

## QuEChERS-Raman spectroscopy method for detecting imidacloprid residue in cucumbers

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**Abstract:** Raman spectroscopy has been more and more frequently used for pesticide residue detection research in recent years, but the development of sample pretreatment technology is relatively lagging behind. In the study, a rapid method had been developed for the determination of imidacloprid residue in cucumbers with the application of Raman spectroscopy technology and Quick Easy Cheap Effective Rugged and Safe (QuEChERS) sample preparation. Three batches of cucumber samples (the concentration of imidacloprid was within the range of 0.2–5 mg/kg) with different preparation steps (acetonitrile extraction, dehydrate extraction, and fading removing impurity) were chosen as experimental objects. Confocal micro Raman spectrometer was utilized with a 780 nm laser to collect three batches of samples of Raman spectra. Six quantitative prediction models of imidacloprid residue were established based on PLS and PCR methods. The results showed that in addition to the PCR model of the samples by two steps preparation, the residual predictive deviation (RPD) of the other five models was higher than 3. The samples which were only extracted with acetonitrile got the best modeling effect. The correlation coefficient of the calibration set and the prediction set were all above 0.99. The root mean square error of prediction (RMSEP) of PLS method was 0.148 mg/kg, and the RPD was 5.52, which obtained the highest precision of the six predictive models. The results could provide a strong basis for the following-up studies.

**Key words:** Raman spectroscopy; QuEChERS; pesticide residue; quantitative analysis

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## QuEChERS-拉曼光谱法测定黄瓜上的吡虫啉残留量

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**摘要:** 采用拉曼光谱技术结合 QuEChERS(Quick Easy Cheap Effective Rugged and Safe)样本前处理建立了黄瓜上吡虫啉残留量的快速检测方法。以进行了不同前处理步骤(乙腈提取、去水萃取、褪色除

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杂)的三批黄瓜样本作为实验对象,利用 780 nm 激光器采集样本的拉曼光谱图,并分别采取偏最小二乘(PLS)和主成分回归(PCR)算法建立了六个黄瓜中吡虫啉含量预测模型。结果表明,仅进行了乙腈( $C_2H_3N$ )提取一步前处理的样本建模效果最优,校正集及预测集的相关系数均在 0.99 以上,其中 PLS 的预测集相对分析误差(RPD)达到 5.52,说明模型具有一定的预测精度,此结果可为后续前处理简化研究提供有力依据。

**关键词:** 拉曼光谱法; QuEChERS; 农药残留; 定量分析

## 0 Introduction

In recent years, food safety incidents caused by excessive pesticide residues in variety of fruits and vegetables occasionally happen. People pay more and more attention to food safety of fruits and vegetables, especially pesticide residues<sup>[1]</sup>.

Currently, chromatography is the most mature method for pesticide residues detection with its high accuracy and stability, but limitations like complex process and slow detection speed restricted the real-time detection. Spectrometry is a popular detection method in food safety detection in recent ten years, and Raman spectroscopy, known as "molecular fingerprints", is based on molecular vibration spectrum to identify substances. Therefore, Raman spectroscopy has been more and more frequently used for pesticide residue detection research. But Raman spectroscopy also has the disadvantages such as its vulnerability to fluorescence interference, and samples' big effect on the spectrum. As a result, nowadays many studies based on Raman spectroscopy to detect pesticide residues need appropriate pretreatment for samples<sup>[2-3]</sup>. However, the development of sample pretreatment technology relatively lags behind the rapid development of instrument analysis technology over the past two decades. Therefore, it is necessary to improve the accuracy and stability of pesticide residues detection for Raman spectroscopy with convenient, high selectivity and green pollution-free sample pretreatment method.

QuEChERS (Quick Easy Cheap Effective Rugged

and Safe) method was put forward by an American chemist in 2003, which is rapidly applied to the analysis of pesticide residues in food. Now it is acknowledged as a rapid, accurate and effective sample pretreatment method for pesticide residues detection by the European standard committee (CEN) and the American association of analytical chemists (AOAC)<sup>[4-6]</sup>. In practical research, QuEChERS method was mostly combined with chromatography<sup>[7-9]</sup>, but the related research on the combined use with spectroscopy technology for fruit and vegetable pesticide residues detection was rare. Xie et al. established a rapid detection method of malathion in legume vegetables using QuEChERS sample preparation and SERS technology<sup>[10]</sup>. Zhai et al. identified and analyzed mixed pesticides of chlorpyrifos, deltamethrin and acetamiprid in apple samples by SERS and QuEChERS preparation technology<sup>[11]</sup>. Huang et al. used SERS technology coupled with a quick pre-treatment method to detect chlorpyrifos (CP) pesticide residue in rice<sup>[12]</sup>.

The procedure of QuEChERS method could be briefly summarized as three steps: acetonitrile extraction, dehydration extraction and fading removing impurity, but it was too complex for the real-time detection. This article aimed to explore the feasibility of simplifying QuEChERS step by step and the pretreatment method for imidacloprid quantitative detection in cucumber based on Raman spectroscopy. Now the research aiming to gradually simplify QuEChERS technology and combined with Raman spectroscopy technology to evaluate the quality of fruit

and vegetable had not been reported.

## 1 Materials and methods

### 1.1 Instruments, materials and reagents

Equipment: DXR laser confocal micro Raman spectrometer (American Thermo Fisher company); Vortex -5 type Vortex mixer (Jiangsu Qilingbeier Instrument Manufacturing co., LTD.); H1650R type refrigerated centrifuge table high speed (Heraeus Germany company); XS105DU type electronic balance (Swiss Mettler Toledo company); Academic Milli -Q type pure water machine (Milliq France).

Materials: several commercially available organic cucumbers.

Reagent (in addition to the other instruction, all were the analytical reagent): the standard substance imidacloprid in methanol solution (China Institute of Metrology Standard Substance, standard value: 1.00 mg/ml, relative expanding uncertainty: 1%).

### 1.2 Sample preparation

We formulated the following preparation plan according to GB 2763 -2014 rules of maximum residue limits of pesticide residues in food: preparation of 20 pesticide samples of imidacloprid residues (GB maximum residue is 1 mg/kg) with mass fraction in the range of 0.2 -5 mg/kg, the distribution range of the mass fraction near the GB maximum residues which has practical significance.

First, the fully shattered clean and dry cucumber slices which was washed with pure water were added in food processor, and crushing was taken as a background solution (Because of the processing of washing and drying etc., the existence of other residues in cucumber could be neglected). Before sample preparation, we took imidacloprid pesticide standard material solution from freezer, and opened the ampoule bottle after shaking up at room temperature. According to the scheme above, the pesticide samples were prepared and set aside.

### 1.3 Sample pretreatment

As previously mentioned, the process of

QuEChERS method could be briefly summarized in three steps: acetonitrile extraction, dehydrate extraction, and fading removing impurity. Three batches of solution samples in this research were obtained from the specific processing steps and shown in Fig.1. Then the under test samples were sent to academy of agricultural sciences and detected with traditional gas chromatography method, and the result was regarded as the true value of modeling at the later stage.

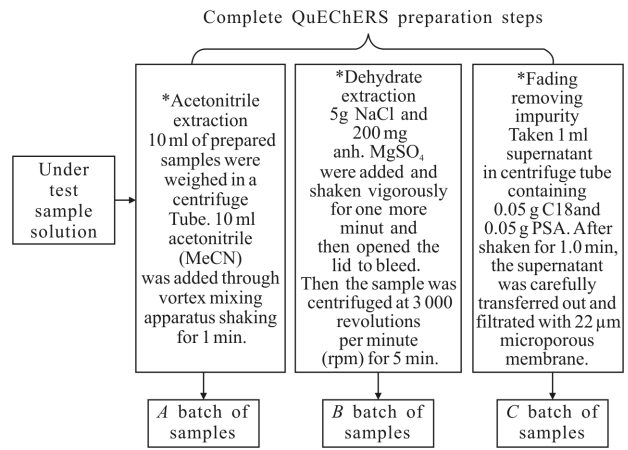


Fig.1 Sample preparation steps

### 1.4 Data collection

200 μL complete sample from 2.3 was injected in liquid pool separately, then we collected spectrums under the relative constant light intensity and the condition of room temperature. It had to be focused on the sample before spectral acquisition, firstly the samples were observed using micro lens and focused on the bottom of liquid, then the stage was adjusted to slowly move down by fine-tuning until gradually blurred the bottom, sometimes the minor impurities in samples could be observed by the correct focus on the cucumber juice samples. We selected an area within the focus scope to process the mapping scanning operation by using Raman spectrometer x-y platform, the specific instruments and measurement parameters were as follows:

Laser wavelength 532 nm (laser energy 10 mW), 780 nm (laser energy 24 mW) (by scanning the three batch samples with two laser scanners respectively, we received

six batch of Raman spectra), grating 400 lines/mm, aperture 50, estimation resolution was 4.7–8.7 cm<sup>-1</sup>, collection exposure time 3 s, fluorescent correction. Mapping scanning unit 30 μm\*30 μm, points 3\*3(i.e., each sample's mapping scanning obtain 9 spectrums).

**1.5 Research methods**

1.5.1 Spectra pretreatment

First the mapping scanning spectrums got from 1.4 was processed. The method employed in this research was manually eliminating singular samples and taking the average which means each sample would get an average spectrum at last in order to represent the sample for further spectral preprocessing and modeling.

The first derivative of spectra(1st derivative) and the second derivative (2nd derivative) were common methods used in spectral analysis of baseline correction and spectral resolution pretreatment. Norris derivation method was used in this paper, this method was put forward by Norris<sup>[13]</sup> and others at the earliest which was often called Norris derivation method. It was based on the theory basis of moving average smoothing method and the direct difference method<sup>[14]</sup>, namely that smooth the original spectrums before derivation.

1.5.2 Model evaluation indices

For building a good model, we must adopt some methods to validate the model's performance. The common evaluation indices were as follows: coefficient of determination(*R*<sup>2</sup>), correlation coefficient (*R*) and root mean square error of calibration/root mean square error of prediction (RMSEC/RMSEP).

In addition to the indices above, the prediction accuracy could be further evaluated using residual predictive deviation (RPD). The RPD was the ratio of standard deviation of independent forecasts sample set and the mean square error of prediction, and its expression was as follows:

$$RPD = SD / RMSEP \quad (1)$$

The parameter SD shown in Eq.(1) refers to the samples' standard deviation<sup>[15]</sup>. It could be concluded

that RPDS considered different models with different prediction true values when measuring model, so it could be more scientific and intuitive to further verify the accuracy of model prediction. The prophase research shown that if the *RPD*>3, the model prediction accuracy was high, it could be used in the actual detection; If 2.5<*RPD*<3, the quantitative analysis with the model was feasible, but its accuracy needs to be improved, only on the actual estimate; If the *RPD*<2.5, the model was difficult to quantitatively analyze.

**2 Experiment and discussion**

**2.1 Laser wavelength selection**

In this study, *A, B, C* three batch samples were scanned with 532 nm and 780 nm laser respectively, sample mapping scanning Raman spectrum was obtained, the average data could be gotten by manually removing singularity in 1.5 samples, finally we got the average spectral curves of the samples. Two wavelength laser spectral curves were shown in Fig.2.

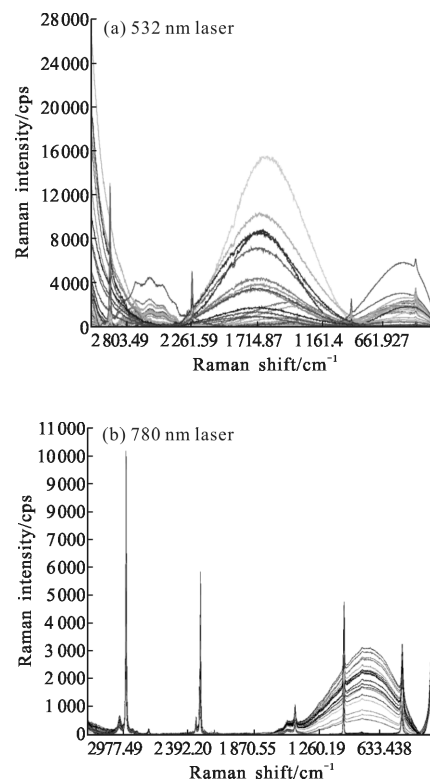


Fig.2 Average Raman spectroscopy

As could be seen from the prophase research, short wave laser could get larger Raman efficiency. In Fig.2, the Raman spectral intensity of 532 nm laser was generally higher than 780 nm laser, which was consistent with the previous research results. It also could be seen, however, from the figure that there were notable fluorescence peak bulges in the spectral curves from 532 nm laser which could be relevant to materials easily generating fluorescence effect in cucumber juice samples; On the other hand 780 nm laser Raman spectrums of samples of *B* batch only had a small bulge in 400–1 400  $\text{cm}^{-1}$  Raman displacement range, the rest of the characteristic peaks were significant, and there was no serious phenomenon of baseline drift, the quality of spectrums was much higher than 532 nm laser. This also coincided with the research of long wave laser which could reduce fluorescence interference. Based on the above discussion, we decided to adopt the sample spectrums got from the 780 nm laser scanning at later stage of the quantitative modeling in this experiment.

According to preliminary research, the most important band present in the Raman spectrum of acetonitrile ( $\text{C}_2\text{H}_3\text{N}$ ) was related to  $\text{C}\equiv\text{N}$  functional group, and it was observed at around 2 250  $\text{cm}^{-1}$ . The other Raman peaks in Fig.2(b) were all similar to the characteristic peaks of acetonitrile( $\text{C}_2\text{H}_3\text{N}$ ). Due to the complexity of the background, the characteristic peak of imidacloprid could not be observed directly, so we considered using some algorithms for quantitative analysis.

## 2.2 Exploration of gradually reduced sample pretreatment feasibility

### 2.2.1 Comparison of mapping scanning spectrums' standard deviation

In this paper, all the Raman spectrums were obtained from mapping scanning, the advantage of mapping scanning was that it could obtain  $n*n$  points' spectrums at the same time in the selected area. Through manual eliminating the singular sample points the method could reduce the possible random error

getting from single point scanning, but if the stability and repeatability of a few pieces of spectrums getting from the same sample were bad, the calculated average spectrum did not have good representation. The standard deviation was a kind of measure standard of the dispersion degree of data distribution which could be used to measure the degree of data values deviating from the arithmetic mean. The smaller the standard deviation was, the less the degree of discrete of the values relative to the average was, and vice versa. In this article the standard deviation would be used to measure the stability and repeatability of mapping scanning spectrums for the three batch samples. The average standard deviations of *A*, *B*, *C* batches of samples were shown in Fig.3.

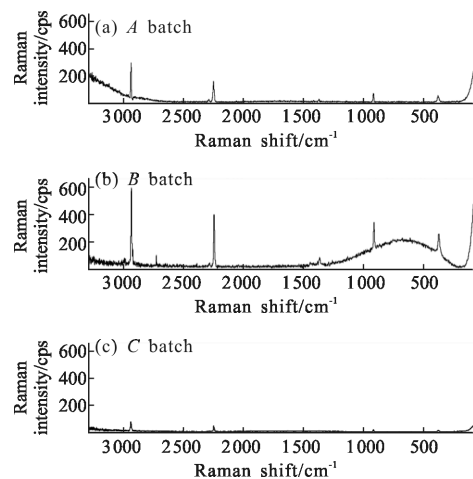


Fig.3 Average standard deviation of the three batches of samples

As could be seen from Fig.3, the standard deviations of three batch samples were all larger in the characteristic peaks. We selected and compared the standard deviation of 2 940  $\text{cm}^{-1}$  Raman shift: *A* batch of the sample standard deviation maintained in 300 cps, *B* batch of the sample standard deviation around 600 cps, *C* batch of sample standard deviation in 100 cps, and the standard deviation of the rest of the characteristic peaks were in line with a certain proportion. By comparing the index of standard deviation we could obtain the resultants of the stability and repeatability of the three batches of samples with *C* batch of samples was optimal, *A* batch of samples

was second, and *B* batch of samples was poor. This conclusion could provide a reference for later modeling results.

2.2.2 Build quantitative models of three batches of samples

SERS spectral data were analyzed using the TQ Analyst software(version 8.0) from Thermo Scientific. The spectral pretreatment method was adopted which smooth 7 points, 3 points difference width Norris derivative when building quantitative model for *A*, *B*, *C* three batches of samples, and the spectral range selection were the evident areas near the five characteristics of peaks. Partial least squares(PLS) and principal component regression(PCR) were selected as two kinds of modeling algorithm.

Figure 4 shows that the results of *A* batch of samples' PLS and PCR modeling. According to the sample quantity we selected three samples (arrow in the figure) randomly as the test samples in the process of verification used for testing the prediction effect of the two models. The predicted results were shown in Tab.1.

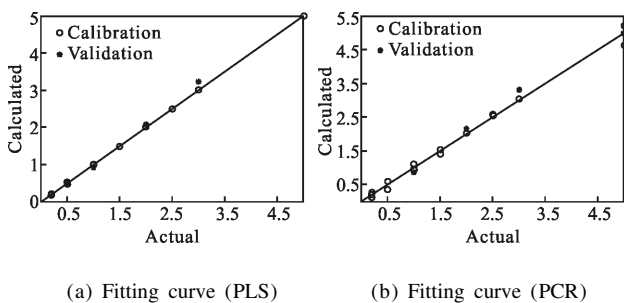


Fig.4 Modeling results of batch A

Tab.1 Predicting results of batch A

Actual/mg · kg <sup>-1</sup>	Algorithm	Calculated /mg · kg <sup>-1</sup>	Residual ratio
1.00	PLS	0.93	0.070
	PCR	0.86	0.140
2.00	PLS	2.09	0.045
	PCR	2.15	0.075
3.00	PLS	3.23	0.077
	PCR	3.31	0.103

The molding process of *A* and *B* samples was ditto. The modeling results and the prediction result

were shown in Fig.5 and Fig.6, Tab.2 and Tab.3. It could be get the information from the residual ratio shown in Tab.1 –3 that each three predictive value relative to the true value of quantitative model had a certain deviation. Overall, in addition to the individual low concentration samples, most of the test samples of residual percentage were within 20%. Among them, the predicted result of batch *A* of samples was optimal, of which the residual percentage almost kept within 10%, *C* batch of samples was second, *B* batch of samples was the worst. Moreover by comparing the predicted results obtained from PLS and PCR, except for the two prediction samples of batch *B*, the residual ratios of the rest of predicted samples obtained from the PLS algorithm were all lower than that from the PCR.

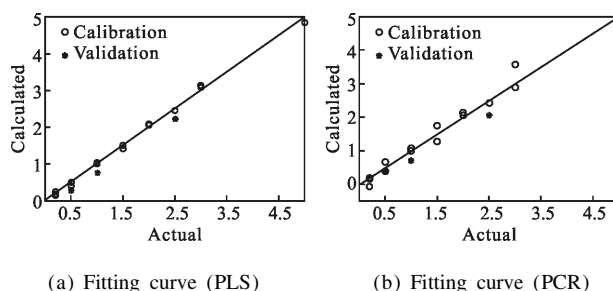


Fig.5 Modeling results of batch B

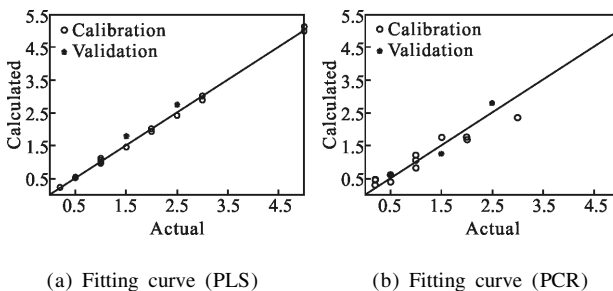


Fig.6 Modeling results of batch C

Tab.2 Predicting results of batch B

Actual/mg · kg <sup>-1</sup>	Algorithm	Calculated /mg · kg <sup>-1</sup>	Residual ratio
0.50	PLS	0.29	0.420
	PCR	0.38	0.240
1.00	PLS	0.76	0.240
	PCR	0.72	0.280
2.50	PLS	2.22	0.112
	PCR	2.06	0.176

**Tab.3 Predicting results of batch C**

Actual/mg · kg <sup>-1</sup>	Algorithm	Calculated /mg · kg <sup>-1</sup>	Residual ratio
0.50	PLS	0.56	0.120
	PCR	0.63	0.260
1.00	PLS	1.78	0.187
	PCR	1.26	0.160
2.50	PLS	2.75	0.100
	PCR	2.82	0.128

As shown in the Tab.4, in addition to the residual ratio of predicted samples, we also adopt correlation coefficient, root mean square error of calibration, root mean square error of prediction and residual predictive deviation as the evaluation indicators of quantitative analysis model of performance.

**Tab.4 Performance indexes of the quantitative models**

Batch number	Algo-rithm	Factors	Calibration set		Prediction set		
			RMSEC /mg · kg <sup>-1</sup>	Rc	RMSEP /mg · kg <sup>-1</sup>	Rp	RPD
A	PLS	8	0.016 4	0.999 9	0.148	1	5.52
	PCR	10	0.123	0.996 6	0.215	0.999 5	3.80
B	PLS	7	0.075 4	0.998 3	0.247	1	3.44
	PCR	10	0.246	0.982 1	0.312	0.998 9	2.72
C	PLS	9	0.063 8	0.999 1	0.223	0.997 9	3.66
	PCR	4	0.286	0.983 4	0.242	0.971 8	3.37

Combined with the data in Tab.4 we got the following three conclusions. First of all was the prediction precision. Besides the PCR models of B batch of sample,  $RPD=2.72 < 3$ , the rest of the model of RPD were greater than 3 which showed that the model had higher prediction accuracy; Followed by a comparison of the two algorithms, the three quantitative models of calibrating the correlation coefficient(Rc) and correlation coefficient of prediction set (Rp) established by PLS were above 0.99, RPD were above 3.5 which was higher than PCR model

results separately; Last, we compared the model results of the three batch samples, because the modeling effect of PLS algorithm was general better than that of PCR, so we only contrasted the PLS models' results: the calibration set correlation coefficient  $Rc(A) > Rc(C) > Rc(B)$ , the forecasting set relative analysis error  $RPD(A) > RPD(C) > RPD(B)$ . Among these  $RPD(A)=5.52$ , the model prediction accuracy was much higher than results of B and C two batches of samples.

### 3 Conclusion

In summary, a simple, rapid and sensitive method for detecting imidacloprid residues quantity in cucumber by using Raman spectroscopy combined with QuEChERS sample pretreatment technology was established. We adopted Norris derivative as the spectral pretreatment method and selected PLS and PCR as the modeling algorithms. Six predicted results obtained by two modeling algorithms were all in good for the samples of different pretreatment. In addition to the PCR model of B batch samples, the remaining five model analysis of the prediction set relative error were all greater than 3 which embodied good prediction accuracy. The model effect of only one acetonitrile extraction step pretreatment of the samples was better than multistep pretreatment of samples. For A batches samples, the root mean square error of prediction of PLS method was 0.148 mg/kg, and the RPD was 5.52. Obviously acetonitrile extraction was simple compared with complete QuEChERS sample preparation steps, and this study could provide a powerful basis for the study in simplifying the sample pretreatment with Raman spectroscopy detection of pesticide residues.

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