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Detection of difenoconazole pesticides in pakchoi by surface-enhanced Raman scattering spectroscopy coupled with gold nanoparticles

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Difenoconazole is an highly effective and broad-spectrum triazole bactericide pesticide and was generally applied to protect and cure foods such as vegetables and fruiter, then pesticide residue may pose a threat to mankind for their contaminations in foodstuffs, the detection and identification of trace pesticides is an urgent need to develop. In this study, we have been presenting a surface-enhanced Raman scattering (SERS) spectroscopy method for detecting difenoconazole in pakchoi using a portable Raman analyzer. The whole experiment for each sample, including sample preparation, solvent extraction and SERS spectra collection, was completed in about 15 min. Density functional theory(DFT) calculations were executed with Gaussian 03 at the B3LYP/6-311G basis sets. Solid, theoretical and SERS spectroscopy of difenoconazole were contrasted to analyze the assignments. Magnesium sulfate, PSA, graphitized carbon and C18 were used to reduce the distractions of chlorophyll, protein and other substances in pakchoi. The original spectra were preprocessed by the methods of MSC, SNV, first derivative, second derivative, smoothing and Normalization and then used to establish the prediction models by the method of Partial Least Squares(PLS), and the prediction model property of SNV is optimal. The correlation coefficient of prediction model (Rp) is 0.9458, root mean square error of prediction (RMSEP) is 3.27mg/L. The higher Rp value and lower RMSEP manifest that the established model of SNV can precisely detect difenoconazole residues in pakchoi. Five unknown pakchoi samples containing difenoconazole pesticide were used to verify the accuracy of the prediction model, and the values of relative

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deviation were calculated to be between 2.42% and 9.95%, and the predicted recovery rates were calculated to be between 94.64% and 109.95%. The T value is 0.475, which is smaller than $t_{0.05, 4}=2.776$. This indicates that it is not obvious difference between the predicted and measured values. This study demonstrates that SERS technique serves an effective approach for detection of difenoconazole in pakchoi quickly and stably.

1. Introduction

In the modern agricultural pest control is a major problem. In order to increase the output of crops, such as rice, cotton, fruits and vegetables, pesticides were applied broadly in agriculture. It was estimated that 20-50% of crops were economized by using pesticides.¹ But the use of pesticides may pose a threat to mammals and environment.² So the detection and identification of trace pesticides is developing. Maximum residue limits (MRL) have been stipulated for the reasonable use of pesticides according to the environmental influences and public security. Bactericide pesticides were one of the most usually used pesticides due to their systemic sterilization.^{3, 4} Difenoconazole, whose molecular formula is $C_{19}H_{17}Cl_2N_3O_3$, is a highly effective and broad-spectrum triazole bactericide pesticide used for growing vegetables and fruits.^{5, 6} The MRL of difenoconazole is 1 mg/L for pakchoi in China. Difenoconazole pesticide was generally applied to protect and cure foods such as vegetables and fruiter for black blain leaves, scab and grey leaf.^{7, 8} To data, analytical methods as gas chromatography-mass spectrometry(GC-MS)^{9, 10} and high performance liquid chromatography(HPLC)^{11, 12} have been reported for detecting difenoconazole pesticide residues. However, although accurate, but these methods are unfit for real time and rapid detection of pesticide because of expensive equipment, long detection time and professional staff. Therefore, the rapid detection and identification of trace difenoconazole pesticide in foods is of

particular interest for consumers and researchers.

Raman spectroscopy is a prodigious spectroscopic method for detection and identification some substances by the researchers in recent few years,¹³ such as dimethylaminochalcone and its cyclic analogs,¹⁴ and 3,4-methylenedioxymethamphetamine (MDMA) by methods of normal Raman spectroscopy, surface-enhanced Raman scattering and DFT.¹⁵ To data, Surface-enhanced Raman Scattering (SERS) method has been widely used for its enhanced sensitivity.¹⁶⁻¹⁸ Electromagnetic enhancement and chemical enhancement were considered to be two main SERS mechanisms. The electromagnetic mechanism provides us with the quantitative explanation, and chemical enhancement are also introduced to explain the spectral changes in SERS.¹⁹ Now research of SERS is still in preliminary stage, and its performance have been proved , such as anthraquinone derivatives on gold electrodes,²⁰ malathion pesticide used a new format of apta-sensing composite particles,²¹ and bacillus thuringiensis (Bt) applied a SERS-barcoded nanosensor.²² Similarly, a novel SERS substrate was developed by capillary monoliths with silver nanoparticles, and the enhancement factor is about 1.2×10^8 for determination of 4-mercaptopyridine and Rhodamine 6G. The substrate was detected phosmet on apples and tea leaves as low as 0.2 mg/kg and 0.5 mg/kg severally with a summary extraction procedure.²³ Carbendazim have reported on silver colloids at different pH values. DFT calculation was used to predict the connection between neutral, protonated or deprotonated species of carbendazim. There is a linear dependence between the relative intensity of the 1230cm^{-1} , 1270cm^{-1} and pH.²⁴ Raman Spectroscopy, Density Functional Theory and SERS were applied to identify Phenethylamines. The Raman Spectroscopy has a very good match with the DFT-calculated Spectroscopy without a scaling factor.²⁵ Cu@Ag/ β -AgVO₃ has an superb SERS property and was used to detect the carbamate pesticides(carbofuran, carbaryl, isoprocarb and propoxur). The substrate is a good choice as a SERS substrate compared to silver

nanoparticles.²⁶ Acephate was detected to the low parts-per-billion range using SERS method, and can be differentiated from urine components and structurally similar pesticides including methamidophos.²⁷ SERS coupled with DFT was used to detect methamidophos (MAP) in vegetables at pH of 13.46, and had a good linear relationship at the range of 0.01 and 1000 ug/mL. The recovery rates were between 86.7% and 96.6 % and the relative standard deviations were between 1.2 and 2.5 %.²⁸ He et al. have developed a SERS method coupled with dendritic silver nanosubstrates for rapid detection and characterization of restricted antibiotics. Dendritic silver nanosubstrates were obtained through a simple replacement reaction and can be kept in deionized water for up to 6 months. The limit of detection for antibiotics could reach the level of 20 ppb.²⁹ Sandpaper was applied as template for vacuum deposition of silver. SERS spectra of triazophos pesticide were collected by swabbing different surfaces, such as Pear, tree leaf, plastic, glass. The characteristic peaks of triazophos at 1001 and 1599 cm^{-1} can be observed on glass, where 5 ng of triazophos spread on 4cm^2 area.³⁰ Therefore, the SERS technology may be used for detection and identification pesticides. While the difenoconazole detection in pakchoi using SERS methods have been scarcely reported.

Here in this report, we aim to use a SERS method coupled with chemometrics method for detecting difenoconazole in pakchoi. DFT calculations were executed with Gaussian 03 at the B3LYP/6-311G basis sets. Samples were prepared to extract difenoconazole pesticide residues in pakchoi. Magnesium sulfate, graphitized carbon, PSA and C18 were applied to reduce the distractions of chlorophyll, protein and other substances in pakchoi. Gold nanoparticles was used to enhance the samples Raman spectra, and the samples was applied to collect their SERS spectra using a portable Raman analyzer and measure their actual values by GC-MS. The original spectra were preprocessed by the methods of MSC, SNV, first derivative, second derivative, smoothing and

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3 90 Normalization and then used to establish the prediction models by the method of PLS. The
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5 91 predicted recovery and paired-samples *T* test were used to verify the performance of the prediction
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11 93 **2. Experimental**
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15 94 **2.1 Reagents and Chemicals**
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17 95 Difenoconazole (99.5%) was gained from the National standards material information center a. The
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19 96 all preparation substrates containing acetonitrile and sodium chloride were bought from merchant
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21 97 sources as analytical pure reagents. OTR202 and OTR103 were purchased from OptoTrace
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23 98 Technologies, Inc. The all materials of GC-MS were purchased from Agilent Technologies co.,LTD.
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25 99 Pakchoi without difenoconazole pesticide was supplied by the experimental base of Jiangxi
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27 100 Agricultural University.
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33 101 **2.2 Sample Preparation**
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36 102 A 100 mg/L stock standard solution was prepared by dissolving 20 mg difenoconazole power into
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38 103 200mL volumetric flask with acetonitrile and used to prepare working solutions of 20, 10, 5, 2, 1,
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40 104 0.5, 0.2 and 0.1 mg/L with deionized water.
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43 105 Pakchoi without pesticide was applied to manufacture experimental samples as follows. 50 g
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45 106 pakchoi was flatted on plastic film. 93 different concentration pakchoi samples were prepared by
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47 107 spraying stock standard solution proportionately and named 1 to 93, two replicates were prepared
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49 108 for each sample. Then the 93 samples were respectively crushed by pulverizer (MJ-BLA25C5,
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51 109 Midea Group, China).
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56 110 The steps were implemented for SERS spectra collection and the measurement of actual values
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58 111 as follows. (1) 10 g homogenized pakchoi sample, 1 g sodium acetate, 10 mL acetonitrile and 5 g
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sodium chloride were blended into a 50 mL centrifuge tube, and mixed for 1 min with a vortex mixer (Vortex-BE1, Beijing Kaiyuan Guochuang Technology Co., Ltd, China), and then a shapely solution was obtained. The solution was centrifuged for 5 min by a centrifugal machine with a speed of 4200 rpm (PGZ1250, Zhangjiagang City Yongda Machinery Co., Ltd, China) and then turned into a green supernatant. (2) 2 mL of the supernatant was shifted into a 15 mL centrifuge tube containing the suitable amount anhydrous Magnesium sulfate, graphon, PSA and C18. The solution of the centrifuge tube was centrifuged by a centrifugal machine for 5 min at a speed of 4200 rpm and then turned into a colourless supernatant, and then filtered. The filtrate may be used to collect Raman spectrum directly. (3) The 1 mL filtrate was condensed by a concentrator (BYDCY-36S, Shanghai Bingyue Electronic Instrument Co. Ltd, China) until the acetonitrile completely evaporated. (4) 1 mL ethyl acetate was used to elute the condensed pesticide, and then the eluted solution was injected into a vial. then its actual value was obtained by a gas chromatograph (Agilent 7890B, Agilent Technologies Co., Ltd, USA).

2.3 SERS collection and GC-MS Measurement

Raman spectra were collected with a portable Raman analyzer equipped with a charge-coupled device (CCD). SERS spectra were collected by Raman Analyzer-V791B. The SERS measurements were carried out with a 785 nm diode laser source, a laser power of 200mW, spectral distinguishability of 4 cm^{-1} , exposure time of 10 s and a detection range of 400 to 1800 cm^{-1} . The Raman apparatus was calibrated using acetonitrile before measurements. The solid Raman spectrum of difenoconazole was collected with solid probe on a glass slide. OTR202 and OTR103 were used for enhancement effects. OTR is the abbreviation of OptoTrace. OTR202 was a gold nanoparticles and OTR103 was an activating agent. OTR202 was used to enhance the Raman signal. The maximum UV-visible absorption peak was appeared at 536 nm. To collect Raman spectra, 500 μL

OTR202, 20 μ L analytical sample and 100 μ L OTR103 were injected into a quartz bottle. Each sample was scanned three times and an average spectrum was produced as eventual spectrum for analysis. The sample measured values were implemented by a gas chromatographon equipped with a flame ionization detector. High purity helium was used for a carrier gas with a 9.7853 psi pressure and a 1.2 mL/min flow rate.

2.4 DFT calculations

All calculations were performed with Gauss View 3.07 software (Gaussian, Inc., Pittsburgh, PA, USA) at the hybrid functional methods RB3LYP and employing 6-31G(d,p) basis set for all atoms.^{31, 32} A scaling factor (0.9816) was used to the calculated spectrum for sufficient clarity between the experimental spectrum and calculated spectrum.

2.5 Data analysis

MATAB R2010a (Matworks Inc., Natick, MA, USA) with a free PLS Toolbox was applied for PLS. The original spectra were pretreated by the methods of MSC, SNV, first derivative, second derivative, Smoothing and Normalization and then used to establish the prediction models by the method of PLS. The prediction model performances were evaluated in terms of the correlation coefficient of the calibration samples model (R_c), Root mean square error of cross validation (RMSECV), R_p and RMSEP. The model with the higher R_p value and the lower RMSEP value is considered to have a better performance. To confirm the recoveries of difenoconazole in pakchoi, five different pakchoi samples contained unknown difenoconazole concentration were prepared by the same analytical procedure. The recoveries were obtained with the predicted concentrations divided by the measured concentrations. The paired-samples t test was implemented on SPASS V17.0 (SPSS Inc., USA), $t_{<t_{0.05, 4}=2.776}$ was considered significant.

3. Results and discussion

3.1 Theoretical and experimental Raman spectra of difenoconazole

Difenoconazole molecules are composed of chlorophenyl ether, dioxypentane, methyl, triazole containing the bands of C-C, C=C, C-O, C-N, C-Cl, C-H and C=N. The experimental Raman spectrum and the DFT-calculated spectrum of difenoconazole for comparison are shown in Fig.1(a) and (b). There are some differences between the experimental and DFT-calculated Raman spectra of difenoconazole in the peak strength, and some peaks of experimental Raman spectrum do not appear in theoretical Raman spectrum. This may be because the material of the theoretical calculation is isolated gaseous molecule form, not considering the mutual interaction with molecules and the disparity between the theoretical calculation simulated orbit and molecular real orbit. Difenoconazole molecules have long chain branch, and there is the inordinate coupling of local vibration between the long chain branch and main structure. But the peak position of experimental Raman spectrum is consistent mainly with the DFT-calculated spectrum.

[Figure 1 about here]

As shown in Fig.1 (a), the distinct peaks at 688, 808, 1086, 1161, 1194, 1363 and 1604 cm^{-1} are observed and attributed as follows. The band at 808 cm^{-1} is tempestuously enhanced and assigned the breathing vibration mode of chlorophenyl ether. The band at 688 cm^{-1} may also be assigned the breathing vibration mode of chlorophenyl ether. The other major peaks observed are due to the C-Cl stretching mode and its breathing vibration mode of 4-chlorobenzene phenyl at 1086 cm^{-1} , the C-O stretching mode and in-plane bending mode of 4-chlorobenzene phenyl at 1161 cm^{-1} , as well as the C-O-C symmetric stretching mode and its breathing vibration mode of 4-chlorobenzene phenyl at 1194 cm^{-1} . The band at 1363 cm^{-1} is assigned the C=N and C-N stretching mode of triazole ring, coupled with the in-plane bending mode of C-H and out-plane bending of CH_2 . The bands at 1585

and 1604 cm^{-1} are assigned the C=C and C-C stretching mode of chlorophenyl ether. These characteristic peaks can be used for qualitative and quantitative analysis of difenoconazole molecules. By comparing of Fig.1(a) and (b), the other Raman peaks of difenoconazole molecules are assigned thoroughly as shown in table 1.

[Table 1 about here]

3.2 SERS spectra analysis of difenoconazole stock solutions

To verify that no interfering signal is generated by acetonitrile solvent, SERS and normal Raman spectra of 10 mg/L difenoconazole solution, as well as SERS spectrum of the background signal for contrast are displayed as shown Fig.2(a), (b) and (c). Fig.2(b) is consistent with the spectrum of acetonitrile and is not observed the characteristic peaks of difenoconazole, and the SERS spectrum of acetonitrile is no overlap with the Raman characteristic peaks of difenoconazole in Fig.2(a). As shown in Fig.2 (a), Some distinct peaks at 507, 633, 696, 808, 1088, 1159, 1194, 1585 and 1604 cm^{-1} can be observed, which manifests that a strong interaction have happened between colloidal gold with difenoconazole molecules. The intensity of 1194 cm^{-1} is sharply enhanced and it is due to the C-O-C symmetric stretching mode and its breathing vibration mode of 4-chlorobenzene phenyl. The band at 808 cm^{-1} is assigned the breathing vibration mode of chlorophenyl ether. These show that the method used SERS for detecting difenoconazole pesticide is feasible.

[Figure 2 about here]

Fig.3 displays the SERS spectra of different concentration difenoconazole solution at the range of 400-1800 cm^{-1} . As shown in Fig.3, the intensities of the characteristic peak strengthen with the increase of difenoconazole pesticide concentration. But it is because difenoconazole molecules and nearby nanoparticles have the interaction force with different absorbability and orientations that the alternative rates of the characteristic peaks are different. The peaks at 507, 633, 696, 1088, 1159,

1585 and 1604 cm^{-1} have a faster change, and the peaks at 808 and 1194 cm^{-1} have a slower variation relatively. It is regarded as the reachable detection concentration of SERS method that the most characteristic peak of lowest concentration is still visible. The bands at 808 and 1194 cm^{-1} can be still observed but very weak in Fig.3(f). These indicate the SERS method used for detecting difenoconazole solution is feasible even below 0.2mg/L.

[Figure 3 about here]

3.3 Detection of difenoconazole pesticide residues in pakchoi

Magnesium sulfate, PSA, graphitized carbon and C18 were used to reduce the distractions of chlorophyll, protein and other substances. The SERS spectra of difenoconazole solutions in pakchoi are displayed in Fig.4 with purification. The characteristic peak at 808 and 1194 cm^{-1} are strengthened and identified easily in the SERS spectra, which is beneficial to detect difenoconazole residues in pakchoi. As shown in both Fig.4(a)-(d), the peaks at 696, 808, 1194, 1585 and 1604 cm^{-1} are obviously observed. As shown in Fig.4(e), the peaks at 808, 1194 and 1604 cm^{-1} are also observed, while the intensities are decreased obviously, and the peaks at 696 and 1585 cm^{-1} is very weak and can not be identified. The peaks at 808 and 1194 cm^{-1} is very weak and are not identified as shown in Fig.4(f), and the other peaks can not be observed. Fig.4(f) is almost coincident with Fig.4(g). These manifest that the SERS method can be used to detect difenoconazole pesticide residues in pakchoi even in concentration below 0.4143mg/L. The intensities of characteristic peaks strengthen with the increase of concentration as shown in Fig.4, and there may exist a linear relationship between the intensities of Raman characteristic peaks and concentrations of difenoconazole solutions extracted from pakchoi. So multivariate methods can be used to establish the prediction model for quantitative analysis difenoconazole pesticide residues extracted from pakchoi with SERS spectra.

[Figure 4 about here]

3.4 Measured values by GC-MS

The 93 samples were measured by GS-MS method for establishing a model between the range of 0.4143~40.2335 mg/L and divided into two subclasses on the basis of their measured values. One subclass was used to build the calibration model and named the calibration set, and the other subclass was used to verify the model reliability and named the prediction set. As shown in Table 2, a division method of 2:1 calibration/prediction was implemented. The calibration and prediction set severally includes 62 and 31 samples, and the scope of the calibration set includes nearly the scope of the prediction set.

[Table 2 about here]

3.5 PLS models with SERS spectra preprocessing

The original spectra were pretreated by the methods of MSC, SNV, first derivative, second derivative, Smoothing and Normalization and then used to establish the prediction models by the method of PLS. These parameters of Rc, Rp, RMSECV and RMSEP were used to verify the model performances. The model with the higher Rp value and the lower RMSEP value is considered to have a better performance. The performances of prediction models with MSC, SNV and Normalization are better than the prediction model performance of original spectra, but the performances of other three pretreated methods are not better than the performance of original spectra, and the prediction model property of SNV is optimal as shown in Table 3. The lower RMSEP and the higher Rp value using 14 latent variable were obtained. Rc is 0.973 and RMSECV is 2.26 mg/L in the calibration set. Rp is 0.9458 and RMSEP is 3.27 mg/L in the prediction set. These show that the established model of SNV can accurately detect the

difenoconazole pesticide residues extracted from pakchoi. The scatter diagrams of Calibration and Prediction set of SNV are shown in Fig.5 (a) and (b).

[Table 3 about here]

[Figure 5 about here]

3.6 Model Verification

Five different pakchoi samples contained unknown difenoconazole concentration were prepared by the methods reported in the chapter "Sample Preparation". Two replicates at all samples were prepared. Three parallel detections have been implemented for each sample. And then the five samples were used to collect their SERS spectra and measure their measured values by GC-MS method. The results of difenoconazole concentration from five different pakchoi samples using GC-MS and SERS method are summarized in Table 4. Table 4 shows that the values of relative deviation were counted to be between 2.42% and 9.95%, with the predicted recovery rates between 94.64% and 109.95%. The high recovery rate and low relative deviation indicates that the results of SERS method is reliable for rapid detection of difenoconazole pesticide residues extracted from pakchoi.

[Table 4 about here]

The measured and predicted values of five pakchoi samples were used to implement the paired-samples *T* test . The *T* value is 0.475, which is smaller than $t_{0.05, 4}=2.776$. This indicates that it is not obvious difference between the measured and predicted values.

4. Conclusions

In this study we have reported the SERS method for the qualitative and quantitative detection of difenoconazole pesticide residues extracted from pakchoi. The limits of detection (LOD) using the SERS method is capable of below 0.4143 mg/L, is lower than the MRL of difenoconazole for

271 pakchoi in China and much higher than the traditional detection method. But the method doesn't
272 need expensive equipment and professional staff, and the SERS method is simple, rapid and
273 inexpensive. The whole experiment for each sample, including sample preparation, solvent
274 extraction and SERS spectra collection, was completed in about 15 min. The original spectra were
275 pretreated by the methods of MSC, SNV, first derivative, second derivative, Smoothing and
276 Normalization and then used to establish the prediction models by the method of PLS, and the
277 prediction model property of SNV is optimal. Five unknown difenoconazole concentration pakchoi
278 samples were used to verify the accuracy of the prediction model. SERS resulted quite accurately
279 with the values of relative deviation were counted to be between 2.42% and 9.95%, and the
280 predicted recovery rates were between 94.64% and 109.95%. The paired-samples t test result
281 indicates that it is not obvious difference between the measured and predicted values. Therefore the
282 SERS method can be used to accomplish an effective approach for the rapid and reliable detection
283 of difenoconazole pesticide in pakchoi. The same method can be easily accepted to other pesticides
284 and agricultural products.

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337 **Tables captions**

338 **Table 1.** Comparison of the theoretical and experimental vibration frequencies of difenoconazole
339 and its assignment.

340 **Table 2.** The measured values of difenoconazole pesticide residues in pakchoi in the calibration and
341 prediction set.

342 **Table 3.** Results for each of the pre-processing method for the calibration and prediction model.

343 **Table 4.** Predicted value and Measured value of difenoconazole in pakchoi.

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345 **Figure captions**

346 **Figure 1.** Raman spectra of difenoconazole(a) experimental and (b) theoretical.

347 **Figure 2.** SERS spectra of 10mg/L difenoconazole solution, (b) normal spectra of 10mg/L

348 difenoconazole solution and (c) SERS of acetonitrile.

349 **Figure 3.** SERS spectra of different concentrations of difenoconazole solutions, (a)~(g): 10, 5, 2, 1,

350 0.5, 0.2, 0.1mg/L.

351 **Figure 4.** SERS spectra of difenoconazole solutions extracted from pakchoi with different

352 concentrations, (a~g):11.6026mg/L, 5.2446mg/L, 2.1346mg/L, 1.1232 mg/L, 0.4143mg/L,

353 0.236mg/L, blank.

354 **Figure 5.** Reference measurement versus Raman prediction in calibration set (A) and prediction set

355 (B).

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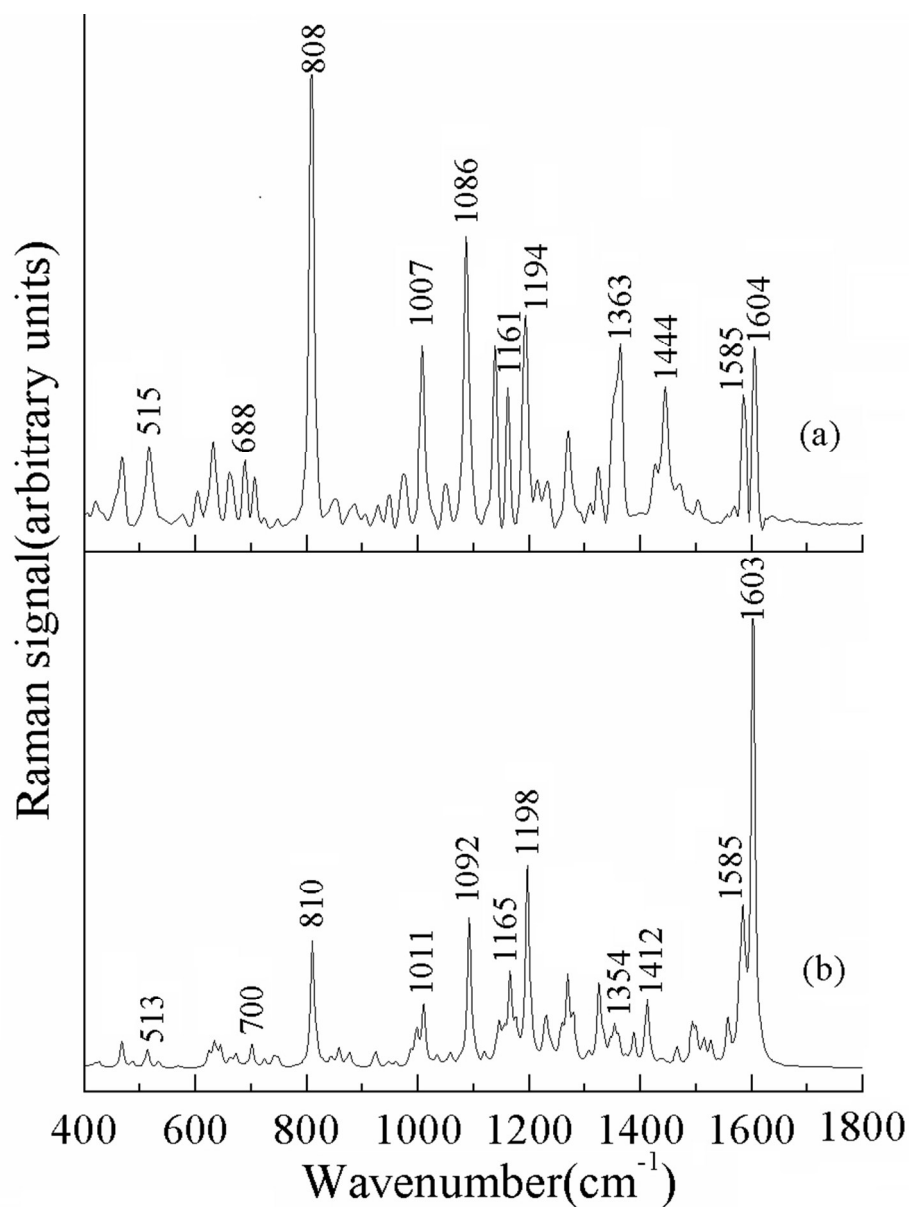


Figure 1. Raman spectra of difenoconazole(a) experimental and (b) theoretical. 244x318mm (96 x 96 DPI)

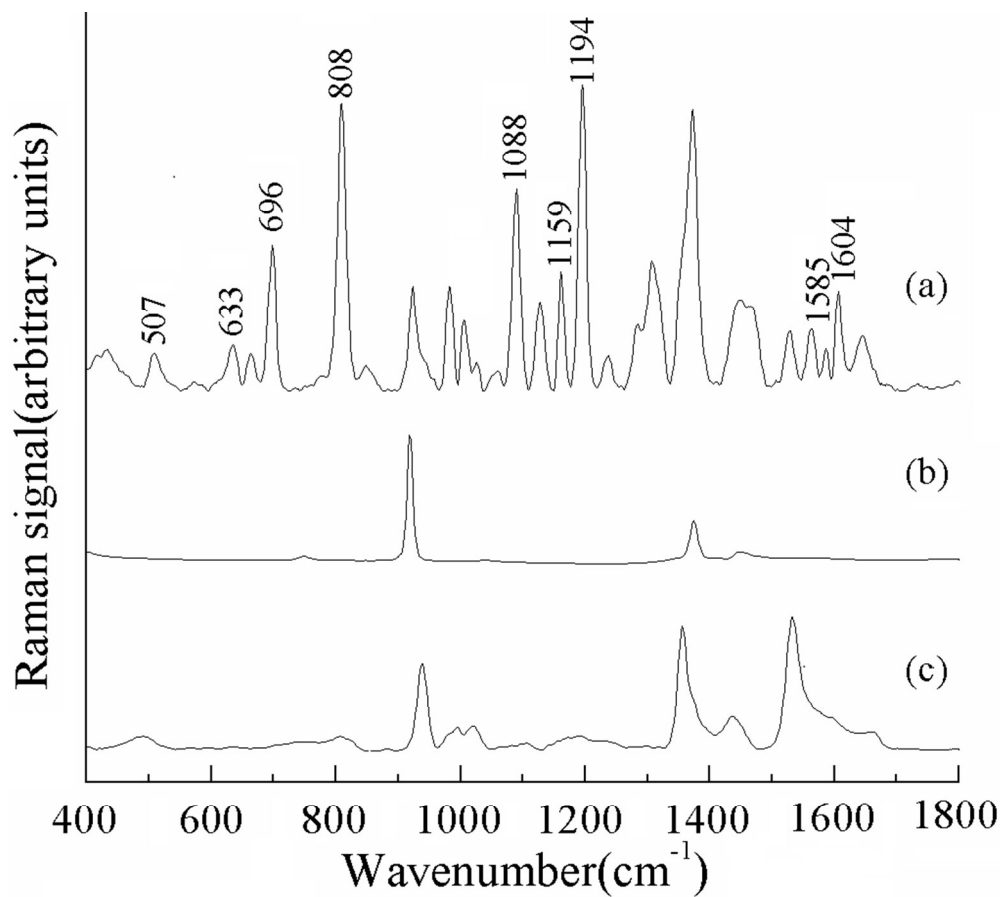


Figure 2. SERS spectra of 10mg/L difenoconazole solution, (b) normal spectra of 10mg/L difenoconazole solution and (c) SERS of acetonitrile
270x241mm (96 x 96 DPI)

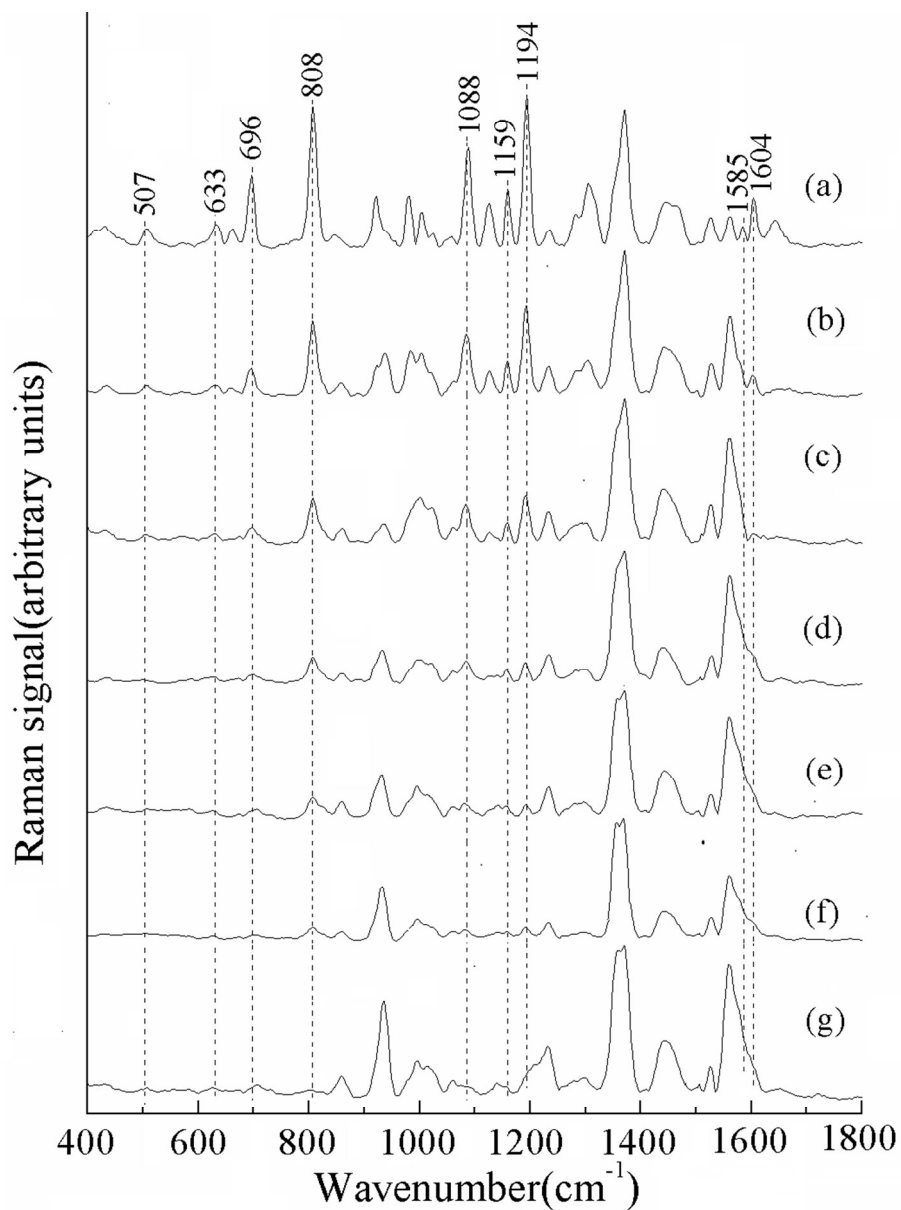


Figure 3. SERS spectra of different concentrations of difenoconazole solutions, (a)~(g): 10, 5, 2, 1, 0.5, 0.2, 0.1mg/L.
278x365mm (96 x 96 DPI)

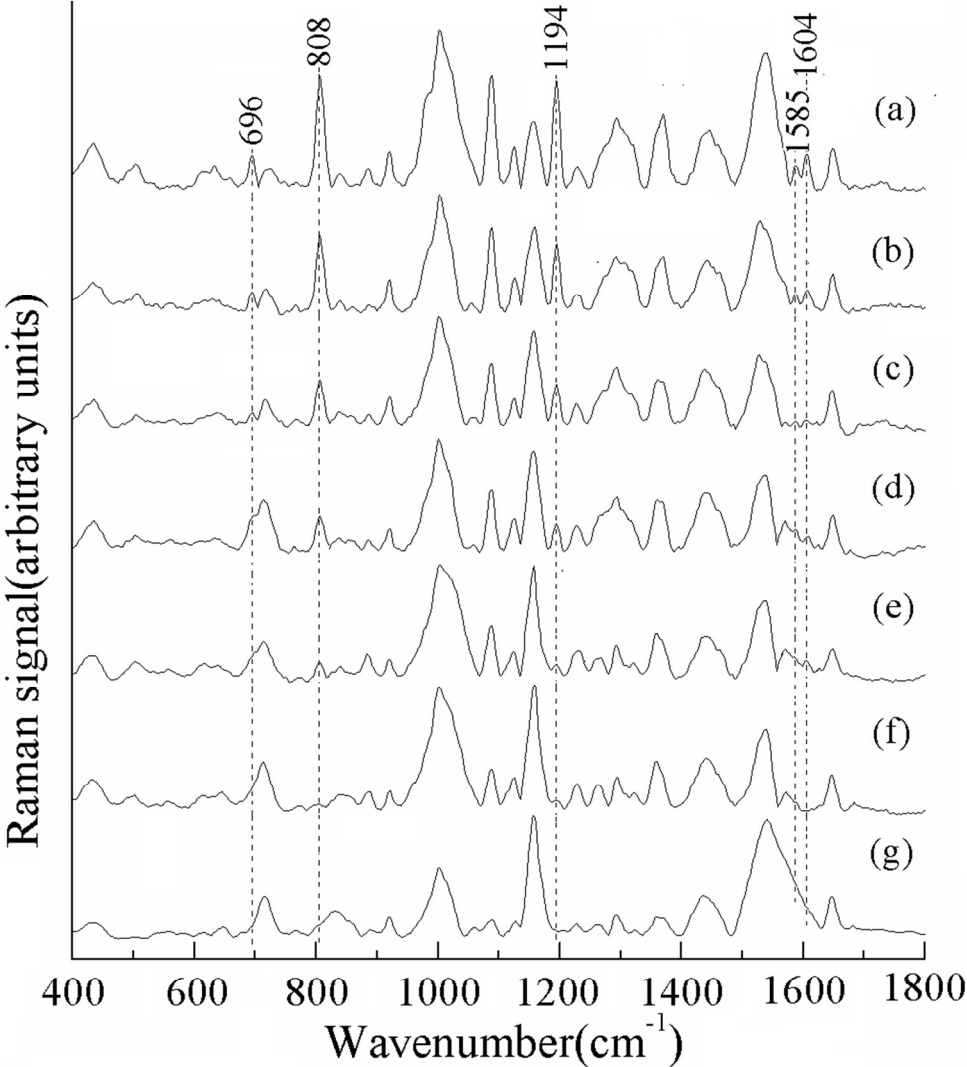


Figure 4. SERS spectra of difenoconazole solutions extracted from pakchoi with different concentrations, (a~g):11.6026mg/L, 5.2446mg/L, 2.1346mg/L, 1.1232 mg/L, 0.4143mg/L, 0.236mg/L, blank. 277x298mm (96 x 96 DPI)

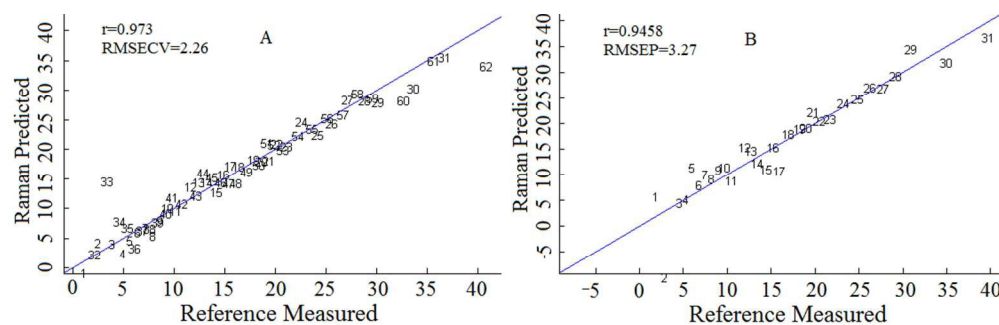


Figure 5. Reference measurement versus Raman prediction in calibration set (A) and prediction set (B).
298x96mm (96 x 96 DPI)

Table 1 Comparison of the theoretical and experimental vibration frequencies of difenoconazole and its assignment

^a Theoretical (cm ⁻¹)	^b Experimental (cm ⁻¹)	^c Assignment
513	515(m)	τ (4-Chlorophenyl Ether)
700	688	ν(3-Chloro-4- Phenyl-4-Chlorophenyl Ether)
810	808	ν(3-Chloro-4- Phenyl-4-Chlorophenyl Ether)
1011	1007	ν _{as} (C-O-C) of Chlorophenyl Ether ,ν(3-Chloro-4-Phenyl)
1092	1086	ν(C-Cl) of 4-Chlorophenyl, ν(4-Chlorophenyl)
-	1138	ν(C-N), ρ(C-H)
1165	1161	ν(C-O) and ρ(C-H) of 4-Chlorophenyl
1198	1194	ν _s (C-O-C), ν(3-Chloro-4- Phenyl-4-Chlorophenyl Ether)
1354	1363	ν(C=N), ν(C-N), ρ(C-H), τ (CH ₂)
1412	1444	ν(C-N), ρ(CH ₂)
1585	1585	ν(C=C), ν(C-C)
1603	1604	ν(C=C), ν(C-C)

^a Calculated wavenumbers at B3LYP/6-311G basis sets of theory.

^b s, strong; m, medium; w, weak.

^c δ, bending; ν,stretching; s, symmetric;τ, out-plane bending; as, asymmetric; ρ, in-plane bending.

Table 2. The measured values of difenoconazole pesticide residues in pakchoi in the calibration and prediction set

Two Subclasses	Number	Units	Range	Mean	Standard deviation
Calibration set	62	mg per kg	0.4143~40.2335	15.7236	9.6726
Prediction set	31	mg per kg	1.1232~39.0324	15.7335	9.7809

Table 3 Results for each of the pre-processing method for the calibration and prediction model

Pre-processing method	Principal components	Calibration		Prediction	
		Rc	RMSECV(mg/L)	Rp	RMSEP(mg/L)
Original spectrum	10	0.9425	3.22	0.936	3.43
MSC	11	0.9712	2.36	0.9453	3.29
SNV	14	0.973	2.26	0.9458	3.27
Normalization	12	0.9725	2.28	0.9466	3.24
first derivative	9	0.9133	2.85	0.8822	3.45
second derivative	9	0.9188	2.72	0.8907	3.3
smoothing	10	0.9327	2.64	0.9184	2.77

Table 4 Predicted value and Measured value of difenoconazole in pakchoi

Sample	Measured value (mg/L)	Predicted value (mg/L)	Relative deviation (%)	Predicted recovery (%)
1	0.6642	0.7303	9.95	109.95
2	1.5258	1.6513	8.23	108.23
3	4.5687	4.3239	-5.36	94.64
4	9.2641	9.4886	2.42	102.42
5	14.3464	13.8632	-3.37	96.63