Surface-Enhanced Raman Spectroscopy Coupled with Dispersive Solid Phase Extraction for the Rapid Detection of Tricyclazole Residues in Rice and Brassica campestris L. ssp. chinensis var. utilis

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Graphical abstract

Matrix 1: Rice

Matrix 2: Brassica campestris
L. ssp. chinensis var. utilis Tsen

D-SPE

32 nm Au nanoparticles

SERS

Note: A and B were tricyclazole data in rice and Brassica campestris L. ssp. chinensis var. utilis Tsen matrix, respectively.
Abstract

An efficient methodology has been developed to determine tricyclazole residue in matrix based on surface-enhanced Raman scattering (SERS) coupled with dispersible matrix solid phase extraction. After pretreatment and test conditions optimization, peaks at 1373 cm\(^{-1}\) and 1317 cm\(^{-1}\) in the SERS spectrum were respectively selected as quantitative peaks for rice and *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen, respectively. The matrix standard curve-external standard method was used to quantitatively conduct a statistical analysis. The correlation between the quantitative peak response and tricyclazole concentration showed a significant linear relationship with a correlation coefficient of $R^2 > 0.99$. The lowest spiked concentration was determined to be the quantitative limit that was below the maximum residue limits of tricyclazole. This study provides a sensitive, stable and rapid approach for the analysis of tricyclazole in above matrix via SERS, and it will be useful complement to quantitative analysis of tricyclazole in complex matrix.

**Keywords:** Surface-enhanced Raman spectroscopy, Tricyclazole, Dispersive solid phase extraction, Rice, *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen
Introduction

Tricyclazole is a systemic fungicide mainly used in the prevention and treatment of rice blast, especially for ear-stem blast\(^1\), and is characterized by a strong internal absorption, resistance to fresh water and a long residual period. Although tricyclazole is a low-toxicity agricultural fungicide\(^1\), it can cause headaches, dizziness, vomiting, muscle tremors and other symptoms after poisoning\(^2\). According to the pesticide registration in China, it can be applied on rice and *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen under some restrictions. To ensure the quality and safety of food, China permitted the tolerance level for residual tricyclazole in rice and cabbage at 2.00 mg/kg. In contrast, the maximum residue limit of tricyclazole in rice is 0.70 mg/kg in Korea, and the limit is 3.00 mg/kg in Japan and the United States. The Codex alimentarius commission (CAC) and Australia have no relevant regulations. At present, chromatography-based techniques are the most common methods for an accurate and sensitive analysis of the presence of chemicals, including tricyclazole\(^3-5\), in vegetables and cereal. In recent years, liquid chromatography-mass spectrometry/mass spectrometry has been widely used to determine the presence of tricyclazole owing to its strong anti-interference ability and high accuracy\(^5\). But the above methods mostly tend to be costly, solvent-consuming, and time-consuming\(^6\). However, quality and safety inspection of agricultural products is moving towards highly sensitive, fast, quantifiable and environmental analysis\(^7\). Therefore, a rapid, green and accurate quantitative method is urgently needed to realize the rapid detection of tricyclazole in the heterogenous matrix.

Surface-enhanced Raman scattering, an emerging molecular fingerprint technology, have
the characteristic of easy to operate, fast to detect, high sensitivity and no use of organic reagents\textsuperscript{8}, and also can meet the needs of on-site testing\textsuperscript{9}. Now, it has been used to detect trace amounts of pesticides in fruits and vegetables\textsuperscript{10-13}, becoming a good alternative to chromatographic methods in screening a large number of samples rapidly\textsuperscript{8,14-15}. Metal nanoparticles are one of the most commonly used SERS substrates, which own a large size distribution, various shapes and minimized combustion value and offer an ensemble-averaged spectrum\textsuperscript{16-17}, have realized the identification of chemicals via SERS\textsuperscript{18-19}. Tang \textit{et al.}\textsuperscript{20} have applied silver nanoparticles as the substrate to determine the tricyclazole content in paddy rice by surface-enhanced Raman spectroscopy. Its quantitative range was from 0.05 to 0.70 mg/L and detection limit was 0.002 mg/L lower than the maximum residue limit in China. However, it was unfeasible to quantitatively analyze tricyclazole residue in matrix once out of the above quantitative range; and the pretreatment in above study was tedious and took a long time. Other studies have also previously investigated the application of SERS in the chemical identification and rapid detection of tricyclazole. Chen \textit{et al.}\textsuperscript{21} studied the density functional theory of tricyclazole surface-enhanced Raman spectra, compared the Raman spectra of tricyclazole molecules and its Ag\textsubscript{3} complex with tricyclazole surface-enhanced Raman spectra, and speculated the adsorption mode between tricyclazole and the enhanced substrate. In addition, Li \textit{et al.}\textsuperscript{22} demonstrated a rapid and sensitive detection of pesticides via surface-enhanced Raman spectroscopy based on a glycidyl methacrylate–ethylene dimethacrylate (GMA–EDMA) porous material, where the mixed standard solutions of tricyclazole and formate could be distinguished by characteristic Raman peaks. Zhao \textit{et al.}\textsuperscript{23} prepared a new simple silver nanoparticle - modified thin polydimethylsiloxane (PDMS)
sheet as SERS substrate to realize measurement of tricyclazole on the surface of apples; its limit of quantitation was 0.1 mg/L. Kim et al.\textsuperscript{24} and Kwon et al.\textsuperscript{25} respectively built high performance plasmonic nanoporous and cellulose paper-based SERS platform, could also accomplished determination of tricyclazole on the surface of apples; the results could meet the maximum residue level (MRL) requirement for apple peels (2000 ng cm\textsuperscript{-2}). However, these methods could not determine the residue of internal tricyclazole, and it is also unknown whether they are suitable for detection of residues with presence of interference matrix. And at present, there are still no reports on the detection and analysis of tricyclazole in \textit{Brassica campestris} L. ssp. \textit{chinensis} var. \textit{utilis} Tsen by SERS.

Co-existing interferences can generate certain signals covering the characteristic peak of the target in SERS process. Therefore appropriate preparation method should be chosen to remove impurities as much as possible\textsuperscript{26}. As we all know, dispersed matrix solid phase extraction (D-SPE) is a fast, simple, accurate and environmentally friendly pretreatment technology, which can effectively remove the matrix interference\textsuperscript{27} and has been successfully used to detect pesticides combined with SERS\textsuperscript{28}. Based on above reports, this study aims to establish highly efficient, rapid and sensitive SERS method to determine tricyclazole in rice and \textit{Brassica campestris} L. ssp. \textit{chinensis} var. \textit{utilis} Tsen employing D-SPE as preparation means, broaden quantitative range of tricyclazole and solve quantification problem of tricyclazole in complex matrix. The established method will meet the maximum residue level (MRL) requirement, and can be useful complement to quantitative analysis of tricyclazole in complex matrix.
Materials and methods

Chemicals, reagents and samples

Tricyclazole (99%) was supplied by Dr. Ehrenstorfer, Inc. (Augsburg, Germany). Chloroauric acid (99%) and other analytical reagents, including trisodium citrate dihydrate, sodium chloride, and magnesium sulfate anhydrous, were purchased from China National Accord Medicines Corporation Ltd. (Shanghai, China). Primary secondary amine sorbent (PSA, 40–60 μm), ostade-cylsilane (C_{18}, 50 μm, 6 μm), graphitized carbon black (GCB, 40 μm) were obtained from Bonna-Agela Technology Co. Ltd. (Tianjin, China). HPLC reagent acetonitrile and methyl alcohol were bought from Thermo Fisher Scientific (Massachusetts, USA). Wahaha pure water was obtained from the Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China).

Rice and Brassica campestris L. ssp. chinensis var. utilis Tsen were purchased from Hualian supermarket (Ji’nan, China) and used as the sample substrates.

Preparation of solutions

Chloroauric acid was dissolved in Wahaha pure water to a final Au^{3+} concentration of 1% (w/w) and stored at room temperature. A solution of sodium citrate was also prepared with Wahaha pure water for the synthesis of Au sol and its concentration was 1% (w/w). Standard stock solutions of tricyclazole (100 mg/L) were prepared with HPLC-grade acetonitrile. Based on the tricyclazole sensitivities of the SERS in the matrix, it was then diluted to the proper concentration, and all solutions were stored in the dark at -4 °C.
Synthesis of Au nanoparticles

A DF-101 magnetic stirrer with constant temperature (Zhengzhou Ketai Ltd., Zhengzhou, China) and DC-0506 low temperature circulating water system (Shanghai Hengping Ltd., Shanghai, China) were used to prepare the Au nanoparticles, based on a typical sodium citrate reduction method reported by Frens. First, a 2 mL chloroauric acid solution was added to 100 mL boiling water and after 10 min, 1.5 mL of sodium citrate solution was added to the mixture. The above mixed solution was stirred for 20 min to prepare the Au nanoparticles. The volume of chloroauric acid solution was then changed to 2.0 mL, 2.5 mL, 3.0 mL and 5 mL to prepare the Au nanoparticles. The five different-sized Au nanoparticles were then cooled with stirring before storage.

Characterization of Au nanoparticles

The optical properties of the Au colloids were analyzed using a U-4100 UV-Vis spectrophotometer (HITACHI, Inc., Tokyo, Japan) with absorbance spectroscopy from 300 to 900 nm. The shapes and sizes of the Au nanoparticles were evaluated with a VEGA 3 XMU scanning electron microscope (TESCAN, Inc., Brno, Czech Republic). The average particle size of the Au nanoparticles was calculated based on the SEM images of 20–50 particles.

Sample preparation

The matrix solid phase dispersion method was applied to extract the tricyclazole from rice and Brassica campestris L. ssp. chinensis var. utilis Tsen and remove impurities from the matrix. Briefly, 10.0 g homogenized rice and Brassica campestris L. ssp. chinensis var. utilis
Tsen were each weighed using a PL2002 electronic balance (Mettler Toledo, Inc., Zurich, Switzerland) and vigorously mixed with 25 mL acetonitrile, 3 g sodium chloride using a MS3 D S25 Vortex mixer (IKA, Inc., Staufen, Germany). The mixture was then centrifuged at 4800 rpm for 5 min with the LDZS-2 high-speed centrifuge (Beijing Jingli Freelance Machine Company, Beijing, China). Next, 0.25 g of PSA sorbent, 0.25 g of C18 sorbent, and 0.75 g of anhydrous MgSO4 were added to 5 mL of the supernatant to remove any pigment, organic acids, lipid compounds, excess water and other non-targeted compounds. The mixture was then vigorously shaken for 30 s and centrifuged at 4800 rpm for 5 min. Finally, the supernatant was filtered through a 0.22 μm filter prior to SERS analysis. The pigment in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen had a strong matrix interference for the SERS measurement, requiring an additional 5 mg GCB in the above purification steps only for the *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen samples.

**SERS measurement**

A QE65 Pro Raman Spectrometer equipped with a Laser-785 (Ocean optics, Inc., Florida, USA) was used to obtain SERS information (300–1700 cm⁻¹) on tricyclazole in the rice and *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen extracts. The laser power used was 300 mW. First, 2 mL gold colloid and 20.0 μL standard or pesticide extract were mixed in a 5 mL centrifuge tube. Then, the mixture was transferred into a quartz plate for the SERS measurements with an exposure time of 5 s per scan.

All obtained SERS spectra were normalized by WiRE 4.0 software (Renishaw plc., London, England). An external standard method was applied to evaluate the linear
relationship between the intensities of a characteristic peak and the analyte concentrations, and to conduct a quantitative analysis of the samples.

Results and Discussion

Characterization of Au nanoparticles

The SEM images of the Au nanoparticles (Fig. 1A–1E) showed that the nanoparticle sizes decreased with an increase in the amount of trisodium citrate (1.5 mL, 2.0 mL, 2.5 mL, 3.0 mL, and 5.0 mL). The calculated average diameters of the five Au nanoparticles were 73 ± 7 nm, 45 ± 5 nm, 32 ± 4 nm, 16 ± 2 nm, and 11 ± 2 nm. Fig. 1F showed the ultraviolet absorption peak of the five Au nanoparticles, which blue-shifted from 541 nm to 520 nm when the amount of trisodium citrate increased from 1.5 mL to 5 mL. The observed shifts of the ultraviolet absorption peak were consistent with a previous report.30

Spectral features of tricyclazole

(1) Characteristic peak of tricyclazole

The major characteristic peaks of the tricyclazole solid standard were 422 cm⁻¹, 552 cm⁻¹, 591 cm⁻¹, 955 cm⁻¹, 1082 cm⁻¹, 1149 cm⁻¹, 1192 cm⁻¹, 1313 cm⁻¹, 1366 cm⁻¹, 1418 cm⁻¹, 1461 cm⁻¹, 1498 cm⁻¹, 1533 cm⁻¹, and 1583 cm⁻¹ (Fig. 2A, trace a), essentially in agreement with the reported literature.21 The most prominent tricyclazole peak appeared at 1366 cm⁻¹ and is attributed to the C-N and C-C stretching modes, as well as the C-C-C bending mode. The other major characteristic peaks were attributed to the C-N stretching and C-C-C bending modes (424 cm⁻¹), C-C, C-N, C-S stretching and C-C-C, C-N-C bending
modes (591 cm$^{-1}$), C-C stretching and H-C-C bending modes (1084 cm$^{-1}$), and C-C stretching mode (1580 cm$^{-1}$). The SERS characteristic peak of the tricyclazole standard solution in Fig. 2 A (trace b) was similar to that of the solid standard, but the Raman shift was slightly changed for all of the peaks. The features at 429 cm$^{-1}$, 562 cm$^{-1}$, 598 cm$^{-1}$, 977 cm$^{-1}$, 1083 cm$^{-1}$, 1150 cm$^{-1}$, 1191 cm$^{-1}$, 1231 cm$^{-1}$, 1318 cm$^{-1}$, 1372 cm$^{-1}$, 1415 cm$^{-1}$ were all enhanced; among them, 1231 cm$^{-1}$ was not found in the spectrum of the solid standard.

(2) Quantitative spectral peak of tricyclazole

The SERS Raman spectra of the tricyclazole varied with the sample substrates. Fig. 2B (trace a) showed the enhanced characteristic peaks of tricyclazole in the rice matrix were mainly 429 cm$^{-1}$, 562 cm$^{-1}$, 597 cm$^{-1}$, 1084 cm$^{-1}$, 1152 cm$^{-1}$, 1192 cm$^{-1}$, 1317 cm$^{-1}$, 1373 cm$^{-1}$, and 1417 cm$^{-1}$. Compared with the SERS Raman spectra of rice matrix (Fig. 2B, trace b), we found that the peak appeared at 562 cm$^{-1}$ had a low degree of overlap with the interference peak, which affected the quantitative analysis. Other peaks were not affected by impurities. Among them, the peak at 1373 cm$^{-1}$ provided the highest response, so it was used as the quantitative spectral peak for tricyclazole. The enhanced characteristic peaks of tricyclazole in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen matrix were mainly 429 cm$^{-1}$, 563 cm$^{-1}$, 597 cm$^{-1}$, 1084 cm$^{-1}$, 1317 cm$^{-1}$, 1373 cm$^{-1}$, and 1417 cm$^{-1}$. Similarly, by comparing Fig. 2C (trace a) with Fig. 2C (trace b), we selected the peak at 1317 cm$^{-1}$ for quantitative analysis in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen.

Optimization of adsorbent dosage

C$_{18}$ and PSA have become the commonly used purification agents for D-SPE with their
The respective advantages of the type and dosage of dispersible adsorbent are crucial for the application of D-SPE. Therefore, the mixing proportion must be carefully considered.

The recommended dosage of C<sub>18</sub> and PSA AOAC 2007.01 were respectively 50 mg per ml of extraction fluid. The amount of pigment in the rice matrix is low, so only PSA and C<sub>18</sub> were chosen for purification. After the recovery test, we found the recovery of tricyclazole added in the rice was between 80% and 120%, meeting the analysis requirement of pesticide residues. When the spiked concentration was 2 mg/kg, it indicated that C<sub>18</sub> and PSA did not adsorb the tricyclazole. Therefore, the PSA and C<sub>18</sub> amounts used in the purification process were each 250 mg.

The matrix of Brassica campestris L. ssp. chinensis var. utilis Tsen is complex and has a significant pigment interference. Therefore, the dosage of GCB should first be considered, and then mixed with the recommended PSA and C<sub>18</sub> amounts for purification. Fig. 3 (a)–(d) demonstrates the ability of varying GCB concentrations to remove the matrix interference. With increasing GCB, the blank matrix interference decreased. After the recovery test, it was found that GCB had a certain adsorption effect on tricyclazole. When the dosage of GCB was 5 mg, the average recovery rate of the 2 mg/kg-spiked tricyclazole was 84.1%. When the dosage of GCB was 10 mg and 15 mg, the recovery of tricyclazole was lower than 65%. This illustrated that the excess GCB obviously affected the accuracy of the experimental results. Thus, 5 mg GCB was selected as the dosage for purification, and the dosages of PSA and C18 each remained unchanged at 250 mg.

Selection of SERS conditions
(1) Effect of gold sol size on the tricyclazole characteristic peak

In this experiment, five kinds of Au nanoparticles (preparation described in Section 2.3) were tested to select an appropriate substrate, and the results are shown in Fig. 4. The abscissa coordinate of Fig. 4 is Raman displacement, and vertical axis is Raman intensity. The different sizes of gold nanoparticles have a significant effect on the Raman signal strength of tricyclazole, which is in agreement with previously reported studies. As the Au nanoparticle size increased, the SERS signal of the main tricyclazole peaks first increased and then decreased. When the gold nanoparticle size was 32 nm, the SERS signal strength of 2 mg/L tricyclazole peaks at 1317 cm$^{-1}$ and 1373 cm$^{-1}$ were all the strongest. Therefore, gold nanoparticles with a size of about 32 nm were selected as the substrate for the SERS measurement.

(2) Determination of the volume ratio

During the test, the higher concentration of matrix or target substance in the solution to be tested was found to make the gold solution change to dark blue. In this unsteady dispersion, the sol particles rapidly gathering and the mixture hardly had any tricyclazole SERS signals. This may be caused by some impurities sticking to the surface of the nano-gold solution with tricyclazole. Therefore, the volume ratio of the sample liquid to the gold sol was set as 1:100, 1:50, 1:20, 1:10 and 1:5 for the experiment. First, 1 mg/L tricyclazole matrix (rice) standard liquid was chosen to test the ratio. When the blending ratio was 1:10, the mixed solution was dark blue and hardly produced signals. The concentration of tricyclazole was then increased and the volume ratio was reduced. The results showed that when the concentration of tricyclazole was 10 mg/L and the blending ratio was 1:50, the mixed solution still changed to
dark blue and hardly produced signals. Similarly, when the matrix was *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen, the mixture would also turn dark blue and hardly produced signals with a tricyclazole concentration of 10 mg/L. So the proportion of sample solution was decreased incrementally, while keeping the concentration of tricyclazole at 10 mg/L, until the solution system remained stable and generate SERS response. When the volume ratio was 1:100, the SERS response of mixture was stable within five minutes and had a linear relationship with a certain concentration range of tricyclazole. Thus, 1:100 was determined to be the proper volume ratio.

(3) Effect of static duration on the stability of the tricyclazole characteristic peak

To investigate the stability of the sample system and understand how the static duration affects the stability of the tricyclazole characteristic peak, SERS spectra of the sample were obtained every 30 s and duration of 5 minutes. As shown in Fig. 5, the SERS response of tricyclazole between 0 to 5 min had good stability. After statistical analysis, the main characteristic peaks’ response at 429 cm\(^{-1}\), 562 cm\(^{-1}\), 598 cm\(^{-1}\), 977 cm\(^{-1}\), 1083 cm\(^{-1}\), 1150 cm\(^{-1}\), 1191 cm\(^{-1}\), 1317 cm\(^{-1}\), 1373 cm\(^{-1}\) produced a relative standard deviation between 2.1% and 6.3%, when the concentration of tricyclazole was 10 mg/L, and 4.7 %, 4.6 % was for characteristic peak at 1373 cm\(^{-1}\), 1317 cm\(^{-1}\) respectively. Thus, the tricyclazole characteristic peaks have stability on the static duration within 5 minutes.

Evaluation of method effectiveness

(1) Linear range, correlation coefficient and quantitative limit

After determining the optimal test conditions, a series of rice matrix and *Brassica*
Campestris L. ssp. chinensis var. utilis Tsen standard solutions (0.5, 1, 2, 5, 10 mg/L and 1, 2, 4, 6, 8, 10 mg/L, respectively) were prepared. The standard curve was then drawn between the Raman peak strength of tricyclazole as the ordinate and the concentration of tricyclazole as the abscissa. The results (Fig. 6 and Fig. 7) showed that the linear relationships were good in the ranges of 0.5–10 mg/L and 1–10 mg/L for rice and Brassica campestris L. ssp. chinensis var. utilis Tsen, respectively. The equations were \( Y = 162.57x + 11.505 \) and \( Y = 126.75x + 11.506 \), respectively. In addition, the correlation coefficients (\( R^2 \)) were all greater than 0.99.

(2) Accuracy and precision of method

The recovery test was carried out at the spiked levels of 0.5, 1, 5 mg/kg in rice and 1, 2, 4 mg/kg in Brassica campestris L. ssp. chinensis var. utilis Tsen. Each treatment was repeated five times. The experimental results (Table 1) showed that at the spiked levels of 0.5, 1 and 5 mg/kg, the recovery of tricyclazole in the rice matrix was between 93.6% and 112.8%, and the relative standard deviation was between 2.8% and 7.5%. At the spiked levels of 1, 2 and 5 mg/kg, the recovery of tricyclazole in Brassica campestris L. ssp. chinensis var. utilis Tsen was from 83.6% to 103.7%, and the relative standard deviation was from 4.8% to 5.9%, which all met the requirements of the pesticide residue analysis. The quantitative limits were 0.5 mg/kg and 1 mg/kg.

Conclusions

The gold nanoparticles used in this study were easily synthesized and could provide significant enhancement effects for the SERS signals of tricyclazole. The D-SPE method applied for sample preparation removed any interfering impurities and shortened the
extraction time of tricyclazole from the matrix. By optimizing the test conditions, tricyclazole could be stably detected via SERS. Through the method feasibility evaluation, we demonstrated the possibility of applying SERS technology for the quantitative analysis of tricyclazole. The quantitative determination in rice and *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen was 0.5 mg/kg and 1 mg/kg, respectively, and all were lower than the maximum residues limits. Overall, the SERS method developed through this study provides a simple, rapid and sensitive approach to analyze tricyclazole in rice and *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen. It satisfies the requirements of maximum residue level (MRL), broaden quantitative range of tricyclazole and solve quantification problem of tricyclazole in complex matrix based on SERS analysis. Thus, it is displayed that the established approach is a good alternative to chromatography, and a useful complement to quantitative analysis of tricyclazole in complex matrix.

Acknowledgments

This research was supported by the Major science and technology innovation engineering projects of Shandong Province (No. 2019JZZY011013), the Agricultural Science and Technology Innovation Project of Shandong Academy of Agricultural Sciences (No. CXGC2017A03), Shandong Provincial Key Laboratory of Food Quality and Safety Inspection Technology Open Project, Shandong province agricultural major application technology Innovation project. We thank Rosalie Tran, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.
References

1. B. H. Lei, *Guizhou agricultural science*, 1996, 5, 59–61.

2. S. Vivek, S. P. Indumathi and T. Radha, *Research Journal of Agriculture and Biological Sciences*, 2009, 5(4), 445–451.

3. J. F. Liu and W. Q. Mei, *Southwest China Journal of Agricultural Sciences*, 2005, 18(6), 836–838.

4. J. G. Yao, “Study on the Residual Behavior of Tricyclazole and Propiconazole in Paddy and Soil”, 2013, Chinese academy of agricultural sciences, Beijing.

5. L. Q. Huang, X. Chen, Z. W. Zhang, L. Ma, X. P. Zhan, J. B. Chen, L. Zhao, *Chinese Journal of Pesticide Science*, 2018, 20(3), 354–362.

6. G. F. Pang, C. L. Fan, Y. M. Liu, Y. Z. Cao, J. J. Zhang, X. M. Z. Y. Li, Y. P. Wu, T. T. Guo, *Journal of AOAC International*, 2006, 89(3), 740–771.

7. B. L. Yuan, X. D. Wang, P. Yang, Z. Z. Fang, Y. J. Huang, J. Chen, J. M. Ye, *Food science*, 2016, 37(02), 198-203.

8. J. M. Liu, L. P. Yan, W. H. Liu and Y. J. Teng, *Fenxi Ceshi Xuebao (Journal of Instrumental Analysis)*, 2016, 35(5), 605–608.

9. P. Hong, L. M. Xu and J. L. Han, *Chinese Journal of Health Laboratory Technology*, 2008, 18(2), 272–274.

10. P. Y. Ma, F. H. Liang, X. P. Li, Q. Q. Yang, D. Wang, D. Q. Song, D. J. Gao, X H. Wang, *Food Analytical Methods*, 2014, 7(9), 1866–1873.

11. H. R. Luo, X. H. Wang, Y. Q. Huang, K. Q. Lai, B. A. Rasco and Y. X. Fan, *Journal of the*
science of food and agriculture, 2018, 98(10), 3892–3898.

12. T. Dong, L. Lin, Y. He, P. C. Nie, F. F. Qu and S. P. Xiao, *Molecules*, 2018, 23(6), 1458.

13. T. T. Pan, D. W. Sun, H. B. Pu and Q. Y. Wei, *Journal of agricultural and food chemistry*, 2018, 66, 2180–2187.

14. W. J. Liu, J. J. Du and C. Y. Jing, *Environmental Chemistry*, 2014, 33(2), 217–228.

15. Y. X. Fan, K. Q. Lai and Y. Q. Huang, *Spectroscopy and Spectral Analysis*, 2014, 34(7), 1859–1864.

16. X. B. Guo, Z. H. Li, J. L. Li, M. Geng and S. S. Huang, *Science and technology of food industry*, 2013, 34(5), 368–371.

17. A. H. Waleed, L. I. Emad, S. S. Michael and A. A. Godwin, *Biosensors and Bioelectronics*, 2017, 91, 664–672.

18. S. G. Huang, Y. Wu, J. P. Hu and M. H. Liu, *Spectroscopy and Spectral Analysis*, 2017, 37(1), 135–140.

19. B. Yu and Z. P. Zhuang, *Journal of Mudanjiang Normal University*, 2016, (3), 43–45.

20. H. R. Tang, D. M. Fang, Q. Q. Li, P. Cao, J. P. Geng, T. Sui, X. Wang, J. Iqbal, Y. P. Du, *Journal of Food Science*, 2012, 77(5), T105–T109.

21. Y. F. Chen, C. B. Shao, M. H. Zuo and Z. P. Zhuang, *Journal of Molecular Science*, 2018, 34(3), 246–250.

22. Q. Q. Li, Y. P. Du, Y. Xu, X. Wang, S. Q. Ma, J. P. Geng, P. Cao, T. Sui, *Chinese Chemical Letters*, 2013, 24, 332–334.

23. H. Zhao, W. Hasi, L. Bao, Y. P. Liu, S. Han, D. Y. Lin, *Journal of Raman Spectroscopy*, 2018, 49(9), 1469–1477.
24. D. Kim, Y. Ko, G. Kwon, Y. M. Choo, J. You, Sensors and Actuators B: Chemical, 2018, 274, 30–36.

25. G. Kwon, J. Kim, D. Kim, Y. Ko, Y. Yamauchi, J. You, Cellulose, 2019, 26(8).

26. J. C. Cui, “Extraction-Integrated Surface Enhanced Raman Spectroscopy Detection Methods Based on Functionalized Graphene Materials”, 2015, Shandong University, Ji’nan.

27. B. R. López, M. R. Nortes, M. J. Robles, G. D. Moreno, L. B. Gilbert, R. J. F. García, A. D. Molina, Journal of Chromatography A, 2016, 1456, 89–104.

28. J. Tang, X. Zhong, T. R. Ni, H. F. Chang, H. Wang and D. W. Zheng, Journal of Food Safety and Quality, 2016, 7(12), 4870–4877.

29. G. Frens, Nature Physical Science, 1973, 241(1), 20–22.

30. S. Wang, J. Cheng and X. Q. Su, Scientia Agricultura Sinica, 2014, 47(11), 2224–2232.

31. W. J. Zhang, H. D. Li, L. P. Fang, Z. Dong, H. X. Du, S. Guan, Z. L. Chen, Chinese Journal of Pesticide Science, 2017, 19(5), 609–661.

32. L. Y. Zhang, R. S. Zhang, F. Y. Gao, Y. R. Zhang, Z. C. Xiong, Z. P. Wu, W. B. Zhang, Chinese Journal of Analysis Laboratory, 2014, 33(9), 1024–1029.
Table 1. Recovery and RSD of tricyclazole in rice and *Brassica campestris* L. *ssp. chinensis* var. *utilis* Tsen (n=5).

| Matrix                          | Spiked (mg/kg) | Recovery (%) | RSD (%) |
|--------------------------------|----------------|--------------|---------|
|                                |                | 1  | 2  | 3  | 4  | 5  |
| Rice                           | 0.5            | 93.6 | 97.6 | 98.3 | 94.6 | 107.7 | 5.1 |
|                                | 1              | 106.8 | 102.0 | 109.4 | 110.9 | 107.2 | 2.8 |
|                                | 5              | 95.4 | 112.8 | 99.4 | 106.1 | 116.9 | 7.5 |
| *Brassica campestris* L.       | 1              | 83.6 | 84.1 | 91.2 | 85.6 | 94.7 | 5.6 |
| *ssp. chinensis* var.          | 2              | 92.9 | 85.3 | 101.1 | 91.6 | 93.1 | 5.9 |
| *utilis* Tsen                  | 4              | 98.8 | 103.7 | 90.5 | 97.1 | 98.0 | 4.8 |
Figure caption

**Figure 1.** Characterization of Au nanoparticles by SEM images of Au nanoparticles synthesized with (A) 1.5 mL, (B) 2.0 mL, (C) 2.5 mL, (D) 3.0 mL, and (E) 5.0 mL of 1% (w/w) sodium citrate solution, respectively. (F) Ultraviolet-visible absorption peak of gold nanoparticles synthesized with (a) 1.5 mL, (b) 2.0 mL, (c) 2.5 mL, (d) 3.0 mL, and (e) 5.0 mL of 1% (w/w) sodium citrate solution.

**Figure 2.** (A) Comparison of the Raman spectrum of the tricyclazole solid standard (trace a) and SERS spectrum of 2 mg/L tricyclazole standard solution (trace b). (B) Comparison of the SERS spectra of 2 mg/L tricyclazole in rice (trace a) and of rice alone (trace b). (C) Comparison of the SERS spectra of 2 mg/L tricyclazole in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen (trace a) and of *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen alone (trace b). All SERS spectra were collected by testing 20 μL sample solution mixed with 2 mL gold-sol.

**Figure 3.** Effects of GCB dosage on removing matrix interference: (a) – (d) were SERS spectra of *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen matrix adding 0 mg, 5 mg, 10 mg and 25 mg GCB, respectively.

**Figure 4.** Influence of gold-sol particle size on the SERS response signal of 2 mg/L tricyclazole.

**Figure 5.** Effect of static duration on the stability of 10 mg/L tricyclazole spectral features.

**Figure 6.** (A) SERS spectra of the tricyclazole matrix standard solution at different concentrations in rice. (B) Linear relationship for the tricyclazole concentration in rice
(as the abscissa) and the Raman intensity (as the ordinate).

**Figure 7.** (A) SERS spectra of varying concentrations of the tricyclazole matrix standard solution in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen. (B) Linear relationship for the tricyclazole concentration (as the abscissa) in *Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen and the Raman intensity (as the ordinate).
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![SERS spectra](image)

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![SERS response signal](image)

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