

# Near-infrared for on-line determination of quality parameter of *Sophora japonica* L. (formula particles): From lab investigation to pilot-scale extraction process

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## ABSTRACT

**Background:** Extraction process of dried flowers of formula particles should be investigated from lab investigation to pilot-scale because of good water absorbing capacity and obscure active pharmaceutical ingredients (API) dissolution. **Objective:** Reliable analysis of on-line near-infrared (NIR) technique and novel application in fascinating modern, traditional Chinese medicine production (formula particles) was proved. **Materials and Methods:** The extraction process of *Sophora japonica* L. (formula particles) was used as an example, the rutin was regarded as API. On-line NIR technology was used to monitor the variation of rutin in the extraction process. High-performance liquid chromatography (HPLC) was used as a reference method to determine the content of rutin during the extraction process. The sample set was selected by Kennard-Stone (KS) algorithm. Different pretreatment methods were compared. The synergy interval partial least square (SiPLS) algorithm was applied. Chemometrics indicators and multivariate detection limits method were mutually used to assess the model. **Results:** According to both errors  $\alpha$  (0.05) and  $\beta$  (0.05), rutin content could be detected by on-line NIR, which was more than 0.181 mg/mL. **Conclusions:** This work demonstrated the feasibility of NIR for on-line determination of rutin in the pilot-scale extraction process of *S. japonica* L. It provided technical support for the NIR application in the extraction process of formula particles.

**Key words:** Formula particles, multivariate detection limits, near-infrared, on-line, *Sophora japonica* L.

## INTRODUCTION

Formula particles are novel modern, traditional Chinese medicine (TCM) products. More than 300 types of formula particles, manufactured by six designated factories of State Food and Drug Administration, are used in Chinese hospitals. Extraction process in formula particles is one of the most popular units. The reports on extraction analysis of formula particles were mostly based on off-line measurement. Once the situation of substandard quality occurred, the extraction failed. To solve this problem, a real-time and on-line technology is urgently needed. However, there is no literature to support on-line monitoring of the extraction process in formula particles.

In 2004, the U.S. Food and Drug Administration issued a "Process Analysis Technology (PAT) Industry Guide,"

the guidance encouraged pharmaceutical companies to develop innovative drugs, manufacture and ensure quality.<sup>[1]</sup> PAT was considered as a quality system, and its purpose was to monitor the raw materials, key intermediate in real time and ensure the quality of the final product. Meanwhile, the on-line technology was mentioned several times in the guidance, which could provide a reference to our TCM production, particularly formula particles and TCM injection.

In the past decade, near infrared (NIR) focused on on-line analysis because it was one of the effective PAT tools. Its wavelength ranges from 780 to 2524 nm. The range mainly contains combination frequencies and overtone frequencies absorption with hydrogen groups. Because of the rich signal in these absorption regions, this technology has advantages as non-destructive, rapid and real-time, etc. In addition, coupled with the optical fiber, on-line NIR was applied to monitor critical quality parameters in the manufacture process of production. NIR technology could predict concentration variance of active pharmaceutical ingredients (API) in real time and provide quality consistency for pharmaceutical products.

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Recently, NIR technology was used in almost every unit of modern TCM production, that is, in the extraction process, the concentration process, alcohol precipitation process, etc. The API have been monitored by on-line NIR in the extraction process of single medical herb.<sup>[2-5]</sup> For TCM injection, on-line NIR method have been developed in the extraction process, that is, Xuebijing injection and Fufang Kushen injection.<sup>[6,7]</sup> These reports showed the reliability analysis of on-line NIR technology in TCM. However, on-line monitoring extraction process in dried flowers (formula particles) has not been reported.

*Sophora japonica* L. (Huaihua in Chinese) was dried flowers of Legumes Cassia, which was collected in summer. It was commonly used as a traditional Chinese herb in bloody diarrhea, vomiting blood, epistaxis, bleeding, hemorrhoids with bleeding, dizziness, headaches, etc. The main ingredient of *S. japonica* L. was rutin, which was recorded in Chinese Pharmacopoeia (2010 Edition).<sup>[8]</sup>

Currently, *S. japonica* L. of formula particles has possessed large sales in China. Therefore, it is necessary to explore the feasibility of on-line NIR technology in the extraction process of dried flowers (formula particles). Regarding the soft texture of *S. japonica* L., the water absorbing property and API (rutin) dissolution should be investigated in the lab. Furthermore, on-line NIR technology was applied to collect the spectra in pilot-scale extraction process. High-performance liquid chromatography (HPLC) was used as a reference method, partial least square (PLS) was used to establish a model. Chemometrics indicators and multivariate detection limits (MDL) were mutually used to assess the model, and prove the reliable analysis of on-line NIR technique in modern formula particles.

## MATERIALS AND METHODS

### Materials

*Sophora japonica* L. was purchased from Lulutong (Hebei) Chinese Herbal Medicine Co., Ltd. (Beijing, China). Rutin reference standard (No. 110809–112940) was supplied by the National Institutes for Food and Drug Control (Beijing, China). Methanol (Fisher Scientific, USA) was HPLC grade. Glacial acetic acid (Beijing Chemical Works, Beijing, China) was analytical grade. Water was supplied by water system (Millipore Corp., Bedford, MA, USA).

### Investigating water absorbing capacity of *Sophora japonica* L

To perform the pilot-scale on-line analysis, the water absorbing capacity of *S. japonica* L. should be investigated because it was a good water-absorbing plant. In the experiment of design (DoE), 100 g *S. japonica* L. was soaked with 10-fold water. After well-soaking, the solution was poured out and collected.

Water capacity was about 750 mL. Therefore, the water absorbing capacity of *S. japonica* L. was 2.5 mL/g.

### Extraction parameter of *Sophora japonica* L. in lab

A total of 100 g *S. japonica* L. was extracted with 10-fold water for three times, once 1.5 h.<sup>[9]</sup> According to the water absorbing capacity of *S. japonica* L., at the first extraction stage, it should be filled with 12.5-fold deionized water. Three-necked flask with condensing equipment was used as extraction container, heated by the temperature controlled electric jacket (Ruide Equipment Co., Ltd. Gongyi, China). *S. japonica* L. was soaked for 30 min and sampled at 15 min and 30 min to investigate the dissolution of rutin vs. soaking time.

During the extraction process, intermediates in the extraction process were removed out in each time of extraction (at the beginning of the heating process (I), at the beginning of the boiling process (II), at the end of boiling process [III]) using a pipette (Dragon Laboratory Instruments Co., Ltd, Beijing, China). Besides investigating the concentration range of rutin, the rutin content of samples could initially be determined by HPLC.

### Extraction parameter of *Sophora japonica* L. in pilot-scale

According to the above water absorbing capacity, 7 kg *S. japonica* L. was firstly extracted with 12.5-fold deionized water in a multi-functional extractor (100 L). Furthermore, solvent capacity in the remaining two was using 10-fold water, once 1.5 h. The speed of stirring paddle was set at 50 rpm. During the extraction process, the NIR spectra were scanned periodically. A good and reasonable sampling interval was necessary for the extraction process.

Table 1 was the sampling interval of extraction according to the content of rutin. At initial heating and boiling time, the content of rutin varied rapidly, so the sampling tiny interval was set. In the second and third stage of the extraction process, the content of rutin varied less than the first extraction stage. Therefore, the interval could be made some adjustment for reducing the amount of work.

The process system included on-line NIR scanning instrument and the extraction equipment with a sampling device [Figure 1]. The whole process could be described as follow: The TCM was added into the tank, and extracted with

**Table 1: The sampling interval of extraction process**

Extraction process	Extraction time		
	Heating	0-1 h	1-1.5 h
1 <sup>st</sup> extraction	2 min	4 min	5 min
2 <sup>nd</sup> extraction	5 min	5 min	5 min
3 <sup>rd</sup> extraction	5 min	5 min	5 min

deionized water. During the extraction process, the extraction solution circulated in the bypass under the action of the pump. In our novel design, 80  $\mu\text{m}$  and 100  $\mu\text{m}$  filters were used to eliminate these interference factors when the extraction solution was passed the filters in bypass, because bubble and solid content could seriously interfere the reality of spectra.<sup>[10,11]</sup>

In addition, temperature was recorded by thermometers in real time. In the whole extraction process, NIR spectra were scanned with optical fiber. After the scanning had stopped, the switch was open, and about 10 mL extraction solution was collected for HPLC analysis.

### Near-infrared equipment and measurement

The spectra were scanned by fiber optic probes. The optical path of NIR light was 2 mm, which was applied with XDS process analyzer and relative software (Foss Co., Denmark). The wavelength range of NIR spectra was from 800 nm to 2200 nm. The parameters of spectra were set in 32 scans for average, and the wavelength increment was 0.5 nm. VISION software was used for data analysis (Vision 3.5, Foss Co., Denmark).

### High performance liquid chromatography reference method

All the samples were diluted with 20% (v/v) methanol-water solution and the rutin content was accurately determined by using HPLC assay.<sup>[12]</sup> Chromatographic analysis was carried out on Agilent 1100 HPLC system (Agilent Technologies, USA). The concentration of rutin was

analyzed by reverse-phase chromatography on an ODS column (250 mm  $\times$  4.6  $\mu\text{m}$ , Dikma, China) in isocratic elution of the mobile phase consisted with methanol, deionized water with 0.1 glacial acetic acid (40:60, v/v), the flow rate was 1.0 mL/min. The column temperature was set 30°C and detection wavelength was at 257 nm. 10  $\mu\text{L}$  extract fluid was injected into HPLC system for analysis.

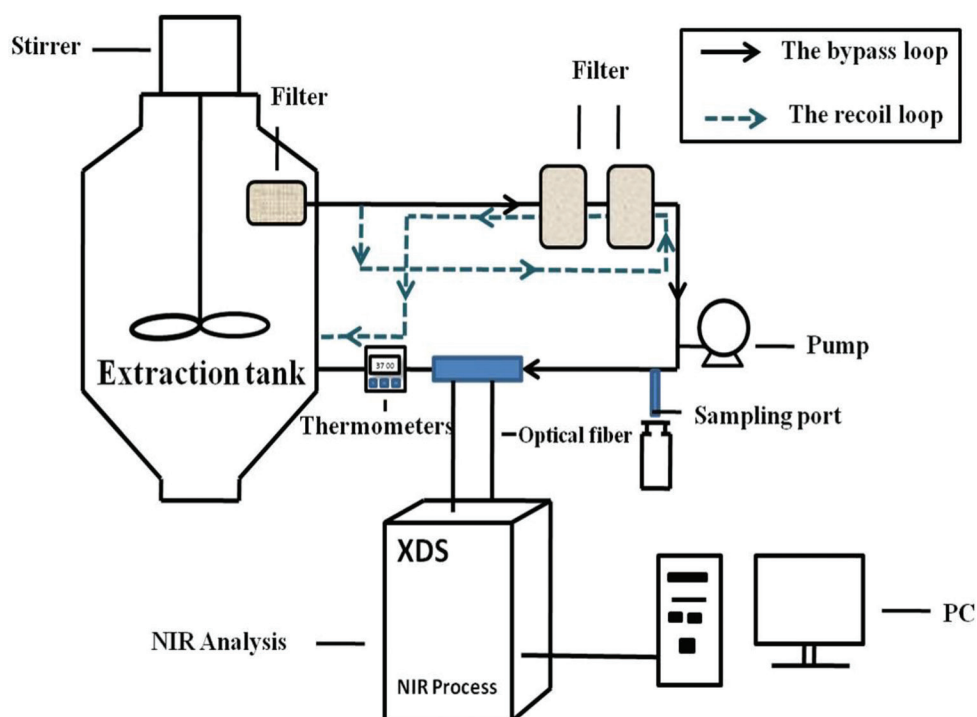
### Software and data analysis

The spectral analysis was completed with the Unscrambler 9.6 software package (CAMO Software AS, Norway). Some algorithms used were performed with MATLAB (MATLAB v7.0, Math Works, MA). Other algorithms were modified on the basis of Norgaard algorithms that developed by us. Samples sets were split according to Kennard-Stone (KS) algorithm (46 calibration sets/23 validation sets). Root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used to evaluate model.

## RESULTS AND DISCUSSION

### Investigating rutin dissolution in lab by high performance liquid chromatography method

The reference values obtained by HPLC were showed in Figure 2. The calibration curve showed good linearity ( $R^2 = 0.999$ ), the line ranged from 0.408  $\mu\text{g}$  to 4.080  $\mu\text{g}$ . Figure 2 showed that the soaking process had no effect on rutin dissolution. At the initial of the heating process, rutin did not exist in the solution. Therefore, the soaking process



**Figure 1:** The extraction platform of traditional Chinese medicine

was not selected in a pilot-scale investigation as described above. Simultaneously, the result demonstrated that the heating process should be investigated in pilot-scale.

### Investigating rutin dissolution in pilot-scale by high performance liquid chromatography method

Figure 3 shows the sample solution in different temperature at the initial of heating. The phenomenon described that rutin did not separate out in large with 60°C. It appeared in large on the chromatogram when the temperature was 86°C. Therefore, 69 samples were collected based on the above result. The average content of rutin in the extraction process was 1.506 mg/mL (minimum value, 0.264 mg/mL; maximum value, 3.759 mg/mL).

### Characteristic of near-infrared spectra in pilot-scale

The raw spectra were shown in Figure 4. As seen in the raw spectra, there was a large fluctuation from 2000 nm to 2200 nm.<sup>[13]</sup> Besides, the aqueous solution heavy absorbance was about in 1950 nm.<sup>[14,15]</sup> Therefore, 800-1900 nm was used to analysis. In detail, 800-1900 nm, first combination-overtone, second combination-overtone, and third overtone can satisfy the analysis acquirement.<sup>[16]</sup>

### Near-infrared pretreatment methods

The spectra were affected by the baseline drift, spectral noise and overlapping peaks, etc., To improve the accuracy of the model performance, pretreatment methods of the spectra were necessary before the model establishment. Several types of pretreatment methods were used in the spectral data set, such as 11-point Savitzky-Golay (SG), 11-point SG and first derivative (SG + 1D), 11-point SG and second

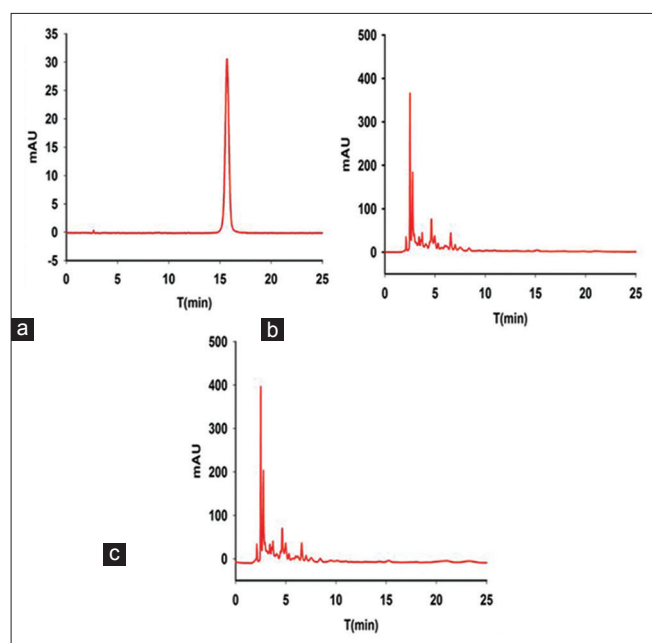
derivative (SG + 2D), multiplicative scatter correction and standard normal variation. Appropriate pretreatment method was selected by leave-one-out cross-validation, the number of latent variable factors was investigated.

The number of the latent variable factors was best with the lowest predicted residual sum of squares (PRESS) value. Table 2 was the result of rutin model with different pretreatment methods, the raw spectra with no pretreatment method had the lowest latent factors, coefficient of determination ( $R^2$ ) closest to 1, the smallest RMSEC and root mean square error of cross validation (RMSECV) value. Figure 5 showed a relationship with latent variable and PRESS in different pretreatment methods. The result showed raw spectra was the best pretreatment method for NIR model.

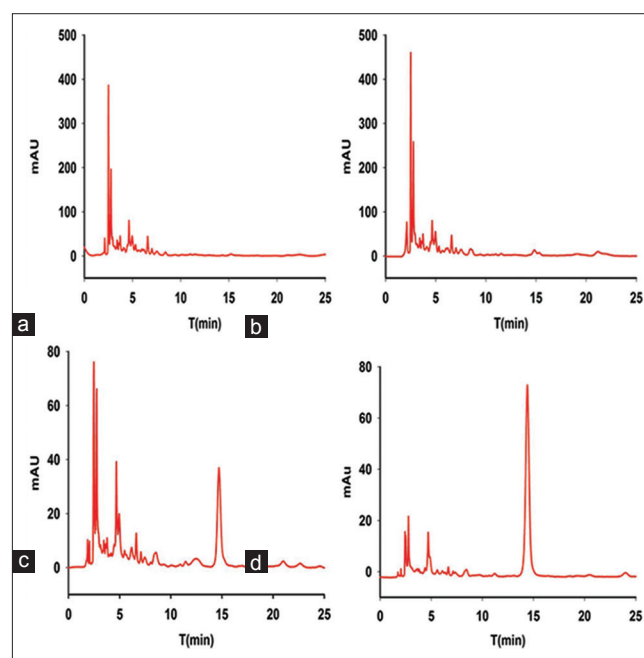
**Table 2: The result of different pretreatment methods**

Methods	Latent factors	Calibration set		Validation set	
		$R^2$	RMSEC	$R^2$	RMSECV
Raw	6	0.994	0.068	0.980	0.131
SG	7	0.976	0.156	0.943	0.209
SNV	7	0.956	0.161	0.913	0.266
MSC	7	0.965	0.158	0.923	0.232
SG+1D	7	0.862	0.329	0.506	0.637
SG+2D	7	0.976	0.148	0.912	0.260

RMSECV: Root mean square error of cross validation; RMSEC: Root mean square error of calibration; SG: Savitzky-Golay; SNV: Standard normal variation; 1D: First derivative; 2D: Second derivative; MSC: Multiplicative scatter correction



**Figure 2:** The chromatograms of rutin reference standard (a), samples in soaking process (b) and samples in heating process (c)



**Figure 3:** The chromatograms of sample solution with 40°C (a), 60°C (b), 86°C (c) and 100°C (d) in extraction process

### Variable selection using synergy interval partial least square model

In the NIR region, the bands were strongly overlapped and interfered. To solve these problems, synergy interval partial least square (SiPLS) was used as a variable selection method.<sup>[17]</sup> The full spectrum was split into some intervals. Several intervals constituted a joint model, joint models with low RMSECV value were regarded, and several good joint models were combined to select the best subinterval combination. As the system was the extraction process of TCM, the parameters of optimal SiPLS model could be used by the reference.<sup>[17]</sup> Therefore, 1010-1080 nm, 1290-1360 nm and 1710-1780 nm was selected by the optical SiPLS model, as described by three blue regions in Figure 6.

### Establishment and prediction of rutin partial least square model

Root mean square error of cross validation, RMSEP, RMSEC and  $R^2$  were used to evaluate the PLS model. The lower the RMSECV, RMSEP, RMSEC value and

the closer to 1 the  $R^2$ , the better the PLS model was. The RMSECV, RMSEP, RMSEC values were 0.054, 0.111, 0.142, respectively. The  $R^2$  was almost more than 0.98, indicating that the model had good accuracy. Figure 7 illustrates the calibration and prediction result in the model, the reference value and the prediction value were almost aligned in a straight line closely. The parameters of the model indicated that the extraction process of *S. japonica* L. could be monitored using on-line NIR in pilot-scale, and the PLS qualitative model was reliable. The on-line NIR could be used in the actual production process.

### Model assessment with multivariate detection limits parameter

Besides evaluating methods using chemometrics indicators, MDL method was proposed in this work. It mainly used for investing the detection ability of rutin, and evaluating the PLS model. False positive and false negative (probability of type I error  $\alpha$ , probability of type II error  $\beta$ ) errors were prevented by it. The theory of MDL could be found in the

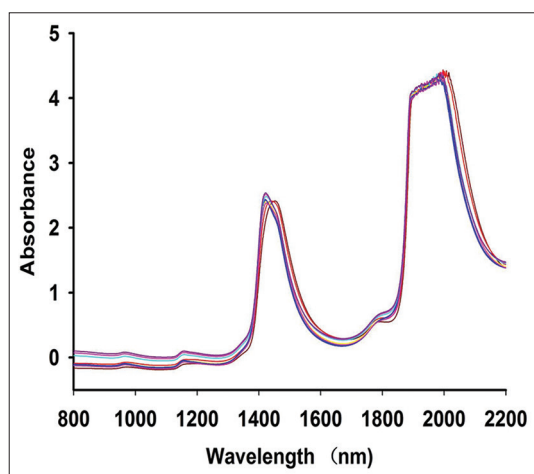


Figure 4: The near-infrared spectra of *Sophora japonica* L.

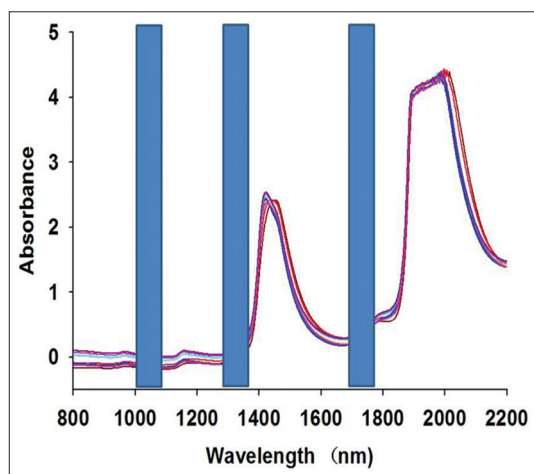


Figure 6: The spectral interval selected by synergy interval partial least square method

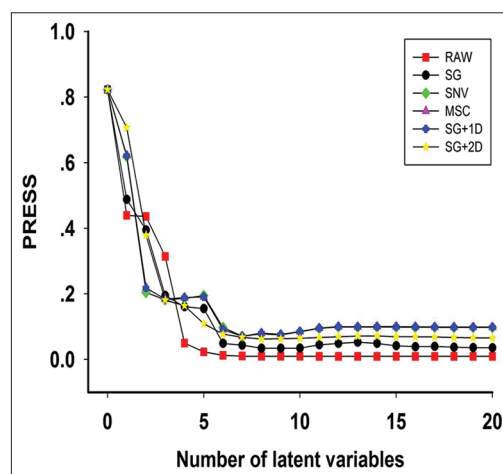


Figure 5: Predicted residual sum of squares plot with different pretreatment methods

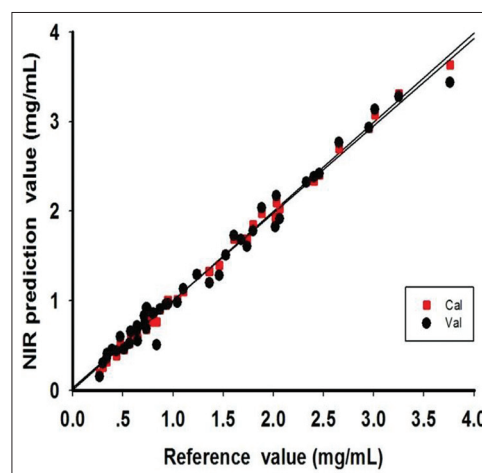


Figure 7: Near-infrared predictions versus the reference method

**Table 3: MDL obtained from PLS model using different error types (mg/mL)**

Method	$\Delta_{0.1,0.1}$	$\Delta_{0.1,0.05}$	$\Delta_{0.1,0.01}$	$\Delta_{0.05,0.1}$	$\Delta_{0.05,0.05}$	$\Delta_{0.05,0.01}$	$\Delta_{0.01,0.1}$	$\Delta_{0.01,0.05}$	$\Delta_{0.01,0.01}$
SiPLS	0.162	0.185	0.228	0.161	0.181	0.218	0.200	0.221	0.259

MDL: Multivariate detection limits; PLS: Partial least square; SiPLS: Synergy interval partial least square

reference.<sup>[18-20]</sup> Final equation of MDL used for on-line NIR model of TCM could be calculated.<sup>[21]</sup> The concentration range of all samples was from 0.264 to 3.759 mg/mL (w/v), considering the both error  $\alpha$  (0.05) and  $\beta$  (0.05), the MDL result showed the rutin value more than 0.181 mg/mL could be reliably detected by NIR [Table 3].

## CONCLUSIONS

Formula particles could satisfy human quick demands of modern life. In this study, aiming to ensure the extraction process successful and reliable, water absorbing capacity and rutin dissolution were investigated in the lab before the pilot-scale extraction. Furthermore, on-line NIR technology was applied to monitor the extraction process of *S. japonica* L. which was one of formula particles. The NIR quantitative model was built, and the change of rutin content was rapid analysis in the extraction process. Based on the chemometrics indicators and MDL method, the result verified the reliability of a quantitative model in the extraction process of *S. japonica* L. This work demonstrated the feasibility of NIR in monitoring the pilot-scale extraction process. It provided technical support to the NIR application of formula particles, which was a novel fascinating application of modern TCM production.

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