Equipment Co., Ltd.

2.2. Reagents, chemicals and materials

HPLC-grade methanol and phosphate acid were purchased from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q water system (Millipore, Bedford, MA, USA) for preparing samples and mobile solution. Other reagents were of analytical grade. All solvents were filtered through 0.22 μm membrane filters before analysis.

The reference standards of rhein, hesperidin, aloe-emodin, honokiol, magnolol and emodin (Fig. 1) were obtained from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of all the standards were not less than 98%.

Dahuang (Lot: LY2010040340, origin: Gansu), Houpu (Lot: 110124, origin: Sichuan), Zhishi (Lot: 100901, origin: Sichuan province) and Mangxiao (Lot: 110307, origin: Hebei) were purchased from Wan Shicheng Co., Ltd. (Shanghai, China). These materials were stored at room temperature in the absence of light in a well-ventilated room. Prof. Bao-Chang Cai authenticated the plant materials, and the voucher specimens were deposited in the Key Laboratory of TCM (Pharmaceutical Department, Nanjing University of TCM).

2.3. Sample preparation

DCQT of 4 mL was transferred to a 10 mL volumetric flask and was diluted with methanol. The mixture was extracted in an ultrasonic bath for 20 min and then the same solvent was added to compensate for the lost volume during the extraction. After centrifugation (10,000 r/min, 10 min), the supernatants were stored at 4 °C and filtered through a 0.22 μm microporous membrane before injecting into the HPLC system for analysis.

![Fig. 1 Structures of main components in DCQT.](image-url)
2.4. Establishment of HPLC methods

2.4.1. Chromatographic conditions

The analysis was performed on an Agilent reversed-phase C$_{18}$ column (250 mm x 4.6 mm, 5 μm) and maintained at 25 °C. The mobile phase consisted of 0.1% phosphate acid in deionized water (A) and methanol (B), the gradient was as follows: 0–5 min, 25% B; 23–30 min, 40% B; 48 min, 80% B; 55–64 min, 85% B; and 69–75 min, 25% B. Elution was performed at a solvent flow rate of 1.0 mL/min, the DAD detector was set at 294 nm to record the chromatograms. The injection volume of each sample and standard solution was 10 μL.

2.4.2. Calibration curves

Methanol stock solution containing hesperidin, aloe-emodin, rhein, emodin, honokiol and magnolol was prepared and diluted to appropriate concentration ranges for the establishment of calibration curves. Seven different concentrations of the six analytes were injected in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the quality of each analyte.

2.5. Preliminary experiment

To our knowledge, because anthraquinone glycosides in Dahuang, active ingredients with purgative functions, are instable to heat and prone to transfer into anthraquinones [7], the decocting time of Dahuang should be shorter than that of other drugs. Therefore, a preliminary experiment was carried out to find the best time point for Dahuang to be added. The following time points were observed: to be decocted for the same time as other drugs (0 min), to be added 3, 5, and 10 min, respectively, before the end of decocting period or only be dissolved in decoction of other drugs for 5 min without cooking. Then the ingredients in the above samples were determined and evaluated. The obtained optimal time point was applied in future experiments.

2.6. Design of experiments

Design expert 7.0 software was applied to generate the matrix and analyze the response surface models. A Box–Behnken design with 3-level and 3-factor was selected for this study because it can evaluate quadratic interactions between pairs of factors while minimizing the number of required experiments. The influence and interactions of three factors were examined in this study: soaking time, the volume of solvent and pressure (Table 1). Ranges for these factors were based on previous studies (data not shown). A total of 17 experiments with factor values were performed. The six responses (the contents of hesperidin, aloe-emodin, honokiol, magnolol, and emodin) were measured for each experiment and the synthetic scores were evaluated based on an established mathematic model [8]. The empirical relationships between three input factors were evaluated from these results. The coded design patterns represent the scaled factor values (high (1), middle (0) and low (−1)) used in each run, in the order of soaking time, pressure and solvent volume, respectively.

2.7. Data analysis

Contents of mark ingredients were obtained according to calibration curves using SPSS software (SPSS for Windows 17.0, SPSS Corporation, USA). Synthetic scores were calculated according to synthetic weights based on AHP combined with CRITIC [9,10]. In this mathematic model, Dahuang was the most important herb like the monarch, so the principal anthraquinones in Dahuang such as emodin, rhein and aloe-emodin were the first critical layer elements, but the influences were negative because the purgative effects decreased when anthraquinone glycosides were transferred into anthraquinones. Using Houpu and Zhishi as assistants, hesperidin in Zhishi, magnolol and honokiol in Houpu were the parameters of second layer; HPLC peaks which had the richest amounts were marked as the third layer; other common peaks were decided as the fourth layer; and uncommon peaks were regarded as the least important factors. Then the obtained contents of mark ingredients and synthetic scores of 17 trials were input into computer and analyzed by Design Expert 7.0 software. The statistical validation of the polynomial equations and response surface analyses plotted in three-dimensional model graphs were provided by the software.

3. Results

3.1. Establishment of HPLC methods

Under the developed method, hesperidin, aloe-emodin, honokiol, rhein, magnolol and emodin (Fig. 1) were respectively separated according to time sequence; a good linearity of each marker ingredient was observed in a relatively wide concentration with the correlation coefficient above 0.999 (Table 2), and LOD and LQD are also listed in Table 2. The precision, reproducibility and accuracy of this method were also satisfactory [10].

3.2. Results of preliminary experiment

Using the above sophisticated HPLC method, samples of different time points were detected and the corresponding spectra were overlaid. Results showed that it could isolate 24 peaks with six known ingredients (Fig. 2), of which the $t_{R}$ (retention time) was 31.2 min (hesperidin), 51.3 min (aloe-emodin), 53.4 min (honokiol), 53.7 min (rhein), 55.4 min (magnolol) and 58.1 min (emodin). When Dahuang was dissolved in decoction of other drugs for 5 min, the contents of hesperidin, magnolol and honokiol were the highest, while the contents of emodin, rhein and aloe-emodin were the lowest (Table 3), suggesting this procedure might be better than other time points. Synthetic scores further confirmed that Dahuang should be dissolved in hot liquid instead of decocted with water (Fig. 3).

| Table 1 | Factors and levels for the Box–Behnken experimental design. |
|---------|-------------------------------------------------------------|
| Level   | $X_1$ (Soaking time (min)) | $X_2$ (Pressure (MPa)) | $X_3$ (Water volume (folds)) |
| −1      | 30   | 0.15  | 6                   |
| 0       | 60   | 0.20  | 8                   |
| 1       | 90   | 0.25  | 10                  |
3.3. Effects of process factors on mark ingredients

According to the contents of six ingredients in 17 Box–Behnken design experiments (Table 4), polynomial equations were available after analysis of software (Table 5). In polynomial equation, $Y$ means the response value (contents of mark ingredients), while the $X_1$, $X_2$ and $X_3$ represent, respectively, three factors which could influence on the decocting process (soaking time, decocting pressure, and water volume). Terms composed of two factors represent the interaction terms, and terms with second-order factors indicate the nonlinear nature of the relationship between the responses and the factors [11,12]. A positive sign indicates a synergistic effect, while a negative sign represents an antagonistic effect. Values of “probability” mean whether model terms are significant, less than 0.05 indicates that model terms are significant. The “lack of fit $F$-value” implies there is a chance that a “lack of fit $F$-value” this large could occur due to noise. Non-significant lack of fit is good—we want the model to fit. “Adeq precision” measures the signal to noise ratio. A ratio greater than 4 is desirable, representing the model can be used to navigate the design space.

The 17 chromatographic fingerprints obtained were superimposed (Fig. 4); meanwhile, the content of six known compositions was calculated by standard curve and put into Design Expert 7.0

| Table 2 Calibration curves of mark ingredients (n=6). |
|--------------------------------------------------------|
| Analyte              | Calibration curve | $r$ | Linear range (µg) | LOQ (ng) | LOD (ng) |
|----------------------|-------------------|-----|-------------------|----------|----------|
| Hesperidin           | $Y=1184.7X−6.8027$ | 1   | 0.056−2.800       | 5        | 9        |
| Aloe-emodin          | $Y=513.86X−0.3438$ | 1   | 0.030−0.600       | 16       | 30       |
| Honokiol             | $Y=1846.2X+3.3171$ | 0.9999 | 0.013−1.300    | 4        | 11       |
| Rhein                | $Y=167.62X−0.4568$ | 0.9997 | 0.042−0.840     | 37       | 50       |
| Magnolol             | $Y=1432.1X−4.0437$ | 1   | 0.020−2.000      | 7        | 20       |
| Emodin               | $Y=2503.8X+3.941$  | 1   | 0.004−0.560      | 2        | 5        |

![Fig. 2 Fingertips of DCQT under different time points when Dahuang was added.](image)

In the spectrum, C was Dahuang to be only dissolved in decoction of other drugs for 5 min without cooking; A represented Dahuang to be decocted for the same time as other drugs (0 min); and B, D and E were the results that Dahuang was added 3, 5, and 10 min, respectively, before the end of decocting period.

| Table 3 Contents of mark ingredients after adding Dahuang at different time points. |
|-----------------------------------------------|
| Time point of later decocting (min) | Hesperidin (mg/g) | Aloe-emodin (mg/g) | Magnolol (mg/g) | Rhein (mg/g) | Honokiol (mg/g) | Emodin (mg/g) |
|------------------------------------------|------------------|------------------|----------------|-------------|----------------|--------------|
| 0                                        | 0.4116           | 0.1052           | 0.0615         | 0.0920      | 0.1047         | 0.0159       |
| 3                                        | 0.4823           | 0.1460           | 0.0515         | 0.0955      | 0.0933         | 0.0197       |
| 5 (soaking)                              | 0.4793           | 0.0989           | 0.0657         | 0.0699      | 0.1227         | 0.0197       |
| 5                                        | 0.3460           | 0.1051           | 0.0618         | 0.0915      | 0.1082         | 0.0192       |
| 10                                       | 0.2708           | 0.1212           | 0.0472         | 0.0796      | 0.0875         | 0.0171       |
software to acquire polynomial equations. Data showed (Table 5) probability values of most responses except rhein were less than 0.05, indicating most responses were significant; the “lack of fit” values for six responses were not significant, indicating the fitness of these models was good; adequate precision of responses were greater than 4, meaning these models could be used to navigate the design space.

We could see from these equations, except aloe-emodin and emodin which were not active ingredients of purgative functions, both pressure and solvent volume were positively relative to the contents of ingredients, indicating that increase of pressure and solvent volume might promote dissolution of active ingredients. Soaking time had a negative relationship to most components except emodin, suggesting herbs should not be soaked for too long periods. Pressure was the most important among all factors. For aloe-emodin and emodin, there were interactions among the three factors, but these interactions were not obvious.

Surface response plots were respectively generated from factors and responses of different marks ingredients as shown in Fig. 5. These surface plots presented that the contents of hesperidin (Fig. 5B), emodin (Fig. 5D) and magnolol (Fig. 5G) might increase as pressure and solvent volume rose; the contents of hesperidin (Fig. 5), honokiol (Fig. 5), magnolol (Fig. 5H) and aloe-emodin (Fig. 5I) might decrease if herbs were soaked for too long periods; the contents of emodin might first increase and then decrease with prolongation of soaking time (Fig. 5C). These plots as well as polynomial equations visually reflected influences of three factors on mark ingredients.

Furthermore, both equations and plots also showed that various factors had different effects on diverse responses, which could be attributed to characteristics of chemical compounds. Based on this

![Fig. 3] Synthetic scores for Dahuang to be added into DCQT at different time points.

**Table 4** The Box–Behnken experimental design with responses.

| No. | Soaking time (min) | Pressure (MPa) | Water volume (folds) | Synthetic scores | Hesperidin (mg/g) | Aloe-emodin (mg/g) | Honokiol (mg/g) | Rhein (mg/g) | Magnolol (mg/g) | Emodin (mg/g) |
|-----|--------------------|----------------|---------------------|------------------|-----------------|-----------------|-----------------|-------------|----------------|---------------|
| 1   | 30                 | 0.15           | 8                   | −25.27           | 0.5563          | 0.1486          | 0.0894          | 0.0935      | 0.1647         | 0.0143        |
| 2   | 90                 | 0.15           | 8                   | −11.71           | 0.6850          | 0.0675          | 0.0728          | 0.0696      | 0.1356         | 0.0100        |
| 3   | 30                 | 0.25           | 8                   | 16.94            | 0.8175          | 0.1032          | 0.1159          | 0.0036      | 0.2117         | 0.0140        |
| 4   | 90                 | 0.25           | 8                   | 21.53            | 1.2176          | 0.1130          | 0.1262          | 0.0036      | 0.2505         | 0.0162        |
| 5   | 30                 | 0.20           | 6                   | −12.78           | 0.5608          | 0.1182          | 0.0605          | 0.0570      | 0.1150         | 0.0148        |
| 6   | 90                 | 0.20           | 6                   | −9.58            | 0.0055          | 0.0183          | 0.0572          | 0.0489      | 0.1122         | 0.0092        |
| 7   | 30                 | 0.20           | 10                  | 20.32            | 1.1043          | 0.1458          | 0.1453          | 0.0050      | 0.2880         | 0.0165        |
| 8   | 90                 | 0.20           | 10                  | 16.76            | 0.7926          | 0.1332          | 0.1194          | 0.0046      | 0.2296         | 0.0168        |
| 9   | 60                 | 0.15           | 6                   | −12.31           | 0.4506          | 0.1093          | 0.0416          | 0.0511      | 0.0781         | 0.0140        |
| 10  | 60                 | 0.25           | 6                   | −20.47           | 0.7594          | 0.1173          | 0.0666          | 0.0823      | 0.1311         | 0.0174        |
| 11  | 60                 | 0.15           | 10                  | 19.59            | 0.8848          | 0.1531          | 0.1522          | 0.0054      | 0.2980         | 0.0212        |
| 12  | 60                 | 0.25           | 10                  | 24.38            | 1.2516          | 0.1394          | 0.1729          | 0.0048      | 0.3300         | 0.0185        |
| 13  | 60                 | 0.20           | 8                   | 15.72            | 0.8917          | 0.1127          | 0.1181          | 0.0037      | 0.2344         | 0.0182        |
| 14  | 60                 | 0.20           | 8                   | −9.78            | 0.7681          | 0.0994          | 0.0803          | 0.0689      | 0.1460         | 0.0198        |
| 15  | 60                 | 0.20           | 8                   | 13.58            | 0.8093          | 0.1085          | 0.0805          | 0.0034      | 0.1418         | 0.0149        |
| 16  | 60                 | 0.20           | 8                   | −16.48           | 0.8412          | 0.1137          | 0.1058          | 0.0895      | 0.2153         | 0.0170        |
| 17  | 60                 | 0.20           | 8                   | −17.53           | 1.0092          | 0.1114          | 0.1088          | 0.1002      | 0.2166         | 0.0168        |

**Table 5** Polynomial equations of components in machine decoction under high pressure.

| Components | Polynomial equations | $P$ value | Lack of fit | Adeq precision |
|------------|----------------------|-----------|-------------|---------------|
| Hesperidin | $Y = -0.9901 - 0.0014X_1 + 3.6735X_2 + 0.14106X_3$ | 0.0027 | 0.0511 | 9.841 |
| Aloe-emodin| $Y = 0.59952 - 0.00491X_1 - 2.4758X_2 - 0.03082X_3 + 0.01515X_1X_2 + 0.000364X_1X_3 - 0.05425X_2X_3 - 1.5 \times 10^{-5}X_1^2 + 4.967X_2^2 + 0.002054X_3^2$ | 0.0023 | 0.0574 | 13.51 |
| Honokiol  | $Y = -0.135081 - 0.000148X_1 + 0.314X_2 + 0.0227438X_3$ | 0.0001 | 0.7883 | 17.534 |
| Rhein     | $Y = 0.22139 - 0.0001X_1 - 0.3133X_2 - 0.137X_3$ | 0.1208 | 0.9268 | 5.228 |
| Magnolol  | $Y = -0.27114 - 0.00021X_1 + 0.61725X_2 + 0.044325X_3$ | 0.0001 | 0.8860 | 14.85 |
| Emodin    | $Y = 0.00012 + 3.35 \times 10^{-5}X_1 + 0.0947X_2 + 0.000405X_3 + 0.0010833X_3 + 2.458 \times 10^{-4}X_1^2 - 0.01525X_3 - 3.98 \times 10^{-6}X_2^2 - 0.053X_3^2 + 0.0001419X_3^3$ | 0.0149 | 0.7107 | 8.919 |

$Y$: contents of mark ingredients, $X_1$: soaking time, $X_2$: decocting pressure, and $X_3$: water volume.
reason, it was difficult for a researcher to make a decision from complicated data. Thus synthetic evaluation should be performed.

3.4. Effects of process factors on synthetic scores

Polynomial equations of synthetic scores were also available after analysis of software. Values of probability less than 0.05 indicated model terms were significant ($P=0.0077$); equation in terms of actual factors was obtained: $Y = -107.82646 + 0.074138 X_1 + 180.22000 X_2 + 8.51156 X_3$ ($Y$: synthetic scores; $X_1$: soaking time; $X_2$: pressure; and $X_3$: water volume). The equation indicated that the three factors were positively relative to synthetic scores in the following sequence: pressure > water volume > soaking time.

As the same as the equation, surface response plots (Fig. 5K and L) also confirmed that pressure was more important than soaking time and water volume.

Finally, from the surface response plots of synthetic scores, we can see when parameters ranged between 30 and 60 min for soaking time, 0.20 and 0.25 MPa for pressure, and 8 and 10 folds for water volume, the synthetic scores were satisfactory.

3.5. Results of verifying tests

In order to verify the reliability of models, tests were further performed according to the obtained ranges of process parameters. In detail, different parameters conditions were combined and the selections of parameters conditions were followed: 30, 45, and 60 min for soaking time; 0.2 MPa and 0.25 MPa for pressure; and 8, 9, and 10 folds for water volume. Altogether, 18 combinations were generated, whose predictive values were calculated according to the polynomial equations of synthetic scores. The top nine combinations in synthetic scores ranking were chosen to carry out verifying tests (Table 6). According to the nine decoction conditions, herbs were cooked and determined by HPLC. The true contents of mark ingredients were counted and compared with predictive values.

Results showed (Table 7) that differences between predictive and true values of the trials were satisfactory with variances less than 30%, demonstrating that the polynomial equations had good predictive ability. Among them, the differences between predictive and true values of No.6 test were the smallest.

So, the optimal conditions of decocting process are listed as follows: the herb materials were soaked for 45 min and extracted with 9 folds volume of water in the decocting machine under the temperature of 140 °C till the pressure arrived at 0.25 MPa, then hot decoction was excreted via the valve to soak Dahuang and Mangxiao for 5 min. Finally, obtained solutions were mixed, filtrated and packed.

4. Discussions

As machine decoction is coming into our daily life, the optimization of the machine decocting method should be paid attention to. So in this paper, we studied the process of the machine decocting method using DCQT as a model.

In terms of the observed responses, many papers about process optimization are often based on contents of one or several components. This method has some limitations and cannot reflect comprehensive information. HPLC fingerprint is widely accepted as a quality evaluation method of TCM [13,14]. It can display the characteristics, complexities and relationships of ingredients. Therefore, in this paper, HPLC fingerprints of DCQT were first successfully established. Some ingredients are known while others are unknown. In order to synthetically evaluate the information of fingerprints, AHP combined with CRITIC was carried out to calculate synthetic scores according to our previous work [9]. We optimized the machine decocting method based on mark ingredients contents and synthetic scores.

In terms of the design method, there are several design methods to optimize process, including the orthogonal design, uniform design and Box–Behnken design. Among these methods, the Box–Behnken design is one of the most efficient methods. One of its advantages is that it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. These designs are useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results are often
Fig. 5  Response surface plots (3D) reflecting the effects of process parameters on mark ingredients and synthetic scores. In this figure, A, C, E, G, I and K respectively reflect the effects of pressure and soaking time on hesperidin, emodin, honokiol, magnolol, aloe-emodin and synthetic scores; B, D, F, H, J and L respectively reflect the effects of pressure and water volume on hesperidin, emodin, honokiol, magnolol, aloe-emodin and synthetic scores.
obtained [15]. Therefore, the Box–Behnken design was carried out in this study.

In terms of factors, variance of some parameters such as soaking time, cooking pressure and the volume of solvent may lead to ingredients alteration and further result in therapeutic differences. Thus, these factors were considered and evaluated based on contents of known ingredients and synthetic values of all peaks in HPLC fingerprints. Results showed that pressure was the most important among all parameters. The possible reasons are as follows: in a closed pot, pressure could accelerate the dissolution of ingredients to increase the extracting efficiency; solvent volume could enlarge the concentration gradient between materials and liquid to increase contents of components; and increment of soaking time could promote the extraction of materials, but too
long period was meaningless. Based on these results, the best parameters for DCQT were finally confirmed.

5. Conclusions

Through this research, we can conclude that HPLC fingerprints combined with the Box–Behnken experimental design can be applied in process study. HPLC fingerprints can express more chemical characteristics than mark ingredients contents, so obtained optimal process is more representative and reliable.

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References

[1] F. Xu, Y. Liu, Z. Zhang, et al., Quasi-MS identification of flavanone 7-glycoside isomers in DCQT by high performance liquid chromatography–tandem mass spectrometry, Chin. Med. 24 (2009) 15–25.
[2] Q. Xia, J.M. Jiang, X. Gong, et al., Experimental study of tong xia purgative method in ameliorating lung injury in acute necrotizing pancreatitis, World J. Gastroenterol. 6 (2000) 115–118.
[3] F.Y. Li, B. Zhang, W.X. Li, et al., The Influences of pre and post processed pieplant and compatibility of pieplant and glauber’s salt on gastrointestinal motility of rat, Guid. J. Tradit. Chin. Med. Pharm. 14 (10) (2008) 72–74.
[4] L. Xu, K.S. Bi, Application of multiple linear regression analytical method to spectrum-activity relationship analysis of mongolian preparation sendeng-4 decoction, Comput. Appl. Chem. 25 (10) (2008) 1189–1192.
[5] L.Y. Zhou, S.W. Liang, S.M. Wang, et al., Studies on the pharmacodynamic fingerprint of cortex fraxini, J. Liq. Chromatogr. Relat. Technol. 34 (2011) 144–149.
[6] C.H. Xiao, Y.R. Lu, Chinese Medicinal Chemistry, Shanghai Scientific & Technical Publishers, Shanghai, 1987, p. 164.
[7] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, et al., Box–Behnken design: an alternative for the optimization of analytical methods, Anal. Chim. Acta 597 (2007) 179–186.
[8] Q.H. Zhao, X. Zhou, R.F. Xie, et al., Comparison of three weighing methods for evaluation of the HPLC fingerprints of cortex fraxini, J. Liq. Chromatogr. Relat. Technol. 34 (2011) 2008–2019.
[10] R.F. Xie, Y.M. Li, Z.N. Shi, et al., Study on spectrum-effect relationship of DaChengQiTang, J. Chromatogr. Sci. 51 (6) (2013) 524–532.

[11] A.E. Ahmed, A.A. Aboelwafa, M.A. Ibrahim, et al., Once daily, high-dose mesalazine controlled-release tablet for colonic delivery: optimization of formulation variables using Box–Behnken design, AAPS PharmSciTech. 12 (4) (2011) 1454–1463.

[12] M. Khajeh, Application of factorial design and box–behnken matrix in the optimization of a magnetic nanoparticles procedure for copper determination in water and biological samples, Biol. Trace Elem. Res. 135 (2010) 355–363.

[13] R.F. Xie, Q.H. Zhao, Z.C. Li, et al., Comparison on HPLC fingerprints between Fraxini cortex and its eye drop, Chin. Herb Med. 5 (2013) 301–306.

[14] Q. Su, P.P. Shang, Y.M. Zhang, et al., HPLC fingerprint and LC–TOF–MS analysis on extract from roots of Gentiana macrophylla, Chin. Herb Med. 4 (3) (2012) 245–251.

[15] J.F. Hao, X.S. Fang, Y.F. Zhou, et al., Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box–Behnken design, Int. J. Nanomed. 6 (2011) 683–692.