

Research Article

Rapid Detection of Tetracycline Residues in Duck Meat Using Surface Enhanced Raman Spectroscopy

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A rapid detection method based on surface enhanced Raman spectroscopy (SERS) was proposed in this paper in order to realize the detection of tetracycline residues in duck meat. Firstly, surface enhanced Raman spectra characteristics of tetracycline aqueous solution, duck meat extract, and duck meat extract containing tetracycline were analyzed. Secondly, the effect of the addition amount of duck meat extract containing tetracycline on SERS intensity and the effect of the adsorption time on SERS intensity were discussed, respectively. Thirdly, SERS intensity ratio at 1272 and 1558 cm^{-1} (I_{1272}/I_{1558}) was used to establish the SERS calibration curve. A good linearity relationship between the tetracycline concentration in duck meat extract and I_{1272}/I_{1558} was obtained, and the linear regression equation and the correlation coefficient (r) were $y = 0.0177x + 0.1213$ and 0.950, respectively. The average recovery of tetracycline in duck meat extract was 101~108% with relative standard deviation (RSD) of 2.4~4.6%. The experimental results showed that the method proposed in this paper was a good detection scheme for the rapid detection of tetracycline residues in duck meat.

1. Introduction

Tetracycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$), which is a kind of broad-spectrum tetracycline antibiotics, is often used to prevent and treat the duck diseases or is added to the duck feedstuff as the additive. However, tetracycline residues content in duck meat easily exceeds the standard within legal limits due to the unsuitable usage of tetracycline, and the excessive tetracycline residues in duck meat may further cause some potential harm to human health, such as kidney toxicity and fetal malformation [1, 2]. Therefore, the regulations for the Maximum Residue Limits (MRL) in meat have been established in China, the EU, and the USA [1, 3]. For instance, tetracycline residues content in duck meat in China and USA cannot exceed 0.1 and 0.2 ppm, respectively [1, 3].

It is highly imperative to detect all the duck products in advance in order to prevent the products containing the tetracycline residues from entering the markets. The conventional detection methods for the antibiotic residues in meat include enzyme-like immunosorbent assay [4–6], microbiological method [7, 8], and physical and chemical

detection method [9]. Although these methods have high detection precision, they cannot meet the rapid, mass, and field detection requirements of tetracycline residues in duck meat owing to the tedious pretreatment process or the time-consuming detection process and so forth. Hence, it is highly needed to investigate a rapid detection method of tetracycline residues in duck meat.

Surface enhanced Raman spectroscopy (SERS) is a highly specific and sensitive method for the detection of chemical and biochemical compounds in chemistry, microbiology, pharmacology, biochemistry, and environmental science [10, 11]. Zhang et al. utilized SERS to analyze prohibited aquaculture drugs including enrofloxacin, furazolidone, and malachite green in fish products [11]. Li et al. applied SERS to detect the residuals of the prohibited and restricted drugs including malachite green and crystal violet in fish muscle, and the lowest concentrations detected were 1.0 and 20 $\mu\text{g}/\text{kg}$, respectively [12]. Ma et al. used SERS to detect sulfamerazine and sulfamethazine in fish, and the detection limits were 0.16 and 0.59 ppm, respectively [13]. Li et al. applied improved surface enhanced Raman scattering on microscale Au hollow

spheres to detect different concentrations of tetracycline aqueous solution, and a good linear response was obtained [14]. Compared to the SERS detection of tetracycline aqueous solution, the SERS detection condition of tetracycline residues in duck meat is more complicated. For instance, the composition of duck meat can have effect on intensities of tetracycline SERS characteristic peaks; even some tetracycline SERS characteristic peaks disappear. Moreover, some tetracycline SERS characteristic peaks can overlap with SERS peaks of the composition of duck meat. So far, the relevant literature about the detection of tetracycline residues in duck meat using SERS was not reported.

The objective of this paper was to investigate the rapid detection method of tetracycline residues in duck meat using SERS. Firstly, SERS spectra characteristics of tetracycline aqueous solution, duck meat extract, and duck meat extract containing tetracycline were analyzed. Secondly, the effect of the addition amount of duck meat extract containing tetracycline on SERS intensity (peak height) and the effect of the adsorption time on SERS intensity were explored, respectively. Lastly, the linear regression equation between the tetracycline concentration in duck meat extract and SERS intensity ratio at 1272 and 1558 cm^{-1} (I_{1272}/I_{1558}) was established to predict the tetracycline residues in duck meat.

2. Materials and Methods

2.1. Materials and Reagents. Sheldrakes were purchased from the vegetable market of Jiangxi Agricultural University. Tetracycline (98.0%) was purchased from Standard Substances Network of China. OTR202 (gold nanoparticles), and OTR103 (gold colloid enhancement reagent) were purchased as SERS enhancement substrate from Opto Trace Technologies, Inc. Ethyl acetate was analytical reagent grade. Ultrapure water was obtained using T10 laboratory water purifier (Hunan Kertone Water Treatment Co., Ltd.) for the preparation of all aqueous solutions. All chemicals are of analytical reagent grade and used without further purification.

2.2. Instruments. RamTracer®-200 portable Raman spectrometer (Opto Trace Technologies, Inc.), T6 series UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.), JK-50B ultrasonic cleaner (Hefei Jinnike Machinery Co., Ltd.), FA1004B electronic weigher (Shanghai Shangping instrument Co., Ltd.), T10 laboratory water purifier, JW-1024 low speed centrifuge (Anhui Jiaven Equipment Industry Co., Ltd.), JJ-2B tissue disintegrator (Jintan Jinnan Instrument Factory), and VORTEX-5 whirlpool mixer (Haimen Kylin-Bell Lab Instruments Co., Ltd.) were used in this study.

2.3. Experimental Methods

- (1) Preparation of duck meat extract: firstly, the breast meats were removed from the sheldrakes and the membranes were eliminated from the breast meats for the following experiment. Secondly, 200 g of duck breast meat was crushed in meat emulsion by using tissue disintegrator, and then the meat emulsion was cryopreserved below -18°C . Thirdly, 5 g of meat

emulsion and 20 mL of ethyl acetate were added into 50 mL centrifuge tube every time. Fourthly, the supernatant was taken out after whirlpool mixing for 2 minutes, oscillating for 10 minutes, and centrifuging (4500 r/min) for 15 minutes. Fifthly, the residues were extracted for two times using the same method, and then two extracted supernatants were mixed together. Lastly, the supernatant was filtered with rapid filter papers.

- (2) Preparation of tetracycline standard solution: 5 mg of tetracycline was added into 50 mL brown volumetric flask, and then 100 mg/L tetracycline standard solution was obtained after ultrasonic dissolving with ultrapure water. Subsequently, 100 mg/L tetracycline standard solution was diluted to different concentrations with ultrapure water during the course of the experiment.
- (3) Preparation of spiked samples: 5 mg of tetracycline was added into 50 mL brown volumetric flask, and then the spiked sample containing 100 mg/L tetracycline was obtained after ultrasonic dissolving with duck meat extract. Subsequently, the spiked sample containing 100 mg/L tetracycline was diluted to different tetracycline concentrations by using duck meat extract in the following experiment.
- (4) 500 μL of OTR202, 20 μL of the analyzed solution, and 100 μL of OTR103 were added to 2 mL sample bottle in turn, and then SERS spectra were collected after this system was well blended. SERS spectra were collected 5 times for each sample, and their average intensities were used as original SERS spectrum intensities of each sample. Spectral range of 400~1800 cm^{-1} was used as the following analysis of SERS spectra.
- (5) The parameters of portable Raman spectrometer were set as follows: laser power was 200 mW, laser wavelength was 785 nm, the scanning range of Raman wavelength was 100~3300 cm^{-1} , spectral resolution was 6 cm^{-1} , the range of Raman intensity was 0~60000 a.u., and the integration time was 10 sec.

3. Results and Discussion

3.1. Pretreatment of SERS Spectra. The original SERS spectrum of duck meat extract containing 12.0 mg/L tetracycline was showed in Figure 1(a). As seen from Figure 1(a), the original SERS spectrum included not only Raman signal of the sample detected but also fluorescence and all other kinds of background signals. Thus, it was indispensable to reduce the effect of the fluorescence and all other kinds of background signals on SERS analysis in order to improve the reliability of SERS qualitative, semiquantitative, or quantitative analysis. Adaptive iteratively reweighted penalized least squares (air-PLS), which need not any user intervention and initial information, is a highly effective background subtraction method [15, 16]. So, air-PLS was employed to remove the fluorescence and all other kinds of background signals in

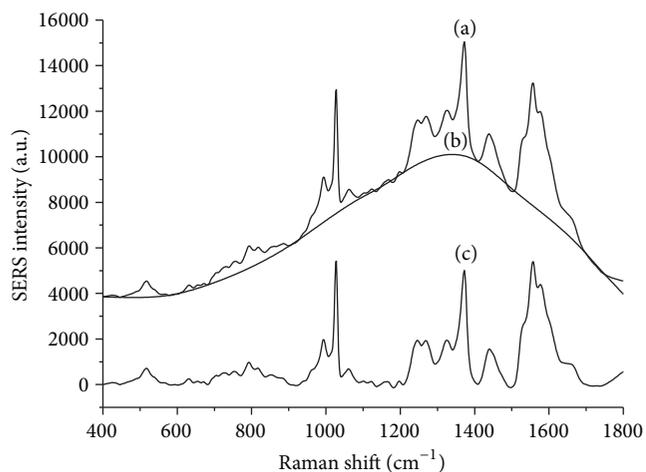


FIGURE 1: Result of background subtraction using air-PLS. (a) Original SERS spectrum; (b) background signals fitted; (c) SERS spectrum subtracted by background signals.

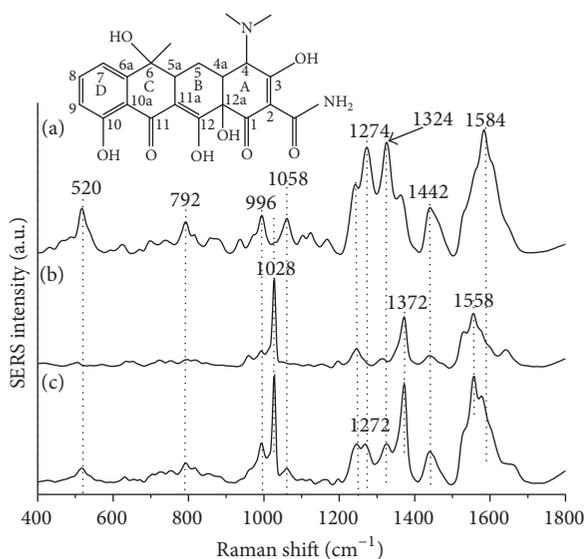


FIGURE 2: SERS spectra of (a) tetracycline aqueous solution, (b) duck meat extract, and (c) duck meat extract containing tetracycline. The inset shows that tetracycline is a derivative of naphthacene according to the chemical structure of tetracycline.

this paper. As showed from Figure 1(c), the fluorescence and all other kinds of background signals on curve (a) were subtracted perfectly by using air-PLS under the condition of keeping the peak shapes of SERS spectra.

3.2. SERS Spectra Characteristics of Samples. The OTR202 has a strong absorption peak at 544 nm according to UV-Vis absorption spectra of OTR202, which is the typical surface plasmon resonance absorption of gold nanoparticles. Moreover, its half-peak width is approximately 79 nm.

SERS spectra of tetracycline aqueous solution, duck meat extract, and duck meat extract containing tetracycline were showed in Figure 2. The major SERS characteristic peaks of tetracycline aqueous solution could be observed at 520, 792,

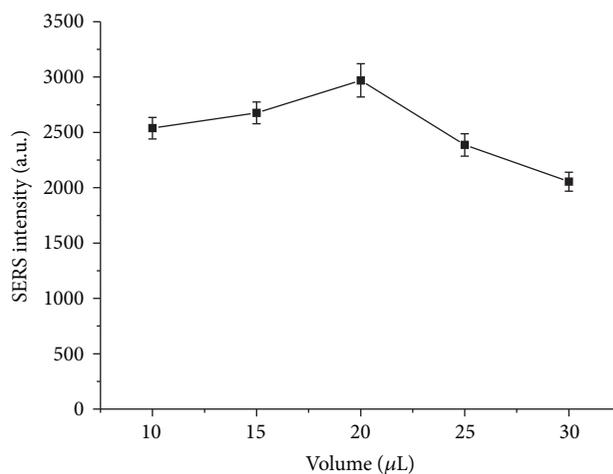


FIGURE 3: Effect of the addition amount of duck meat extract containing tetracycline on SERS intensity.

996, 1058, 1274, 1324, and 1584 cm^{-1} ($\pm 3 \text{ cm}^{-1}$) in Figure 2(a). SERS characteristic peak of tetracycline aqueous solution at 1274 cm^{-1} has blue-shifted to 1272 cm^{-1} on SERS spectrum of duck meat extract containing tetracycline, which was probably caused by the charge transfer that took place between gold nanoparticles and tetracycline [13]. The main SERS characteristic peaks of duck meat extract could be seen at 1028, 1372, and 1558 cm^{-1} ($\pm 3 \text{ cm}^{-1}$) in Figure 2(b). As showed in Figure 2(c), SERS characteristic peaks of tetracycline aqueous solution at 520, 1058, and 1274 cm^{-1} , which did not overlap with SERS characteristic peaks of duck meat extract, appeared on SERS spectrum of duck meat extract containing tetracycline. So, SERS characteristic peaks at 520, 1058, and 1272 cm^{-1} could be selected as SERS characteristic peaks for the detection of tetracycline residues in duck meat extract. Tetracycline is a derivative of naphthacene according to the chemical structure of tetracycline in Figure 2(a). The characteristic peak at 1058 cm^{-1} was attributed to stretching vibration of CO3 [17, 18]. The characteristic peak at 1272 cm^{-1} was ascribed to bending vibration of CH4,4a,5a, OH12, and amid-NH and stretching vibration of CO10, CO3, CH7,8,9, amid-NC, C4aC5, and benzene ring D [17, 18]. In conclusion, it was entirely feasible, at least in theory, to detect tetracycline residues in duck meat using SERS according the above analysis.

3.3. Effect of Addition Amount of Samples on SERS Intensity. To analyze the effect of the addition of the samples on SERS intensity, SERS intensity at 1272 cm^{-1} was investigated under the condition of the different addition amounts (10, 15, 20, 25, and 30 μL) of duck meat extract containing tetracycline and the fixed volumes of OTR202 (500 μL) and OTR103 (100 μL). As showed from Figure 3, SERS intensities at 1272 cm^{-1} were different when different volumes of duck meat extract containing tetracycline were mixed with gold nanoparticles. The possible reason was that their mixture ratio had the significant impact on adsorption effect and then affected SERS intensity. SERS intensity at 1272 cm^{-1} was strongest

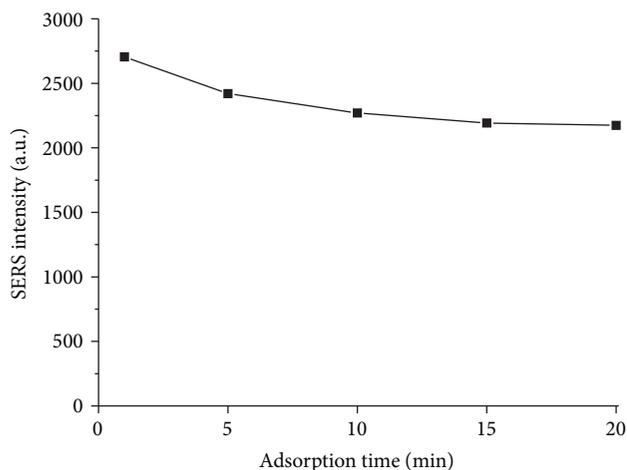


FIGURE 4: Effect of adsorption time on SERS intensity.

while the addition amount of duck meat extract containing tetracycline was 20 μL . This showed that adsorption effect on the detected sample was best when the volume of duck meat extract containing tetracycline was 20 μL . Therefore, 20 μL was determined as the addition amount of duck meat extract containing tetracycline.

3.4. Effect of Adsorption Time on SERS Intensity. In order to study the effect of adsorption time on SERS intensity, the change of SERS intensity at 1272 cm^{-1} with adsorption time was explored on the condition of 500 μL of OTR202, 20 μL of the same concentration of the sample detected, and 100 μL of OTR103. With the increases of adsorption time between duck meat extract containing tetracycline and SERS active substrate, SERS intensities of the mixture solution at 1272 cm^{-1} gradually reduced (Figure 4). This might be because the aggregation was produced to some extent after gold nanoparticles were mixed with duck meat extract containing tetracycline, and the enhancement effects, which were produced by combining active hotspots determining SERS enhancement effects with duck meat extract containing tetracycline, decreased gradually along with the increases of adsorption time. SERS intensity at 1272 cm^{-1} was strongest when adsorption time was 1 min. This indicated that SERS enhancement effects, which were produced by combining active hotspots with duck meat extract containing tetracycline, were best when adsorption time was 1 min. Therefore, 1 min was ascertained as the best adsorption time.

3.5. SERS Calibration Curve and Predicted Results. SERS spectra of duck meat extract containing different tetracycline concentrations were showed in Figure 5. SERS characteristic peak of tetracycline aqueous solution at 1272 cm^{-1} could be observed on SERS spectrum of duck meat extract containing tetracycline (4 mg/L), and the peak position at 1272 cm^{-1} was stable. This illustrated that OTR202 and OTR103 could be used as SERS active substrate for the detection of tetracycline residues in duck meat. Thus, 1272 cm^{-1} could be selected as the characteristic peak of semiquantitative or quantitative analysis of tetracycline residues in duck meat.

TABLE 1: Predicted results of tetracycline in duck meat ($n = 5$).

Spiked (mg·L ⁻¹)	Detected (mg·L ⁻¹)	Recovery (%)	Relative standard deviation (%)
10	10.639 ± 0.321	106.39	4.597
15	16.201 ± 0.431	108.01	3.510
20	20.114 ± 0.354	100.57	2.376

In order to decrease the effects of external factors on SERS intensity at 1272 cm^{-1} and take full advantage of ratio effect, SERS intensity ratio at 1272 and 1558 cm^{-1} (I_{1272}/I_{1558}) was used to establish the SERS calibration curve between the tetracycline concentration in duck meat extract and I_{1272}/I_{1558} . SERS characteristic peak at 1558 cm^{-1} was caused by duck meat extract. As showed from the inset in Figure 5, the linear regression equation was $y = 0.0177x + 0.1213$, and the correlation coefficient (r) was 0.950, in which x and y represented the tetracycline concentration in duck meat extract and I_{1272}/I_{1558} , respectively. This illustrated that a good linearity relationship between the tetracycline concentration (4~25 mg/L) in duck meat extract and I_{1272}/I_{1558} was obtained.

The SERS calibration curve obtained from above was applied to predict the tetracycline concentration in duck meat extract, and the predicted results were showed in Table 1. The average recovery of tetracycline in duck meat extract was 101~108% with the relative standard deviation (RSD) of 2.4~4.6%, which indicated that this method had a good precision and satisfactory reproducibility. The detection limit of tetracycline in duck meat extract could reach 1.120 mg/L ($S/N = 3$). The experimental results showed that the pretreatment method adopted in this paper was more simple and rapid than the conventional detection methods such as enzyme-like immunosorbent assay, microbiological method, and physical and chemical detection method. However, this method still needs to be improved owing to the disadvantage of its high detection limit, which is possibly because surface enhancement factor of SERS substrate is affected by many factors, such as particle size, shape, and uniformity of the distribution of nanoparticles [19], and the composition of duck meat can decrease intensities of tetracycline SERS characteristic peaks in duck meat extract. So, it will be a good selection, in future studies, to explore a pretreatment method with less effect on intensities of tetracycline SERS characteristic peaks in duck meat extract or a nanoparticle with the better enhancement effect to decrease the detection limit. Also, the method proposed in this paper could provide the important technical support for achieving the rapid detection of tetracycline residues in duck meat.

4. Conclusions

A simple and rapid method using OTR202 and OTR103 as SERS enhancement substrate was developed for the detection of tetracycline residues in duck meat. The effects of addition amount of samples and adsorption time on SERS intensity were analyzed, respectively. A good linearity relationship between the tetracycline concentration in duck meat extract

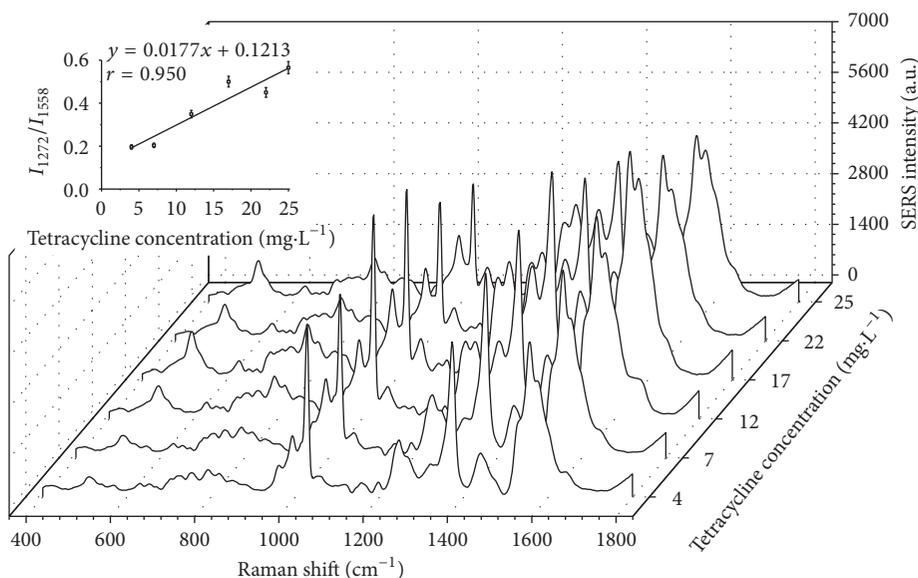


FIGURE 5: SERS spectra of duck meat extract containing different tetracycline concentrations. The inset shows the linear relationship between the tetracycline concentration and SERS intensity ratio at 1272 and 1558 cm^{-1} (I_{1272}/I_{1558}).

and I_{1272}/I_{1558} was obtained, and the linear regression equation and r were $y = 0.0177x + 0.1213$ and 0.950, respectively. The pretreatment method in this paper was simple and rapid, and the detection limit of tetracycline in duck meat extract could reach 1.120 mg/L. Therefore, the method proposed in this paper was a good detection scheme for the rapid detection of tetracycline residues in duck meat.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Jinhui Zhao and Ping Liu contributed equally to this work and should be considered co-first authors.

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