

Rapid Determination of Methyl Salicylate and Menthol in Activating Collaterals Oil by Near Infrared Spectroscopy

Yao Wanqing¹, Peng Mengxia¹, Chen Ziyun¹, Wang Yanxia¹, Lin Tao¹

¹(School of Chemistry and Environment, Meizhou, Guangdong 514015)

Abstract: The near infrared spectra of 10 kinds of commercial collaterals oil samples were collected by liquid transmission analysis module, and the contents of methyl salicylate and menthol in collaterals oil were determined by gas chromatography-mass spectrometry (GC-MS). The quantitative analysis model of methyl salicylate content (model 1) and menthol content (model 2) was established by correlating spectral information with measured values by partial least square method (PLS) in chemometrics. Model 1 was used to detect the content of methyl salicylate in activating oil. The predicted results showed that the absolute error was in the range of -0.098~0.082%, and the relative error was in the range of -9.986~8.195%. Model 2 was used to detect menthol in activating collaterals oil. The predicted results showed that the absolute error was in the range of -0.173~0.194%, and the relative error was in the range of -7.25~9.69%. A new method for rapid and accurate determination of methyl salicylate and menthol in activating collaterals oil was established.

1 Introduction

Active collateral oil is a compound external oil Chinese medicine preparation [1] composed of holly oil (main component methyl salicylate), menthol, turpentine, camphor, etc. It has the effects of relaxing muscles and activating collaterals, dispelling wind and removing blood stasis, and is clinically used for the treatment of various traumatic injuries, rheumatic osteodynia, backache and tautness of bones and muscles. At present, there are many kinds of active oils on the market, among which the famous ones are "Huang Daoyi" and "Shi Malong". The composition, efficacy, production technology and quality control of different brands of activating oils are different [2]. However, with the rapid development of online e-commerce industry, it is difficult to judge the quality of collaterals oil on online sales platform, with different curative effects, and the composition of collaterals oil is complex, which is easy to cause unpredictable adverse drug reactions.

In Chinese Pharmacopoeia, menthol and methyl salicylate in activating collaterals oil were detected by packed column gas chromatography with high accuracy [3]. Many researchers mainly explore how to optimize the experimental conditions for simultaneous, simple, accurate and rapid determination and quality evaluation of the main components in collaterals oil or medicinal oils containing similar components. Li Meigui et al. [4]

used gas chromatography to determine menthol in activating oil, and calculated the content of methyl salicylate in activating oil by the method of one measurement and multiple evaluation. Zhang Run Rong et al. [5] used capillary gas chromatography to simultaneously determine the contents of eight components in activating collaterals oil. Pu Yiqiong et al. [6] studied the establishment of capillary gas chromatography external standard method for the simultaneous determination of camphor, menthol, borneol and methyl salicylate in rubber paste. Shan Tingting et al. [7] simultaneously determined the contents of camphor, menthol, borneol and methyl salicylate in Shangshi Zhitong ointment by GC. Zhao Xin et al. [8] used gas chromatography-mass spectrometry (GC-MS) for the first time to analyze the chemical components of activating collaterals oil. The above methods can accurately determine the content of main components in activating collaterals oil or similar medicinal oils, but they all need to explore the conditions in the early stage of instruments. Moreover, instruments and equipment are expensive, sample pretreatment is tedious and time-consuming. To achieve multi-index quality control, enough reference substances and specialized technicians are needed, which is difficult to achieve in actual production. After decades of development, fast, efficient and environmentally friendly near infrared spectroscopy technology has been widely used in agriculture, medicine, food and chemical industry, and has made some progress

Fund projects: Guangdong province's 2019 rural science and technology correspondent's science and technology assistance project, and jiaaying university's key scientific research project in 2019 (2019KJZ02).

Author brief introduction: Yao Wanqing (1983-), Woman(Han), Mainly engaged in applied chemistry, analytical chemistry, chemistry teaching and research. E-mail: 627744294@qq.com.

in drug analysis and nondestructive testing of food^[9-11].

In this paper, near infrared spectroscopy (NIR) combined with Partial least square (PLS) in chemometrics, taking GC-MS test sample data as the standard value, established a fast and accurate method for simultaneous analysis of methyl salicylate and menthol, which provided a stable, fast and reliable rapid batch detection method for drug administration.

2 Instruments and Reagents

2.1 Instruments and Reagents

NICOLETTIS 10 Fourier transform infrared

spectrometer, American Thermofisher Scientific Co.; TQ Analyst 9.0 software, Ominic 9.0 spectrogram processing software, American Thermo Fisher Scientific company; QP2010 Ultra gas chromatography-mass spectrometer, Shimadzu Corporation of Japan.

2.2 Reagents and Samples

Methyl salicylate standard: Shanghai Civic Chemical Technology Co., Ltd.; Menthol standard substance: Shanghai Teng Zhu biotechnology co., Ltd.; Anhydrous ethanol and acetone: Guangdong Guanghua Sci-Tech Co., Ltd., all of which are analytically pure. See table 1 for test samples.

Table 1 Experimental samples

No.:	Medicine name	Lot number	Manufacturer
HLY1	Lion Malong activating collaterals oil	20150518	Shimalong Pharmaceutical Co., Ltd.
HLY2	Lion Malong activating collaterals oil	20160884	Shimalong Pharmaceutical Co., Ltd.
HLY3	Flying eagle activating collaterals oil	C1511004	Hongkong Ouhua Medicine Limited
HLY4	Huang Daoyi activating collaterals oil	50901	Wong To Yick Wood Lock Ointment Limited
HLY5	Huang Daoyi activating collaterals oil	80312	Wong To Yick Wood Lock Ointment Limited
HLY6	Huoluo oil	20180102	Nanchang Huang Daoyi Kaiqing Medicine Technology Co., Ltd
HLY7	Tianqi Huoluo Oil	20171101	Jiangxi Jidantang Biology Science And Technology Co., Ltd
HLY8	Huoluo oil	20180402	Jiangxi Yuzhentang Industry Co., Ltd
HLY9	Huoluo oil	20180302	Yongfeng County Kangqi Biology Science And Technology Co., Ltd
HLY10	Miao medicine activating collaterals oil	20180401	Jiangxi Renyi Biotechnology Co., Ltd.

3 Experimental Method

3.1 Determination of Methyl Salicylate and Menthol in Activating Collaterals Oil by GC-MS

Chromatographic conditions: Rxi-5Sil MS(30.0m×0.25mm×0.25μm) Shi Ying capillary column; Carrier gas: 99.999% helium; Sample size: 0.2μ l; Temperature rising procedure: the column initial temperature is kept at 60°C for 4min, raised to 90°C at 5°C/min for 2min, raised to 115°C at 5°C/min for 3min, and then raised to 250°C at 10°C/min for 6 min. Split ratio: 100: 1; Pressure: 57.4kPa; Total flow: 15mL/min; Column flow: 1.00 ml/min; Linear speed: 36.6 cm/s; Purge flow rate: 2.0mL/min.

Mass spectrometry conditions: electron bombardment (EI) ionization source; Electron energy is 75eV; Ion source temperature: 230°C; Interface temperature: 260°C; Quality scanning range: 30 ~ 400 amu; Scanning period: 1 second.

Drawing of standard curve: Using absolute ethyl alcohol as solvent, prepare standard solutions of methyl salicylate and menthol with concentrations of 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0mL/mL. The chemical structure of activating oil was determined by searching the spectrum library, and the standard working curve was drawn by peak area normalization method.

Measurement of samples: Take raw liquid of activating collaterals oil, take absolute ethyl alcohol as solvent, and prepare it into 0.1mL/mL. GC-MS conditions are the same as when drawing standard curve, and 10 kinds of active oil samples are determined. According to the peak area of each index, the contents of methyl salicylate and menthol in the sample were calculated and converted on the standard working curve.

3.2 Collection of Near Infrared Spectra of Active Oil

Measurement conditions of NIR: Taking air background as reference, liquid transmission analysis module and sample tube with 6 mm optical path were used to scan at 10000~4000cm⁻¹ with resolution of 8cm⁻¹ and scanning

times of 32 times.

NIR acquisition method: Take 1 mL of active oil samples in liquid sample tubes, and collect the near infrared spectra of different samples under the same temperature and humidity. Three parallel samples (reloaded) were taken from each sample, and five near infrared spectrograms were collected from each parallel sample, and 15 spectrograms were collected from each sample, totaling 150 spectrograms.

3.3 Establishment of Near Infrared Quantitative Analysis Model

Twelve near infrared spectrograms of 8 kinds of activating oil samples were selected as training sets, and the other three were internal verification sets. A total of 120 spectrograms of training set and internal verification set were imported into TQ Analyst 9.0 stoichiometry software. PLS method was used to model, and the optical path difference was set as a constant. Different modeling spectral wavelength ranges and spectral preprocessing methods were combined and verified, and the abnormal points were eliminated and the model was optimized, from which the best preprocessing method and spectral wavelength range were selected. According to the correlation coefficient R, root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), root mean square error of cross validation (RMSECV) and the prediction effect of the model, the optimal model is optimized.

Using Ominic 9.0 software to process the spectrogram, using TQ Analyst 9.0 stoichiometry software of spectrum analyzer, combining the infrared spectral data of samples with their chemical measured values, the quantitative model was established by partial least square method, and then appropriate mathematical processing was carried out. According to the generalized square distance-Mahalanobis distance, the outliers are eliminated, and the model is optimized by preprocessing the spectrogram and selecting the modeling spectral range. According to the correlation coefficient r, root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and root mean square error of cross validation (RMSECV), the optimal model of each component is determined.

3.4 Application of Quantitative Model

A total of 30 near infrared spectrograms of HLY2 and HLY5 samples from different batches from the same manufacturer, which are not involved in modeling, are imported into the model built in 2.3 for external detection, and the content predicted by the model is compared with the actual content determined by GC-MS. Meanwhile, F test is carried out on the model to detect and evaluate the prediction performance of the built model.

4 Results and Discussion

4.1 Determination Results by GC-MS

According to the method of 2.1, the mass spectra of 10 kinds of Woo Lok oil samples were determined. The chemical structure of Woo Lok oil was determined by spectrum library searching, and the contents of methyl salicylate and menthol in the samples were obtained by peak area normalization method. The results are shown in Table 2.

Table 2 The determination results by GC-MS

	Methyl salicylate/(mg/ml ⁻¹)	Menthol/(mg/ml ⁻¹)
HLY1	247.7	200.3
HLY2	285.4	227.7
HLY3	411.6	319.5
HLY4	488.8	375.7
HLY5	445.3	344
HLY6	695.1	525.7
HLY7	285.2	227.5
HLY8	102	94.3
HLY9	336	264.5
HLY10	259.7	209

From Table 2, it can be seen that the content of these two main chemical compositions in different samples of Woo Lok oil varies widely. From the same manufacturer, the content of HLY1 and HLY2 is relatively consistent, and so is that of HLY4 and HLY5. But HLY8 is much different from other samples in composition. The variation range of the sample's composition is wide, which may be applied to NIR quantitative analysis model.

4.2 Near Infrared Spectrogram Analysis of Woo Lok Oil Samples

Woo Lok oil is a traditional Chinese medicine with complex composition, and the collected near infrared spectrum is the superposition of the near infrared spectrum of all samples. There are obvious differences in the composition, color and density of Woo Lok oil for different brands and qualities, and the spectrogram is also different.

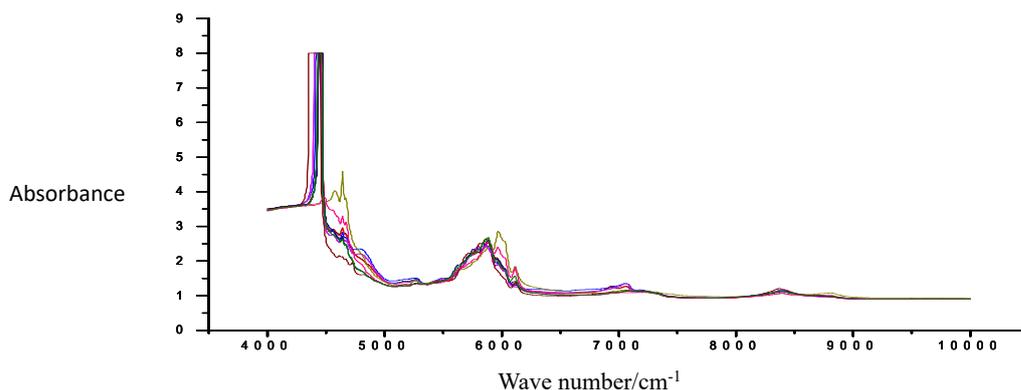


Figure 1 Near-infrared spectrum of all samples

The spectrum of each Woo Lok oil sample is averaged, and the average spectrogram is obtained, as shown in Figure 1. There are obvious differences in the spectrum of Woo Lok oil samples from different manufacturers, which are caused by the different composition of main ingredients in the samples produced by different manufacturers, and they are represented as a whole in the near infrared spectrum. It provides reliable and abundant data information for us to establish the quantitative model of main ingredients of Woo Lok oil by NIR and PLS.

4.3 Establishment of Quantitative Analysis Model

Through the PLS method of TQ Analyst software, the spectrum of the sample training set and the internal

prediction set selected in 2.3 are imported, and the abnormal samples appearing in the process of near infrared modeling are properly eliminated to improve the prediction accuracy of the prediction mode⁷¹². The vibration information of chemical bonds in the composition of different ingredients leads to the variation of the best spectral band of each ingredient. As R_C , R_P , R_V of the established model approach 1, RMSEC, RMSEP and RMSECV become smaller. The more similar the data, the prediction is more accurate and the model is more stable. Through repeated optimization, the best spectral pretreatment method and the best spectral band of the two main ingredients listed in Table 3 are obtained. The quantitative analysis model of methyl salicylate in the sample (Model 1) is shown in Figure 2, and the quantitative analysis model of menthol in the sample (Model 2) is shown in Figure 3.

Table 3 Optimal NIR model parameters of methyl salicylate and menthol

Composition	Pretreatment method	Band of modeling(cm^{-1})	RMSEC	R_C	RMSEP	R_P	RMSECV	R_V	Performance index
Methyl salicylate	Original spectrum (SG light filtering)	9068.22~8278.29	0.122	0.9969	0.144	0.9929	0.453	0.9681	91.2
		6309.02~5639.42							
		4985.63~4495.53							
Menthol	Original spectrum (No light filtering)	8524.63~8209.44	0.119	0.9943	0.154	0.9986	0.684	0.8084	87
		7128.07~6727.63							
		6169.04~5934.69							
		5890.84~5660.34							
		4755.22~4492.99							

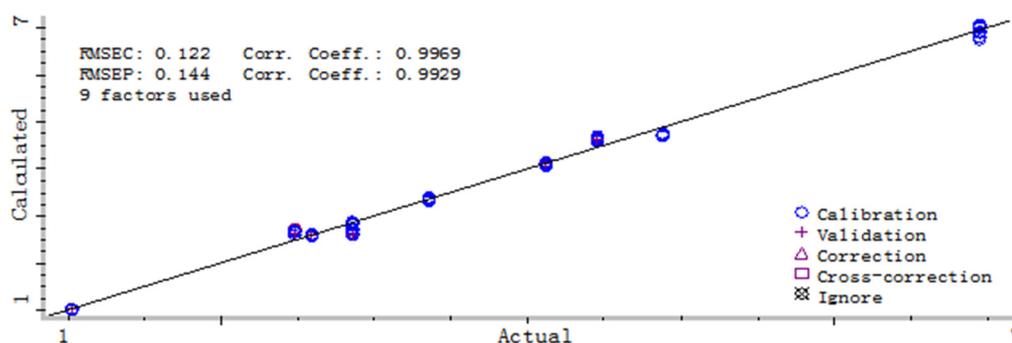


Figure 2 Quantitative analysis model of methyl salicylate

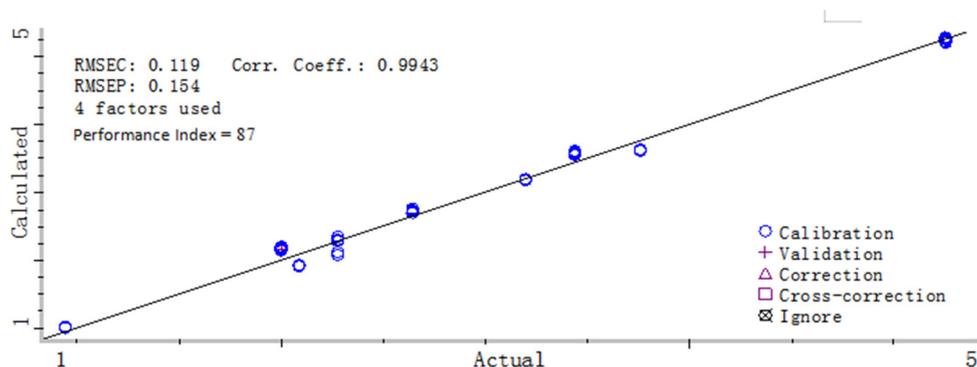


Figure 3 Quantitative analysis model of menthol

As can be seen from Table 3, except for the cross-validation coefficient R_V of the menthol model, the correlation coefficients of calibration, prediction and cross validation are all above 0.9. The root mean square error of calibration, root mean square error of prediction and root mean square error of cross validation of methyl salicylate and menthol models are 0.122, 0.144, 0.453 and 0.119, 0.154, 0.684 respectively, and the performance index is 91.2 and 87, respectively. From the cross validation correlation diagram in Figure 4, it can be seen that there is a good linear relationship between the

predicted value and the standard value of methyl salicylate model, with absolute error ranging from -0.098% to 0.082% and relative error ranging from -9.986% to 8.195%. Although the linear relationship of menthol model is moderate (Figure 5), the absolute error between the predicted value and the measured value of the model is in the range of -0.173 ~ 0.194%, and the relative error is less than 10% in the range of -7.25 ~ 9.69%. Therefore, the model meets the requirements of near infrared quantitative analysis model and can be used for detecting menthol content.

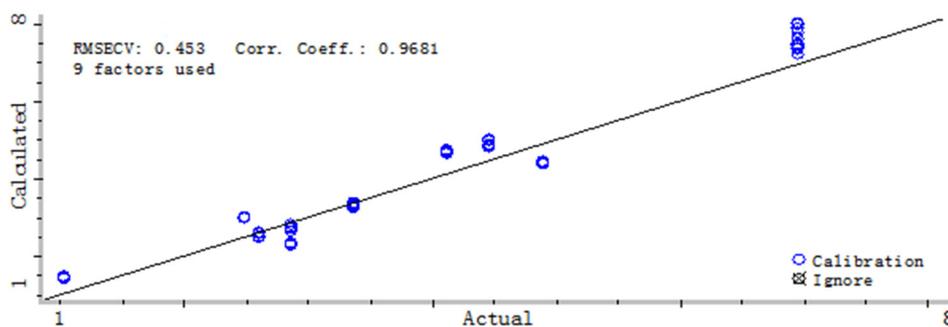


Figure 4 Cross validation correlation diagram between NIR predicted values and standard values of methyl salicylate

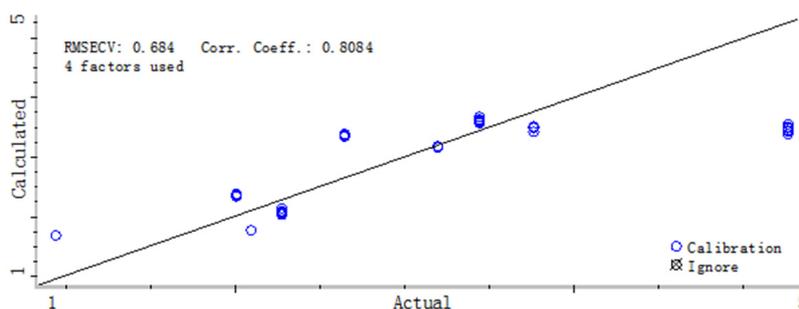


Figure 5 Cross validation correlation diagram between NIR predicted values and standard values of menthol

4.4 Application of the model

According to the method of 2.4, the model was verified by external application. The results are shown in Table 4. There is no significant difference between the standard values of methyl salicylate and menthol in Woo Lok oil measured by GC-MS and the values predicted by the

established near infrared model. The relative error is all less than 10%, and the mean relative error is about 3%. The results show that the model has a good prediction effect, meets the requirements of the near infrared quantitative analysis model, and can be used to detect the contents of methyl salicylate and menthol.

Table 4 Variation range and deviation between predicted values and standard values in the validation sets

Composition	Variation range of GC-MS standard values/%	Variation range of NIR predicted values /%	Absolute error/%	Relative error/%	Mean relative error/%
Methyl salicylate	1.02~6.95	1.01~6.93	-0.098~0.082	-9.986~8.195	3.62
Menthol	0.94~5.26	0.97~5.27	-0.173~0.194	-7.25~9.69	3.39

The predicted value of the model and the measured value of GC-MS went through F test to check the significance of difference between the model testing method and GC-MS determination method. The results are shown in Table 5: Methyl salicylate $F=1.01 < F_{\alpha=0.05}=3.20$, menthol $F=1.04 < F_{\alpha=0.05}=3.20$. The test values of the two models are all less than the critical value, indicating that there is 95% probability that the difference on precision between the predicted values of these two models and measured values by GC-MS is not significant, and it can meet the requirements of rapid determination of its content.

Table 5 E-test Results

	Methyl salicylate	Menthol
s^2 (GC-MS)	17.11	9.06
S^2 (Model Prediction)	17.28	8.72
F	1.01	1.04
$F_{\alpha=0.05}$	6.390	

5 Conclusions

In this study, the spectral information of Woo Lok oil samples was collected by near infrared spectroscopy, and the difference in spectral information was analyzed by partial least square method. The spectral information was correlated with the measured values of the corresponding samples, and for the first time the near infrared quantitative analysis model of methyl salicylate and menthol in Woo Lok oil was established. The established model has good predictive ability and stability. The method has the characteristics of being quick, easy, accurate, environment friendly and green. It can be applied to the detection of large quantities of Woo Lok oil products, and also provides a new research idea and method for the large-quantity multi-index quality evaluation and control of other traditional Chinese medicine oils.

Acknowledgement

Foundation item: Project of science and Technology Assisting Agriculture of rural science and Technology Commissioner in Guangdong Province (163-2019-XMZC-0009-02-0065), Science and Technology Program of Guangdong Province (2020B121201013): Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural

Resources in Mountainous Areas, Guangdong Engineering Technology Developing Center of High-Performance CCL

References

- Li Qifeng, Xiao Fei, Wang Zhenhua, et al. Study on Content Determination Method and Sample Determination of HX Woo Lok Oil. *Journal of China Prescription Drug*. 2016, 14(08): 19-21.
- Lin Zi, Wang Hui. Literature Review of the Ingredients and pharmacological effects of Woo Lok Oil. *Journal of Clinical Medical Literature*. 2016, 3(38): 7691-7692.
- Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People's Republic of China* (2005 Edition 1). Beijing: China Medical Science Press. 2010: Appendix 38-39.
- Li Meigui, Ou Aiping, Lu Lijia. Quantitative analysis single marker used to simultaneously determine the content of menthol and methyl salicylate in Shi Ma Long Woo Lok oil. *Journal of North Pharmacy*. 2015, 12(2): 4-5.
- Zhang Runrong, Ye Xuefeng, Chen Haiyan. Determination of Eight Components in Huoluo Oil by Capillary Gas Chromatography. *Traditional Chinese Drug Research and Clinical Pharmacology*. 2016, 27 (3). 413-417.
- Pu Yiqiong, Zhang Tong, Xiang Le, et al. Determination of camphor, menthol, borneol and methyl salicylate in rheumatism analgesic plaster by capillary gas chromatography. *Chinese Traditional Patent Medicine*. 2009, 31(8): 1224-1226.
- Shan Tingting, Cao Hong, Xing Junbo, et al. Determination of Camphor Menthol Synthetic Borneol and Methyl Salicylate in Shangshizhitong Adhesive Plaster by GC. *Pharmaceutical Journal of Chinese People's Liberation Army*. 2014, 30(6): 525-529.
- Zhao Xin, Zhu Ling, Xiao Yuping, et al. GC-MS Analysis of Huoluo Oil. *Chinese Journal of Experimental Traditional Medical Formulae*. 2011, 17 (15): 116-118.
- Yao Wanqing, Peng Mengxia, Liu Ting. Near Infrared Spectroscopy Combined with Chemometrics and Its Application in Chemical Analysis. *Journal of Jiaying University*. 2018, 36 (5): 17-27.

10. Yao Wanqing, Peng Mengxia, Liu Ting. Nondestructive Identification of Golden Pomelo Varieties in Meizhou Based on Near Infrared Spectroscopy. *Food Research and Development*. 2019, 40 (18): 178-182.
11. Chen Ziyun, Peng Mengxia, Ruan Yundan. Rapid Determination of Limonene in Orange Essential Oil by Near Infrared Spectroscopy Model. *Food Research and Development*. 2019, 40 (18): 178-182.
12. Chu Xiaoli, Lu Wanzhen, Wang Yanbin. Establishment and Application of Calibration Model for NIR Spectroscopy. *Physical Testing and Chemical Analysis (Part B: Chemical Analysis)*. 2004, 32 (6): 255-261.