

Classification and detection of testosterone propionate and nandrolone residues in duck meat using surface-enhanced Raman spectroscopy coupled with multivariate analysis

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ABSTRACT There is a critical need for a rapid and simple method of qualitative and quantitative analysis of testosterone propionate (TP) and nandrolone (NT) residues in duck meat. In this study, we applied surface-enhanced Raman spectroscopy (SERS) coupled multivariate analysis for the classification and detection of TP and NT residues in duck meat. A total of 294 duck meat extract samples were obtained from duck breast meats based on a LC-MS/MS sample preparation method with slight modification including 102 duck meat extract samples without TP and NT, 43 duck meat samples containing TP, 47 duck meat extract samples containing NT, and 102 duck meat extract samples containing TP and NT. Raw Raman spectra were pretreated by using adaptive iteratively reweighted penalized least squares (airPLS), normalization and first derivative, and then the score values of first 10 principal components were selected as the inputs of the developed models. A particle

swarm optimization–support vector classification (PSO-SVC) model was created to classify all the duck meat samples into the 4 groups (i.e., control group, TP group, NT group, and TP combined with NT group) with the classification accuracies of 99.49 and 100% for training set and test set, respectively. Furthermore, 2 least squares support vector regression (LS-SVR) models were developed to predict the TP values in samples with a determination coefficient (R^2) value of 0.9316, root mean square error (RMSE) value of 2.1739, and ratio of prediction to deviation (RPD) value of 3.2189 for the test set, and NT values in samples with an R^2 value of 0.9038, RMSE value of 2.2914, and RPD value of 2.9701 for the test set. Surface-enhanced Raman spectroscopy technology, in combination with multivariate analysis, has the potential to become the qualitative and quantitative analysis tool for TP and NT residues in duck meat extract.

Key words: surface-enhanced Raman spectroscopy, testosterone propionate, nandrolone, duck meat, multivariate analysis

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INTRODUCTION

Testosterone propionate (TP) and nandrolone (NT) are the members of the androgenic–anabolic steroids group of hormones containing mother nucleus structure of perhydrocyclopentanophenanthrene (Quan et al., 2011). Owing to their cost-effective feature, they were commonly used to promote the growth and food conversion of food-producing animals including duck in the past (Zhang et al., 2016). However, the TP and NT

residues in edible animal tissues enter easily into human body through the food chain, and may cause metabolic disorder, dysplasia, teratogenic and/or carcinogenic of human body (Hou et al., 2016; Zhang et al., 2016). In view of this fact, the EU and China have banned their use in food-producing animals owing to the potential health risks caused by TP and NT (Pedersen and Andersen, 2011; Malone et al., 2011; Zhang et al., 2016). In addition, some hormones are allowed the restricted use in food-producing animals in some other countries. For example, United States has established the regulation for maximum residue limit of TP in the muscle of cattle with 0.64 $\mu\text{g}/\text{kg}$. Up to now, their illegal or abuse use has been existed in food-producing animals. Therefore, it is important to monitor the TP and NT residues in duck meat with regard to food safety issues.

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Until now, many diverse methods have been investigated to detect effectively TP and NT residues in food-producing animals, including high-performance liquid chromatography (HPLC) (Jeong et al., 2017), gas chromatography-mass spectrometry (GC-MS) (Combalbert et al., 2010), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Pedersen and Andersen, 2011; Malone et al., 2011), enzyme-linked immunoassay (ELISA) (Ma et al., 2010). Although these methods can provide the precise detection, HPLC, GC-MS, and LC-MS/MS demand expensive instruments and complex operations. ELISA possesses the high specificity, yet it is time-consuming with tedious steps. Hence, it is necessary to develop a rapid and simple assay for the detection of TP and NT residues in duck meat.

Surface-enhanced Raman spectroscopy (SERS), as a powerful vibrational spectroscopic method that can be used to detect molecules or biomolecules in food samples owing to the molecular fingerprint specificity and the potential single-molecule sensitivity (Song et al., 2016; Chen et al., 2019; Cialla et al., 2012), has been used to detect the trace amounts of antibiotics and hormones in food-producing animals (Wu et al., 2015; Yang et al., 2018; Song et al., 2019). A rapid SERS method was developed to determine the trace diethylhexyl phthalate as an environmental hormone in energy drinks by using a portable Raman system (Yuan et al., 2018). A SERS method combined with principal component analysis and support vector machine was applied to rapid classify sulfadimidine and sulfapyridine in duck meat with the classification accuracy of 90.44% for the test set (Xu et al., 2020). The silver nanowires exhibiting high sensitivity and activity were used as a SERS substrate to detect malachite green, crystal violet, furazolidone, and chloramphenicol in fish (Song et al., 2016). Giving its excellent features, SERS technology is a great promise and power to achieve hormones detection in food-producing animals as a rapid and sensitive analytical assay.

The objective of this article was to apply SERS coupled with multivariate analysis to develop classification and regression models for qualitative and quantitative analyses of TP and NT residues in duck meat. To achieve this objective, duck meat extract samples were obtained from duck breast meats based on a LC-MS/MS sample preparation method with slight modification, and their SERS spectra were measured by a portable Raman spectrometer to establish a particle swarm optimization-support vector classification (PSO-SVC) model to classify all the duck meat samples into the 4 groups and a least squares support vector regression (LS-SVR) model to predict the TP and NT residues in duck meat.

MATERIALS AND METHODS

Synthesis of Gold Nanoparticle Colloids

The colloidal gold nanoparticles (Au NPs) were synthesized based on a typical trisodium citrate reduction

method with slight modification (Frens, 1973). In short, 0.9 mL of 1% trisodium citrate (Precision Scientific Instruments Co., Ltd., China) was added into 100 mL of boiling 0.01% HAuCl₄ solution (HAuCl₄·3H₂O, R_t²49% gold, Sigma-Aldrich) with continuously stirring for 9 min. Next, they were cooled to room temperature, and then kept in a brown bottle with stopper at room temperature. UV-Vis absorption spectra of the synthesized Au NPs were measured by a UV-vis spectrometer (T6, Beijing Purkinje General Instrument Co., Ltd., China), and its surface plasmon resonance peak was about 529 nm with the particle diameter of around 44 nm in accordance with the relationship between surface plasmon resonance peak and particle diameter (Haiss et al., 2007).

Preparation of Standard Solutions

About 10.0 mg of TP (99.0%, purchased from Nanchang Precision Scientific Instruments Co., Ltd., China) or NT (98.1%, purchased from Standard Substances Network of China) were, respectively, dissolved in 100 mL of acetonitrile to obtain 100 mg/L TP or NT standard solutions.

Sample Preparation

Duck breast meats were removed from their carcasses after the sheldrakes were purchased from the vegetable market of Jiangxi Agricultural University. The membranes were removed from duck breast meats before duck breast meats were mashed (tissue disintegrator, JJ-2B, Jintan Jinnan Instrument Factory, China) into meat muds. Next, a simple and rapid method based on LC-MS/MS method with slight modification (Huo et al., 2006; Pedersen and Andersen, 2011; Malone et al., 2011; Wang et al., 2014) was used to extract TP and NT from duck breast meats. In brief, around 5 g of duck breast meat muds, spiked with 2 mg of TP or NT with 20 mL of acetonitrile, were vigorously whirled (VOTRER-5 whirlpool mixer, Haimen Kylin-Bell Lab Instrument Co., Ltd., China) for 1 min, oscillated ultrasonically (JK-50B ultrasonic cleaner, Hefei Jinnike Machinery Co., Ltd., China) for 10 min and centrifuged (JW-1024 low speed centrifuge, Anhui Jiawen Instrument Equipment Industry Co., Ltd., China) for 10 min (4,200 r/min). Subsequently, the obtained supernatants were merged together and vigorously whirled with 2 g of magnesium sulfate for 1 min after the residue was extracted with 20 mL of acetonitrile once more. Then, they were evaporated to around 10 mL with nitrogen at 60°C (HSC-24B nitrogen evaporation, Tianjin Heng Ao Technology Development Co., Ltd., China), centrifuged for 10 min (4,200 r/min) and filtered using 0.45 μm filter membrane. The aforementioned solution was whirled with 10 mL of hexane for 1 min, oscillated ultrasonically for 10 min and centrifuged for 10 min (4,200 r/min). Finally, the acetonitrile phase was pipetted to evaporate to nearly dry with nitrogen at 30°C and fixed the volume to 20 mL with methanol, and duck

meat extract samples containing 100 mg/L of TP or NT could be obtained by the above steps. The duck meat extract samples containing different concentrations of TP or NT were obtained by diluting the above obtained duck meat extract using duck meat extract without TP and NT. The duck meat extract samples containing different concentrations of TP and NT were obtained by diluting the duck meat extract containing different concentrations of TP or NT using duck meat extract without TP and NT. A total of 294 duck meat extract samples were obtained and divided into 4 groups, that is, 102 duck meat extract samples without TP and NT (control group), 43 duck meat samples containing TP (TP group), 47 duck meat extract samples containing NT (NT group), and 102 duck meat extract samples containing TP and NT (TP combined with NT group).

Spectral Measurement

A portable Raman spectrometer (QE65 Pro, Ocean Optics Co., Inc.), equipped with a Raman coupled fiber probe for 785 nm with SMA-SMA connectors to connect with a 785 nm diode laser with a fixed power output of 650 mW (LASER-785-LABADJ-S, Ocean Optics Co., Inc.), was used to scan the samples to obtain the SERS spectra in this study.

About 500 μL of Au NPs colloid, 10 μL of duck meat extract and 100 μL of magnesium sulfate solution (0.1 mol/L) were added into 2 mL quartz sample vials to measure the SERS spectra of samples. Each sample was only scanned one time.

Spectral Data Processing

All Raman spectra were first baseline-corrected based on adaptive iteratively reweighted penalized least squares (**airPLS**) to reduce the baseline variability and fluorescence background in the region of 400 to 1,800 cm^{-1} , and normalized to a range of 0 to 1 to remove laser power fluctuation and the interference of background noises using MATLAB R2010b (The MathWorks, Inc., Natick, MA) (Li et al., 2016). The first derivative spectra of Raman peak intensities vs. Raman shifts were calculated to remove the baseline offsets with Savitzky–Golay algorithm from the normalized Raman spectra using The Unscrambler X 10.4 (CAMO Software AS, Norway).

Next, principal component analysis (**PCA**) as an unsupervised method was performed to extract key features and compress the data dimension with singular value decomposition algorithm and cross validation using The Unscrambler X 10.4. PCA scores and loadings were calculated to maximize the variance in the pre-treated Raman data, and the first 10 PCA scores were used as the inputs of classification and regression models.

The PSO-SVC model was developed to classify all the duck meat samples into the 4 groups, that is, control group, TP group, NT group, and TP combined with NT group using MATLAB R2010b. Where, two-thirds of samples ($n = 196$) were randomly selected from all

the samples to build the classification model, and the rest ($n = 98$) were used to test the model performance evaluated with accuracy, sensitivity, and specificity.

For the samples classified as TP, NT, and/or TP combined with NT groups, we further established 2 LS-SVR models to predict the contents of TP and NT in samples, respectively. Where, 99 samples belonging to NT and TP combined with NT groups were used as training set of the LS-SVR model for predicting the NT contents in samples, and the remaining 50 samples were used as test set. About 97 samples pertaining to TP and TP combined with NT groups were used as training set of the LS-SVR model for predicting the TP contents in samples, and the remaining 48 samples were used as test set. The LS-SVR model performance was evaluated with the determination coefficient (R^2), root mean square error (**RMSE**), and ratio of prediction to deviation (**RPD**).

RESULTS AND DISCUSSION

SERS Spectra Characteristics of Samples

As evident from Figure 1, there was Raman peak at 812 cm^{-1} on SERS spectra of NT group and TP combined with NT group, but there was no Raman peak at 812 cm^{-1} on SERS spectra of magnesium sulfate-aggregated Au NPs, control group, and TP group. Therefore, this Raman peak, which was ascribed to C-H asymmetric swaying vibration of outside of the benzene ring and C-H bending vibration on benzene ring, could be used to judge whether there existed NT residues in duck meat or so. In addition, Raman peak at 842 cm^{-1} was visible on SERS spectra of TP group and TP combined with NT group instead of on SERS spectra of magnesium sulfate-aggregated Au NPs, control group, and NT group. So, this Raman peak, assigned to CH_3 symmetrically inwardly deformed vibration, could be selected to discriminate whether there was TP residues in duck meat or so.

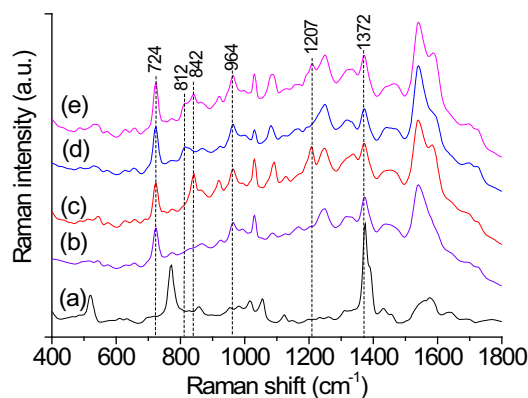


Figure 1. Representative SERS spectra of (a) magnesium sulfate-aggregated Au NPs and the 4 groups of samples: (b) controls, (c) TP, (d) NT, as well as (e) TP combined with NT group. The SERS spectra have been vertically offset. Abbreviations: Au NPs, gold nanoparticles; NT, nandrolone; SERS, surface-enhanced Raman spectroscopy; TP, testosterone propionate.

As far as SERS spectra of TP combined with NT group were concerned, only Raman peak at 812 cm^{-1} has a certain degree of difficulty to distinguish whether there was NT residues in duck meat, owing that Raman peak at 812 cm^{-1} was easily hidden by Raman peak at 842 cm^{-1} when the contents of NT in samples of TP combined with NT group were relatively low. Therefore, multivariate analysis method was investigated to classify all the samples into 4 groups and predict the TP and NT contents in samples.

Raman Spectral Pretreatment

To obtain the better classification performance, the classification accuracies of PCA-SVC models based on 3 spectral pretreatment methods (i.e., airPLS, airPLS and normalization, as well as airPLS, normalization, and first derivative) were compared. The PCA numbers with no less than 90% of cumulative variance contribution could represent most of information of the original variables. Based on this criterion, the first 8, 7, and 10 PCA scores, which accounted for 91.20, 90.16, and 90.30% of the spectral variance, respectively, were selected as the inputs of PCA-SVC models based on 3 pretreatment methods that airPLS, airPLS and normalization, as well as airPLS, normalization, and first derivative, respectively. Classification accuracies of PCA-SVC models with 3 spectral pretreatment methods for the classification of TP and NT in duck meat were showed in Table 1. All the PCA-SVC models for 3 spectral pretreatment methods performed well in classifying all the samples into 4 groups with good classification accuracies. Relatively speaking, airPLS, normalization, and first derivative were used as the spectral pretreatment method of PCA-SVC model with a better classification accuracy. Therefore, they were selected as the optimal spectral pretreatment method, which had the classification accuracies of 99.49 and 100% for training set and test set, respectively.

PCA Feature Extraction

Raman data size can be reduced to simplify the computational complexities for the development of the effective prediction model by using PCA without compromising prediction accuracies (Li et al., 2016).

Table 1. Classification accuracies of PCA-SVC models with 3 spectral pretreatment methods for classification of TP and NT in duck meat.

Pretreatment methods	Training set	Test set
AirPLS	99.49%	97.96%
AirPLS and normalization	96.43%	96.94%
AirPLS, normalization, and first derivative	99.49%	100%

Abbreviations: AirPLS, adaptive iteratively reweighted penalized least squares; NT, nandrolone; PCA, principal component analysis; SVC, support vector classification; TP, testosterone propionate.

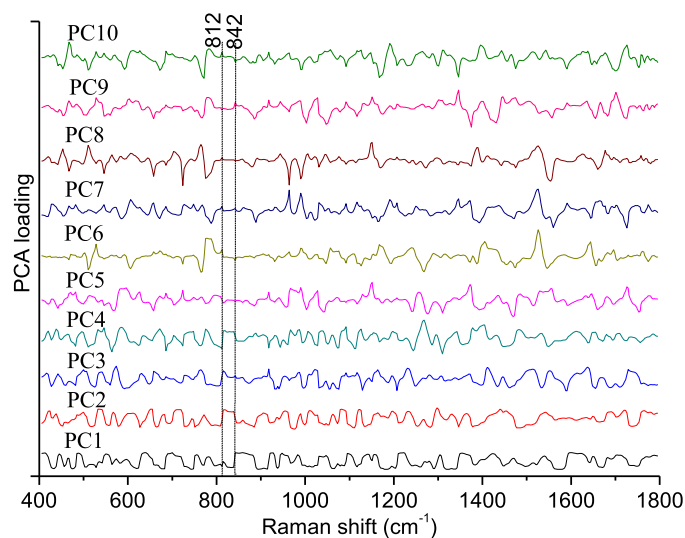


Figure 2. PCA loadings of the first 10 principal components.

Figure 2 showed the PCA loadings of first 10 principal components describing 90.30% of the spectra variances of the data set. The peaks at 812 cm^{-1} were presented on loading curves of PC6, PC7, PC8, and PC10, and the troughs at 812 cm^{-1} were seen on loading curves of PC1 and PC9. The loading curves of PC3, PC7, and PC9 had the peaks at 842 cm^{-1} , and the loading curves of PC5, PC6, PC8, and PC10 had the troughs at 842 cm^{-1} . The peaks and troughs at 812 and 842 cm^{-1} on many of those PC loading curves existed in the raw Raman spectra, which showed these 2 peaks weighed more in PCA and played an important role in the following modeling.

Support Vector Classification Analysis

Support vector classification was utilized to establish the classification model using the first 10 PCA scores as the inputs. C-support vector classification and radial basis function (RBF) were selected as the type and the kernel function of SVC model, respectively. Two important parameters of SVC model, that is, the penalty parameter C and the kernel parameter g, were optimized automatically to improve the performance of SVC classification model by using PSO algorithm, which was an effective method of searching optimal or near-optimal solutions. The values 4.5981 and 0.4156 were obtained as the optimal values of C and g by using PSO algorithm, respectively.

Table 2 summarized the values of sensitivity and specificity for each group using PCA-SVC model for training set and test set. Sensitivity was defined as the probability of correctly classifying a Raman spectrum as belonging to a certain group. Specificity was defined as the probability of correctly classifying a Raman spectrum as not belonging to a certain group. For training set, the sensitivity value of 98.53% was obtained in the case of TP combined with the NT group, and the

Table 2. Sensitivity and specificity values of PCA-SVC model for classification of TP and NT residues in duck meat for training set and test set.

Groups	Training set		Test set	
	Sensitivity/%	Specificity/%	Sensitivity/%	Specificity/%
Control	100%	100%	100%	100%
TP	100%	100%	100%	100%
NT	100%	99.39%	100%	100%
TP combined with NT	98.53%	100%	100%	100%

Abbreviations: NT, nandrolone; PCA, principal component analysis; SVC, support vector classification; TP, testosterone propionate.

sensitivity values of 100% were obtained in the case of other 3 groups. Specificity value was 99.39% in case of NT group, and specificity values were 100% in case of other 3 groups. As far as test set was concerned, sensitivities and specificities values of each group are both 100%, which showed that the adopted classification method had the ability to classify correctly the duck meat samples containing TP and NT into 4 groups.

Least Squares Support Vector Regression Analysis

Least squares support vector regression, a multivariate method, can reduce the computational complexity and improve the speed by comparison of support vector regression (SVR) (Si et al., 2017). It was utilized to establish the prediction model with RBF as the kernel function to predict the TP and NT contents in duck meat extract. As shown in Table 3, LS-SVR prediction model for predicting TP contents in duck meat extract obtained the better results with an R^2 value of 0.9603, RMSE value of 1.6869, and RPD value of 4.2084 for the training set, and an R^2 value of 0.9316, RMSE value of 2.1739, and RPD value of 3.2189 for the test set. In addition, LS-SVR prediction model for predicting NT contents in duck meat extract obtained the better results with an R^2 value of 0.9693, RMSE value of 1.396, and RPD value of 4.9653 for the training set, and an R^2 value of 0.9038, RMSE value of 2.2914, and RPD value of 2.9701 for the test set. As showed from Figure 3, there were the good linear relationships between actual values of TP or NT in duck meat extract and their predicted values to exhibit the good predictabilities. The minimum detection limits of TP and NT in duck meat extract could reach 0.1 and 0.5 mg/L, respectively. All in all, SERS coupled with LS-SVR displayed the good potential to predict the TP and NT contents in duck meat extract.

Table 3. Static summary of LS-SVR prediction models for detection of TP and NT contents in duck meat extract.

Samples	Training set			Test set		
	R_t^2	RMSEC	RPD	R_p^2	RMSEP	RPD
Samples containing TP	0.9603	1.6869	4.2084	0.9316	2.1739	3.2189
Samples containing NT	0.9693	1.396	4.9653	0.9038	2.2914	2.9701

Abbreviations: LS-SVR, least squares support vector regression; NT, nandrolone; TP, testosterone propionate; RPD, ratio of prediction to deviation.

CONCLUSIONS

In this report, SVC model was developed to classify all the duck meat samples into the 4 groups (i.e., control group, TP group, NT group, and TP combined with NT group) with the classification accuracies of 99.49 and 100% for training set and test set, respectively. Furthermore, 2 LS-SVR models, exhibiting the good predictabilities, were created to predict the TP values in samples with an R^2 value of 0.9316, RMSE value of 2.1739, and RPD value of 3.2189 for the test set, and NT values in samples with an R^2 value of 0.9038, RMSE value of 2.2914, and RPD value of 2.9701 for the test set. Before establishing the classification and regression models, the raw Raman spectra were pretreated by using airPLS, normalization and first derivative, and then first 10 principal components were selected as the inputs of the developed models. The results of the report suggested that SERS technology, in combination with multivariate analysis, has the potential to become the qualitative and quantitative analysis tool for TP and NT residues in duck meat extract.

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DISCLOSURES

The authors declare no competing or financial interests.

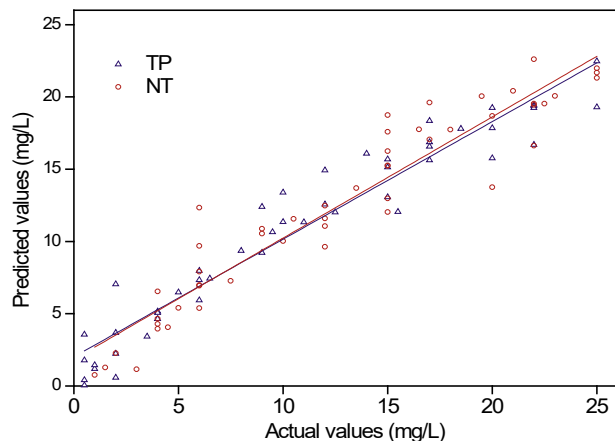


Figure 3. The relationship between actual values of TP or NT residues in duck meat extract and predicted values for the test set with the LS-SVR model. Abbreviations: LS-SVR, least squares support vector regression; NT, nandrolone; TP, testosterone propionate.

REFERENCES

- Chen, C. P., W. F. Liu, S. P. Tian, and T. T. Hong. 2019. Novel Surface-enhanced Raman spectroscopy techniques for DNA, protein and drug detection. *Sensors* 19:1712.
- Cialla, D., A. März, R. Böhme, F. Theil, K. Weber, M. Schmitt, and J. Popp. 2012. Surface-enhanced Raman spectroscopy (SERS): progress and trends[J]. *Anal. Bioanal. Chem.* 403:27–54.
- Combalbert, S., M. Pype, N. Bernet, and G. Hernandez-Raquet. 2010. Hernandez-Raquet. Enhanced methods for conditioning, storage, and extraction of liquid and solid samples of manure for determination of steroid hormones by solid-phase extraction and gas chromatography-mass spectrometry. *Anal. Bioanal. Chem.* 398:973–984.
- Frens, G. 1973. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature* 241:20–22.
- Haiss, W., N. T. K. Thanh, J. Aveyard, and D. G. Fernig. 2007. Determination of size and concentration of gold nanoparticles from UV-Vis spectra. *Anal. Chem.* 79:4215–4521.
- Hou, C., J. Qiu, Y. Y. Cheng, L. Y. Zhao, and S. M. Yang. 2016. Determination of steroid hormones in meat using ultra-high performance liquid chromatography QTRAP mass spectrometry. *Chin. J. Anal. Lab.* 35:750–754.
- Huo, T. M., L. Y. Wang, L. Q. Liu, X. G. Chu, and C. L. Xu. 2006. Rapid time-resolved fluoroimmunoassay for diethylstilbestrol residues in chicken liver. *Anal. Biochem.* 357:272–276.
- Jeong, K. H., M. Y. Jeong, H. Park, M. A. Hossain, D. G. Kim, K. Lee, and J. W. Kang. 2017. Development of new analytical methods using high performance liquid chromatography for animal hormones; gonadorelin, progesterone, oxytocin and estradiol. *Korean J. Vet. Res.* 40:253–258.
- Li, X. Z., T. Y. Yang, S. Q. Li, D. L. Wang, Y. T. Song, and K. D. Yu. 2016. Different classification algorithms and serum surface enhanced Raman spectroscopy for noninvasive discrimination of gastric diseases. *Raman Spectrosc.* 47:917–925.
- Ma, D. Z., Y. W. Diao, J. X. Yang, B. D. Wang, and Y. H. Yu. 2010. ELISA detection of steroidal hormones in Water Resources, foods and feed. *Chin. J. Appl. Chem.* 27:1466–1469.
- Malone, E., C. Elliott, G. Kennedy, D. Savage, and L. Regan. 2011. Surveillance study of a number of synthetic and natural growth promoters in bovine muscle samples using liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* 28:597–607.
- Pedersen, M., and J. H. Andersen. 2011. Confirmatory analysis of steroids in muscle using liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* 28:428–437.
- Quan, C., F. H. Su, H. F. Wang, and H. M. Li. 2011. Development of anabolic-androgenic steroids purity certified reference materials for anti-doping. *Steroids* 76:1527–1534.
- Si, G. Q., J. Q. Shi, Z. Guo, L. X. Jia, and Y. B. Zhang. 2017. Reconstruct the support vectors to improve LSSVM sparseness for mill load prediction. *Math. Probl. Eng.* 2017:4191789.1-4191789.12.
- Song, J., Y. Q. Huang, Y. X. Fan, Z. H. Zhao, W. S. Yu, B. A. Rasco, and K. Q. Lai. 2016. Detection of prohibited fish drugs using silver nanowires as substrate for surface-enhanced Raman scattering. *Nanomaterials* 6:175.
- Song, D., R. Yang, F. Long, and A. Zhu. 2019. Applications of magnetic nanoparticles in surface-enhanced Raman scattering (SERS) detection of environmental pollutants. *J. Environ. Sci.* 80:14–34.
- Wang, G., Y. Y. Sun, X. S. Tang, Q. X. Zhang, B. X. Yin, D. S. Fang, H. Zhang, and W. Chen. 2014. Detection of estrogens in animal products by DSPE-LC-MS/MS. *Sci. Technol. Food Industry* 35:282–285.
- Wu, X. L., P. Fu, W. Ma, L. G. Xu, H. Kuang, and C. L. Xu. 2015. SERS-active silver nanoparticle trimers for sub-attomolar detection of alpha fetoprotein. *RSC Adv.* 5:73395–73398.
- Xu, N., M. H. Liu, H. C. Yuan, S. G. Huang, X. Wang, J. H. Zhao, J. Chen, T. Wang, W. Hu, and Y. X. Song. 2020. Classification of sulfadimidine and sulfapyridine in duck meat by surface enhanced Raman spectroscopy combined with principal component analysis and support vector machine. *Anal. Lett.* 3:1–12.
- Yuan, X., M. Li, X. Guo, Y. Wu, Y. Ying, Y. Wen, and H. Yang. 2018. Raman rapid detection of environmental hormone. *Sensors Actuators B: Chem.* 262:44–49.
- Yang, N., T. T. You, Y. K. Gao, C. M. Zhang, and P. G. Yin. 2018. Fabrication of a flexible gold nanorod polymer metafilm via a phase transfer method as a SERS substrate for detecting food contaminants. *J. Agric. Food Chem.* 66:6889–6896.
- Zhang, X. G., Y. J. Zheng, Y. T. Zeng, J. Yang, and G. W. Yang. 2016. Determination of 19 hormones in food by liquid chromatography-tandem mass spectrometry. *Food Industry* 37:281–286.