

Rapid detection of sulfamethazine and ofloxacin residues in duck meat using synchronous fluorescence spectroscopy coupled with chemometric methods

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ABSTRACT Rapid detection of antibiotic residues in duck meat is of great significance for strengthening food safety and quality supervision of duck meat and fighting against inferior products in the duck meat market. The objective of the current paper was to evaluate the potential of synchronous fluorescence spectroscopy (SFS) coupled with chemometric methods for the rapid detection of sulfamethazine (SM2) and ofloxacin (OFL) residues in duck meat. The SFS spectral data from duck meat containing different concentrations of SM2 and OFL were preprocessed by baseline offset. The detection conditions, including the adding amounts of β -mercaptoethanol solution and o-phthalaldehyde solution, as well as the reaction time, were optimized by a single factor experiment for obtaining a better detection effect,

and their optimal values were 400 μL , 25 μL , and 40 min, respectively. By comparing 2 chemometric models based on peak-height algorithm and peak-area algorithm, the prediction model based on peak-height algorithm was a better quantitative model with correlation coefficient for the prediction set (**R_p**) of 0.9031 and 0.9981, the root mean error for the prediction set (**RMSEP**) of 7.9509 and 0.5267 mg/kg, recovery of 81.7 to 155.1% and 96.4 to 111.2%, and relative standard deviation (**RSD**) of 4.1 to 6.7% and 2.9 to 6.8% to predict SM2 and OFL residues in duck meat, respectively. Overall, the results of this investigation showed that SFS technique was an effective and rapid tool for the detection of SM2 and OFL residues in duck meat.

Key words: synchronous fluorescence spectroscopy, sulfamethazine, ofloxacin, duck meat, chemometric method

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INTRODUCTION

Duck, as an important economic poultry, is widely reared in many countries, such as China, Vietnam, Thailand, and India. In these countries, antibiotics, such as sulfonamides and fluoroquinolones, are extensively used as prophylactic, therapeutic, and growth-promoting agents in the breeding industry (Chen et al., 2017; Bacanli and Basaran, 2019; Yi et al., 2019; Samokhvalov, 2020). However, their excessive and unreasonable use may cause the presence of sulfamethazine (SM2) and ofloxacin (OFL) residues in duck tissues, which can give rise to a series of public health concerns on toxic effects, allergic reactions and antimicrobial resistance, even cancer (Chen et al., 2017; Bacanli and Basaran, 2019). Besides, a significant portion of them in the body is excreted in urine and feces into the environment, and

antibiotics of the continuous accumulation in the environment are toxic to plants and aquatic organisms (Frade et al., 2014). Recently, it often hits the news that antibiotic residues in duck meat become increasingly serious, which has aroused much concern among the public. In an attempt to guarantee the quality of duck products and environmental protection, the Ministry of Agriculture and Rural Affairs of the People's Republic of China has restricted or prohibited the use of sulfonamides and fluoroquinolones in food-producing animals (Ministry of Agriculture of the People's Republic of China, 2015; Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2019). There were many analytical methods for the detection of sulfonamides and fluoroquinolones residues in animal tissues, such as ultra-performance liquid chromatography (Song et al., 2017; Wang et al., 2017) and liquid chromatography-mass spectrometry (Zhang et al., 2016; Chen et al., 2020). Although these methods were highly useful, they were too laborious, time-consuming, and expensive to make them suitable for the quick detection on-site environment. Consequently, it is necessary to investigate an approach for detecting rapidly and efficiently whether there are these antibiotic residues in duck tissues or not.

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SM2 and OFL belong to sulfonamides and fluoroquinolones, respectively. These 2 antibiotic residues in duck meat, which is one of the most popular foods in China, can result in the potential negative consequences on the human body through food chain.

Fluorescence spectroscopy, photoluminescence spectroscopy, is now a popular method for qualitative and quantitative analysis (Lakowicz, 2006). For the “conventional” fluorescence excitation spectroscopy, the excitation wavelengths (λ_{exc}) (or the emission wavelengths (λ_{emiss}) are continuously changed to form an excitation (or emission) spectrum under the condition of keeping λ_{emiss} (or λ_{exc}). As far as synchronous fluorescence spectroscopy (SFS) is concerned, the λ_{emiss} and λ_{exc} are scanned simultaneously on the condition of keeping a constant wavelength difference ($\Delta\lambda$) between them to obtain a SFS spectrum (Genis et al., 2019; Samokhvalov, 2020). Compared with the conventional fluorescence spectroscopy, the SFS is more suitable for the analysis of multicomponent mixtures since it has the characteristics of simplifying the spectrum, improving the selectivity, and reducing the light scattering interference (Xu and Wang, 2006; Huyan et al., 2018). There were previously published studies on the application of the fluorescence technique for analyzing SM2 and OFL residues in animal derived food (Ren and Guo, 2007; Deng et al., 2016). To date, there was no report about the simultaneous detection of SM2 and OFL residues in duck meat based on the SFS technology. So, it's an utmost meaningful work to investigate the rapid detection method of SM2 and OFL residues in duck meat based on SFS.

Herein, we set out to investigate the potential of applying SFS coupled with chemometric methods to rapidly detect SM2 and OFL residues in duck meat with the accelerated solvent extraction. The SFS detection conditions of SM2 and OFL residues in duck meat were optimized by the single factor experiment. Two chemometric models based on peak-height algorithm and peak-area algorithm were established and compared for the quantitative analysis. The current study provided a significant basis for further applying SFS and chemometric methods to quantitatively analyze sulfonamides and fluoroquinolones residues in meat.

MATERIALS AND METHODS

Materials and Reagents

The ducks were purchased from a supermarket in Nanchang city of Jiangxi province. SM2 (99.4%) was supplied by Dr. Ehrenstorfer, GmbH (Augsburg, Germany). OFL ($\geq 99\%$) was purchased from Yuanye Biological Technology Co., Ltd. (Shanghai, China). O-phthalaldehyde (99.0%) and β -mercaptoethanol (99.0%) were obtained from Aike Reagent Co., Ltd. (Chengdu, China). Boric acid ($\geq 99.5\%$) and phosphoric acid ($\geq 85.0\%$) were provided by Xilong Scientific Co., Ltd. (Shantou, China). Acetic acid glacial ($\geq 99.5\%$) was bought from Damao Chemical Reagent Factory (Tianjin, China). Ethylenediamine tetraacetic acid disodium

salt (Na_2EDTA), potassium dihydrogen phosphate (KH_2PO_4), and di-Potassium hydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$) were purchased from Xilong Scientific Co., Ltd. (Analytical grade, Shantou, China). Trichloroacetic acid (Analytical grade) was obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

Phosphate buffer (containing 0.4 mmol/L of Na_2EDTA and 10% trichloroacetic acid solution) was prepared by dissolving 1 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 4 g of KH_2PO_4 , 75 mg of Na_2EDTA , and 50 g of trichloroacetic acid in a 500 mL volumetric flask with approximately 500 mL of ultrapure water.

A total of 0.01 mol/L o-phthalaldehyde solution was obtained by dissolving 0.134 g of o-phthalaldehyde in a 100 mL volumetric flask with nearly 100 mL of ultrapure water.

A total of 0.1 mol/L β -mercaptoethanol solution was prepared by diluting 704 μL of β -mercaptoethanol in a 100 mL volumetric flask with about 100 mL of ultrapure water.

BR buffer solution was made of 0.04 mol/L of boric acid, 0.04 mol/L of phosphoric acid, and 0.04 mol/L of acetic acid glacial.

Solutions Preparation

Approximately 7.5 mg of SM2 (or OFL) was dissolved in 50 mL of ultrapure water to obtain 150 mg/L of SM2 (or OFL) stock solution. Nearly 0.6 mL of SM2 stock solution was diluted to 15 mL with ultrapure water to obtain 6.0 mg/L SM2 working solution. About 1.0 mg/L OFL working solution was obtained by diluting 0.1 mL of OFL stock solution to 15 mL with ultrapure water. These solutions were stored at 4°C before use.

Sample Preparation

SM2 and OFL were extracted from duck meats based on the accelerated solvent extraction with slight modification (Teng et al., 2018), as described next.

- Preparation of the working solution containing SM2 and OFL: Different volumes of SM2 stock solution (including 0.083, 0.167, 0.333, 0.500, 0.750, 1.000, 1.500, 1.750, 2.000, 2.250 and 2.500 mL) and OFL stock solution (including 0.042, 0.083, 0.167, 0.250, 0.333, 0.417, 0.500, 0.583, 0.667, 0.750 and 0.917 mL) were diluted to 4 mL with ultrapure water to obtain the working solution containing different concentrations of SM2 and OFL.
- Preparation of spiked duck meat samples: Duck breasts were homogenized with tissue disintegrator (JJ-2B, Jintan Jinnan Instrument Factory, China) after they were removed from the whole ducks by using a scalpel. Approximately 5 g of homogenous duck breast was spiked with the working solution containing different concentrations of SM2 (2.50–75.00 mg/kg) and OFL (1.25–27.50 mg/kg), and

then they were vigorously whirled by VOTRER-5 whirlpool mixer (Haimen Kylin-Bell Lab Instruments Co., Ltd., China) for 1 min.

- c. Extraction of SM2 and OFL from duck meat: Firstly, 10 mL of phosphate buffer was added into the spiked samples, and then they were vigorously whirled for 1 min, shaken by KJ-201BS Oscillator (Kangjian Medical Supplies Co., Ltd. Jiangsu, China) for 15 min and centrifuged by JW-1024 centrifuge (Jia Wen Instrument and Equipment Co., Ltd. Anhui, China) at 4,500 r/min for 15 min. Subsequently, the above steps were repeated once more time after the residues were added with 10 mL of phosphate buffer, and then the abovementioned supernatants were merged together. Third, after the obtained supernatant was filtered by 0.45 μm filter membrane, its volume was fixed to 25 mL with phosphate buffer. In the same way, duck meat extracts without SM2 and OFL were obtained in the similar steps to the above mentioned method by using duck breasts without SM2 and OFL.

Measurement of Three-Dimensional SFS Spectra

In order to obtain and analyze their three-dimensional SFS spectra, 200 μL of the analyzed sample solution (i.e., 6.0 mg/L SM2 standard solution, 1.0 mg/L OFL standard solution, duck meat extract sample without SM2 and OFL, as well as duck meat extract sample containing 6.0 mg/L SM2 and 1.0 mg/L OFL), 800 μL of BR buffer, 100 μL of β -mercaptoethanol solution and 100 μL of o-phthalaldehyde solution were added into quartz cuvettes and mixed well. Next, their three-dimensional SFS spectra, which had λ_{exc} in the range of 240 to 490 nm and $\Delta\lambda$ in the range of 20 to 300 nm with an interval of 10 nm, were performed on a fluorescence spectrometer (Cary Eclipse, Varian Co., Palo Alto, CA) with 700 V of the photomultiplier tube voltage, 10 nm of the excitation slit width and 5 nm of the emission slit width.

Qualitative Measurement

First, in order to investigate the effect of the adding amounts of β -mercaptoethanol solution on fluorescence intensity, different volumes (i.e., 25, 100, 200, 400, 500, 600, and 700 μL) of β -mercaptoethanol solution were mixed with 200 μL of duck meat extract containing 6.0 mg/L SM2 and 1.0 mg/L OFL, BR buffer (used to

fix the total volume to 1.2 mL) and 100 μL of o-phthalaldehyde solution, respectively.

Second, for the analysis of the effect of the adding amounts of o-phthalaldehyde solution on fluorescence intensity, different volumes (i.e., 25, 100, 200, 300, and 400 μL) of o-phthalaldehyde solution were blended with 200 μL of duck meat extract containing 6.0 mg/L SM2 and 1.0 mg/L OFL, BR buffer (used to fix the total volume to 1.2 mL) and 400 μL of β -mercaptoethanol solution, respectively.

Third, based on the above-optimized results, the mixture solutions of 200 μL of duck meat extract (containing 6.0 mg/L SM2 and 1.0 mg/L OFL), 575 μL of BR buffer, 400 μL of β -mercaptoethanol solution and 25 μL of o-phthalaldehyde solution were used to select the optimal reaction time.

Five parallel samples for each concentration level were performed during the above 3 optimization processes. In the first 2 processes, SFS spectra at 150 nm and 210 nm of $\Delta\lambda$ were measured with 700 V of the photomultiplier tube voltage, 10 nm of the excitation slit width and 5 nm of the emission slit width at 40 min of reaction time, respectively. In the third process, SFS spectra at 150 nm and 210 nm of $\Delta\lambda$ were measured with 700 V of the photomultiplier tube voltage, 10 nm of the excitation slit width and 5 nm of the emission slit width in the range of 0 to 100 min (interval 4 min) of reaction time, respectively.

Quantitative Measurement

Two hundred μL of duck meat extracts containing different concentrations of SM2 and OFL were mixed with the mixture solution of 575 μL of BR buffer, 400 μL of β -mercaptoethanol solution and 25 μL of o-phthalaldehyde solution, respectively. Next, SFS spectra of samples at 150 nm and 210 nm of $\Delta\lambda$ were measured at 40 min of reaction time with 700 V of the photomultiplier tube voltage, 10 nm of the excitation slit width and 5 nm of the emission slit width. Five parallel samples for each concentration level were performed, and 11 concentration levels of duck meat samples containing SM2 and OFL were obtained for quantitative analysis. Statistical results of samples were shown in Table 1. Six concentration levels of samples were selected for the training set and the rest were used for the prediction set.

Data Analysis

Baseline offset of raw SFS spectra was performed to eliminate the baseline drift by using the Unscrambler X

Table 1. Statistical results of samples of SM2 and OFL residues in duck meat.

Sample set	Concentration/(mg/kg)		Mean/(mg/kg)		Std dev/(mg/kg)	
	SM2	OFL	SM2	OFL	SM2	OFL
Training set	2.50–75.00	1.25–27.50	41.25	13.96	30.93	10.62
Prediction set	10.00–60.00	5.00–20.00	27.50	11.50	19.69	6.02

Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

10.4 (CAMO, Norway) before the prediction model was established. Next, the peak areas of SM2 ($\Delta\lambda = 150$ nm, λ_{exc} at the region between 270 and 315 nm) and the peak areas of OFL ($\Delta\lambda = 210$ nm, λ_{exc} at the region between 275 and 315 nm) were calculated by LabSpec 5 (HORIBA Jobin Yvon, Paris, France). Subsequently, the intensities of fluorescence excitation peak at 292.5 nm ($\Delta\lambda = 150$ nm) and at 295 nm ($\Delta\lambda = 210$ nm) were respectively used to correlate SM2 and OFL concentrations in duck meat to establish the prediction models based on peak-height method. Additionally, fluorescence excitation peak areas were used to correlate SM2 and OFL concentrations in duck meat to build up the prediction models based on peak-area method. The correlation coefficient for the training set (\mathbf{R}_T), correlation coefficient for the prediction set (\mathbf{R}_P), root mean square error for the prediction set (\mathbf{RMSEP}), recovery, and relative standard deviation (\mathbf{RSD}) were calculated to evaluate the model performance. The \mathbf{RMSEP} and recovery were calculated by using the following formula:

$$\sqrt{\frac{\sum_{i=1}^n (y_{i,\text{predicted}} - y_{i,\text{actual}})^2}{n}} = \mathbf{RMSEP} \quad (1)$$

$$\frac{y_{i,\text{predicted}}}{y_{i,\text{actual}}} \times 100 = \% \text{ Recovery} \quad (2)$$

where $y_{i,\text{predicted}}$ is the predicted concentration of No. i sample in duck meat, $y_{i,\text{actual}}$ is the actual concentration of No. i sample in duck meat and n is the total sample numbers for the prediction set.

RESULTS

SFS Characteristics of Samples

SM2 and OFL have different fluorescence characteristics owing to the fact that they have different fluorescence groups. In order to achieve the purpose of simultaneous detection of these 2 antibiotics, it is necessary that both SM2 and OFL can simultaneously produce obvious fluorescence characteristics, so as to obtain fluorescence information containing these 2 antibiotics. On the one hand, SM2 is a weak fluorescent substance. But, it can be derivatized by a derivatizing agent like o-phthalaldehyde owing to its primary amine group, and its derivative has a fluorescence excitation peak at $\Delta\lambda/\lambda_{\text{exc}}$ of 150/292.5 nm (Figure 1A). On the other hand, OFL has excellent fluorescence properties because its molecule has a conjugate and rigid structure (Li et al., 2017). It could be seen that OFL had fluorescence excitation peaks at $\Delta\lambda/\lambda_{\text{exc}}$ of 170/330 nm and 210/295 nm (Figure 1B). It was clear that SFS spectra of SM2 and OFL standard solutions had a prominent difference at $\Delta\lambda = 150$ nm and $\Delta\lambda = 210$ nm (Figure 2).

The compositions of duck meat are complex, and some components such as tryptophan have strong fluorescence properties. Therefore, the duck meat extracts were treated with trichloroacetic acid and an organic phase needle filter to weaken the effects of the protein, fat, and other substances in the current paper. Duck meat extract without SM2 and OFL had fluorescence excitation peaks at $\Delta\lambda/\lambda_{\text{exc}}$ of 80/447.5 nm, 90/285 nm, 100/320 nm, and 150/370 nm (Figure 1C).

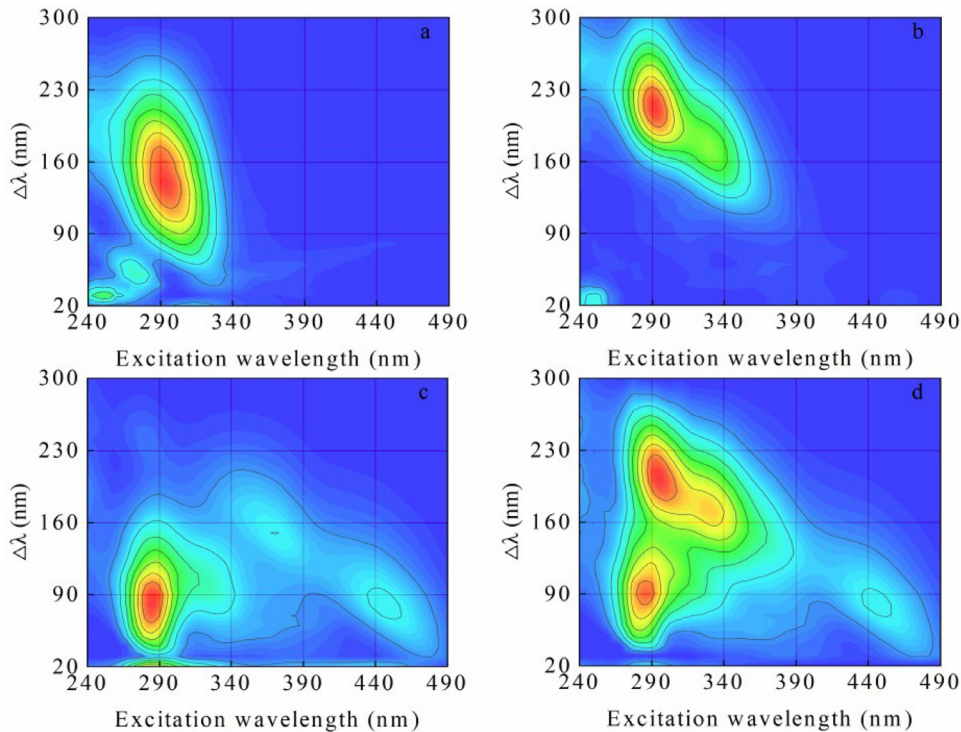


Figure 1. Contour plots of three-dimensional SFS: (A) SM2 standard solution; (B) OFL standard solution; (C) duck meat extract without SM2 and OFL; (D) duck meat extract containing SM2 and OFL. Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

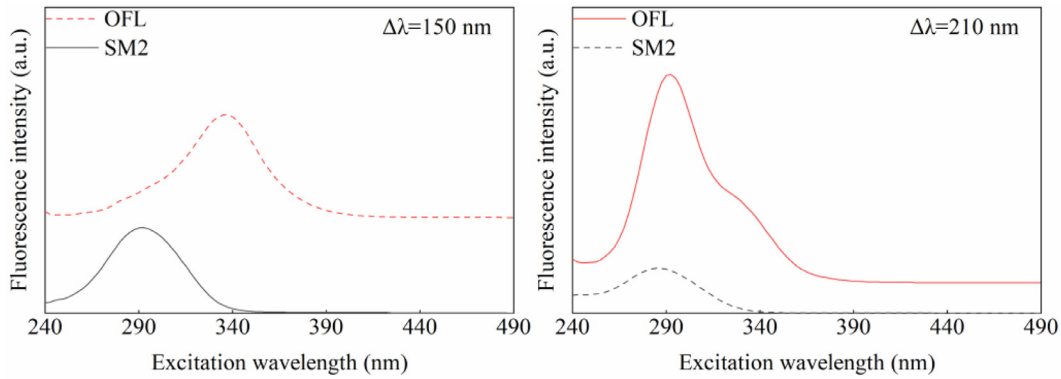


Figure 2. SFS of SM2 and OFL standard solutions at $\Delta\lambda = 150$ nm and $\Delta\lambda = 210$ nm. Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

As seen from Figure 1D, SM2 derivatives and OFL presented their fluorescence excitation peaks in duck meat extracts containing SM2 and OFL.

In summary, the fluorescence signals had the clear difference between SM2 and OFL in duck meat extract and duck meat background, and $\Delta\lambda/\lambda_{\text{exc}}$: 150/292.5 nm and 210/295 nm could be used to simultaneously detect SM2 and OFL residues in duck meat.

Optimization of SFS Detection Conditions of SM2 and OFL Residues in Duck Meat

The effects of the adding amounts of β -mercaptoethanol solution and o-phthalaldehyde solution, as well as reaction time on fluorescence intensities of duck meat

extract containing SM2 and OFL were investigated by a single factor experimental method.

To analyze the effect of the adding amounts of β -mercaptoethanol solution on fluorescence intensity, the intensities of characteristic peaks of SM2 and OFL were investigated under the condition of only changing the adding amounts of β -mercaptoethanol solution. As shown in Figure 3A, the fluorescence intensities of SM2 derivative gradually increased with the adding amounts of β -mercaptoethanol solution in the range of 25 to 400 μL . One of the probable reasons was that more SM2 derivatives were formed to enhance the fluorescence intensities with the adding amounts of β -mercaptoethanol solution.

The different adding amounts of β -mercaptoethanol solution in the range of 400 to 700 μL had little influence on the fluorescence intensities of SM2 derivative. A

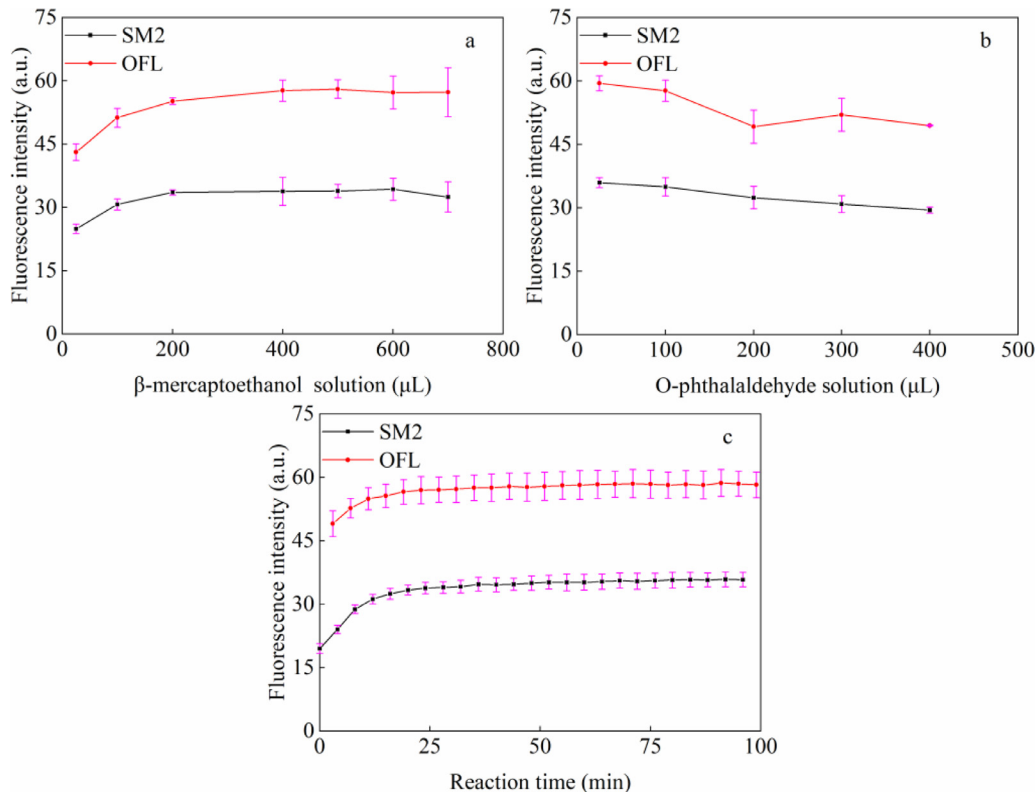


Figure 3. Effects of difference conditions on the fluorescence intensities: (A) addition amounts of β -mercaptoethanol solution; (B) addition amounts of o-phthalaldehyde solution; (C) reaction time.

possible explanation for this phenomenon was that the effect of the adding amounts of β -mercaptoethanol solution on the yield of SM2 derivatives was weakened. Additionally, the fluorescence intensities of OFL increased as the increase of the adding amounts of β -mercaptoethanol solution in the range of 25 to 400 μ L. It was universally acknowledged that the fluorescence intensities of fluoroquinolones were pH-dependent (Kaur et al., 2012). The change of the adding amounts of β -mercaptoethanol solution in the process could affect the pH value and consequently enhance the fluorescence intensities of OFL. The adding amounts of β -mercaptoethanol solution in the range of 400 to 700 μ L exerted a little impact on the fluorescence intensities of OFL. This phenomenon may be explained that the effect of the adding amounts of β -mercaptoethanol solution on the pH was weakened. Considering all of this evidence, it seemed that the fluorescence intensities of SM2 derivatives and OFL were the strongest when adding β -mercaptoethanol solution of 400 μ L. Therefore, 400 μ L was chosen as the adding amounts of β -mercaptoethanol solution.

As the increase of the adding amounts of o-phthalaldehyde solution, the fluorescence intensities of SM2 derivatives showed a downward trend (Figure 3B). The probable reason for the decrease of fluorescence intensities was that excess o-phthalaldehyde could lead to the rapid degradation of the SM2 derivatives during the formation of isoindole derivative (Stobaugh et al., 1983; Jacobs et al., 1986). In the section that follows, the situation of the fluorescence intensities of OFL would be argued. The fluorescence intensities of OFL presented a downward trend as the increase of the adding amounts of o-phthalaldehyde solution in the range of 25 to 200 μ L. This result could be attributed to the change in pH caused by the addition of o-phthalaldehyde solution. The previous literature showed the strongest fluorescence intensities of OFL could be obtained at the pH 4 of the system (Ren and Guo, 2007). The pH value of the system increased with the increase of the adding amounts of o-phthalaldehyde solution in the acidic system, and this further weakened the fluorescence intensities of OFL.

When adding amounts of o-phthalaldehyde solution were in the range of 200 to 400 μ L, the fluorescence

intensities of OFL have been finitely affected by the adding amounts of o-phthalaldehyde solution. A possible explanation for this might be that the effect of the adding amounts of o-phthalaldehyde solution on the pH of the system was weakened. The evidence presented here suggests that when the adding amount of o-phthalaldehyde solution was 25 μ L, the fluorescence intensities of SM2 derivatives and OFL were the strongest. Therefore, the optimum amount of o-phthalaldehyde solution was determined to be 25 μ L.

Reaction time is one of the important factors affecting the fluorescence intensities of the analyte. As could be seen from Figure 3C, fluorescence intensities of SM2 derivative rapidly increased along with the increase of reaction time in the range of 0 to 40 min. Its fluorescence intensities remained unchanged after 40 min. Besides, fluorescence intensities of OFL showed a little raising along with the increase of reaction time in the range of 0 to 30 min and its fluorescence intensities almost remained unchanged after 30 min. Taken together, fluorescence intensities of OFL reached the maximum at 30 min and still maintained nearly the maximum value at 40 min, and fluorescence intensities of the SM2 derivatives reached the maximum at 40 min. Thus, 40 min was selected as the acquisition time.

Establishment of Prediction Model

Peak-height algorithm was used to establish the quantitative model to predict the SM2 and OFL residues in the duck meat. To verify the reliability of the prediction model based on peak-height algorithm, the correlations were checked between the actual and predicted values of SM2 and OFL residues in the duck meat for the prediction set. As seen from Figure 4 and Table 2, a good linear relationship for the prediction model of SM2 residues in the duck meat was obtained with an R_p value of 0.9031, RMSEP value of 7.9509 mg/kg, recovery of 81.7 to 155.1% and RSD of 4.1 to 6.7%. In addition, a good linear relationship for the prediction model of OFL residues in the duck meat was obtained with an R_p value of 0.9981, RMSEP value of 0.5267 mg/kg, recovery of 96.4 to 111.2% and RSD of 2.9 to 6.8%. The experimental

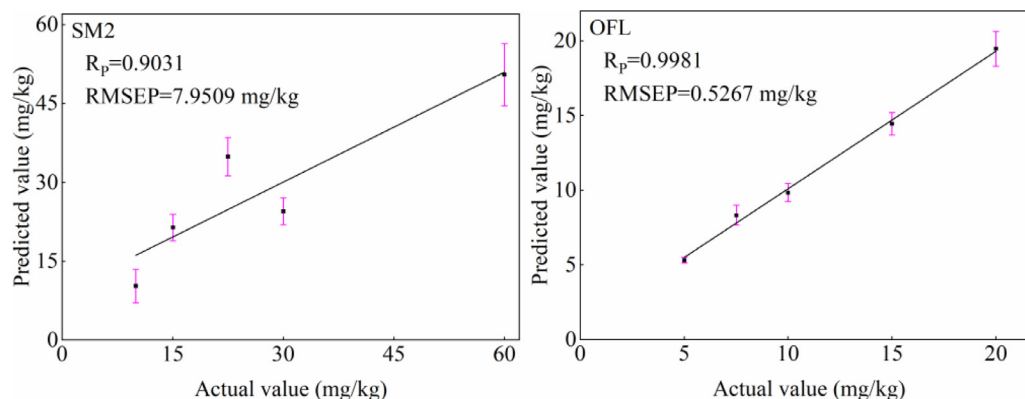


Figure 4. Plots of the relationship between the actual and predicted values of SM2 and OFL residues in duck meat in the prediction samples based on peak height algorithm. Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

Table 2. Prediction results of SM2 and OFL residues in duck meat based on peak-height algorithm.

No.	Actual value/(mg/kg)		Predicted value/(mg/kg)		Recovery/%		RSD/(n = 5, %)	
	SM2	NEO	SM2	NEO	SM2	NEO	SM2	NEO
1	10.0	5.0	10.3	5.3	102.8	106.6	6.6	2.9
2	15.0	7.5	21.4	8.3	143.0	111.2	4.2	6.8
3	22.5	10.0	34.9	9.9	155.1	98.5	5.0	5.4
4	30.0	15.0	24.5	14.5	81.7	96.4	4.1	4.6
5	60.0	20.0	50.5	19.5	84.2	97.4	6.7	5.5

Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

results showed that it was probable to analyze quantitatively the SM2 and OFL residues in the duck meat using SFS technology coupled with peak-height algorithm. The detection limits of SM2 and OFL residues in duck meat could reach 2.5 mg/kg and 1.25 mg/kg, respectively.

DISCUSSION

The linear functions based on peak-height algorithm and peak-area algorithm were established and their prediction effects were compared for the prediction of SM2 residues in duck meat, respectively. As shown in Table 3 by comparison of these 2 linear functions, the linear function based on peak-height algorithm (i.e., $y = 0.5017x + 19.049$) could obtain a good linear relationship between fluorescence intensities and SM2 concentrations in duck meat with an R_T value of 0.9191 for the training set.

Additionally, the good linear relationships between fluorescence intensities and OFL concentrations in duck meat could both be obtained by using peak-height algorithm and peak-area algorithm. These 2 algorithms could both obtain R_T values of no less than 0.9982 for the training set.

To summarize, this evidence indicated that the prediction models of OFL residues in duck meat had a better linear relationship than those of SM2 residues in duck meat based on both peak-height algorithm and peak-area algorithm. To ensure the good linear relationship of the prediction model of SM2 residues in duck meat, the peak-height algorithm was selected to establish the prediction model of SM2 and OFL residues in duck meat.

Previous studies reported the fluorescence detection method of a single antibiotic SM2 or OFL in food. It has been reported that the ofloxacin residues in musculature of crucian, eel, carp, and tilapia was determined by spectrofluorimetry at the excitation wavelength of 294 nm and the emission wavelength of 496 nm (Ren and Guo, 2007). Wang (2009) reported

Table 3. Prediction models for SM2 and OFL residues in duck meat.

Prediction object	Method	R_T	Linear function
SM2 in duck meat	Peak height	0.9191	$y = 0.5017x + 19.049$
	Peak area	0.8492	$y = 19.258x + 771.89$
OFL in duck meat	Peak height	0.9983	$y = 7.3206x + 10.705$
	Peak area	0.9982	$y = 223.72x + 350.1$

Abbreviations: OFL, ofloxacin; SM2, sulfamethazine.

that a fluorescence spectrophotometry assay was developed to detect sulfamethazine residues in egg with the recoveries of the spiked sample of 89.20 to 96.80%. In the current study, SM2 and OFL residues in duck meat were detected simultaneously by using synchronous fluorescence spectroscopy. In conclusion, the experimental results showed that the adopted method based on peak-height algorithm could meet the requirement of rapid simultaneous detection of SM2 and OFL residues in duck meat and had the certain reference value for subsequent research.

CONCLUSIONS

To conclude, a novel method was established for the identification of SM2 and OFL residues in duck meat by using SFS combined with chemometric methods, with the purpose of rapid quantitative detection of these 2 antibiotics in duck meat. We investigated SFS spectra characteristics of duck meat extract, SM2 derivative and OFL, and discovered that they could be effectively distinguished based on the fluorescence characteristic peaks. Certain factors such as the additional amounts of β -mercaptoethanol solution and o-phthalaldehyde solution as well as the reaction time could affect the intensities of SM2 and OFL residues in duck meat. Thus, the effects of these 3-factors on fluorescence intensities were investigated using single factor experimental method to obtain the optimal SFS detection conditions. Furthermore, the performances of the 2 prediction models based on peak-height algorithm and peak-area algorithm were compared, and the prediction model based on peak-height algorithm was selected as the analytical model. The prediction model of SM2 residues had the RMSEP of 7.9509 mg/kg, recovery of 81.7 to 155.1%, RSD of 4.1 to 6.7%, respectively. Also, the prediction model of OFL residues had the RMSEP of 0.5267 mg/kg, recovery of 96.4 to 111.2%, RSD of 2.9 to 6.8%, respectively. The experimental results showed that this method could meet the needs of the rapid detection of SM2 and OFL residues in duck meat. The implemented method simplified the detection step and provided technical support to rapidly detect antibiotic residues in duck meat.

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DISCLOSURES

The authors declare no competing or financial interests.

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