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Research Article

Electro-analytical Chemistry and Chemo-metrics for Meat Products in the Quantitative Determination of Hazardous Substances

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Abstract: In this study, differential pulse stripping voltammetry meat tissues examined three drug residues salbutamol and ractopamine hydrochloride oxidation peak on the glassy carbon electrode serious overlapping and partially overlapping with clenbuterol, among the three components there is a strong interaction, the mixture is difficult to determine the content of each single component directly. Based on the optimization of the experimental conditions and based on the introduction Chemometrics mixing its components resolved to avoid the tedious separation and purification steps, simplifying the measurement process.

Keywords: Chemical metering analytics, electric chemistry, voltammetry

INTRODUCTION

With large-scale animal husbandry towards the development of livestock breeding are becoming increasingly mixed together, thus leading to cross contamination between a variety of different viruses and bacteria, making the prevention of animal diseases increases the difficulty, in the early stages, the main discovery of antibiotics on farmed prevent bacteria and viruses have a very good effect, thereby breeding owners widespread use of antibiotics for prevention of animal diseases (Ni *et al*., 2006). However, with the widespread use of antibiotics, many new problems were discovered, such as their side effects and drug resistance of animals. Particularly, some unscrupulous companies are seriously overweight leads to the overuse of antibiotics in animal's residues that do not meet national food safety regulations and regulations on food import and export trade of the country, not only to the main farming, but also to the country to bring considerable losses (Fang and Chen, 2007).

Clenbuterol common name, English name clenbuterol hydrochloride, are the best kind of adrenal hormones, white or almost white crystalline powder, odorless, tasteless. Clenbuterol as a best agonist, selectively acts on the adrenal receptors at therapeutic doses, has the effect of relaxation of airway smooth muscle for the treatment of asthma to animals fed feed containing clenbuterol, the animal can activate adenosine cyclase enhance lipolysis and promote animal muscle, particularly skeletal muscle protein synthesis to accelerate animal growth rate, increase lean (Zou and Mo, 1997). U.S. Food and Drug Administration and the World Health Organization recommend clenbuterol in animal tissue MRLs for: Meat 0.2 ug/Kg, liver 0.6 ug/Kg, kidney 0.6 ug/Kg, fat 0.2 ug/Kg, milk 0.05 ug/Kg.

Salbutamol and ractopamine hydrochloride (Ractopamine hydrochloride), β2 belong to the selective β2-agonists; can promote animal tissue transfer of nutrients from fat to muscle tissue, increase lean body brittle. When our country for Clenbuterol takes pressure against the trend, criminals instead use salbutamol and ractopamine hydrochloride as a substitute for clenbuterol (Peilong and Yipeng, 2013).

Since the 20th century, a large number of articles reported that diets supplemented with an activating agent may improve feed conversion rate and increase lean meat, it is also known as "nutritional weight distribution agents." However, due to its use as a growth promoter in large quantities, use a long time and if the drug round in the internal organs of the higher residues of drugs, including human consumption, high drug concentrations organ will appear tachycardia, palpitations other acute poisoning. Since the drug residues in animals cause severe poisoning after eating people, has caused concern around the world, the European Union as early as 1988 on the prohibition of the use of clenbuterol substances, in 1991 the United States Department of Agriculture has taken coercive measures prohibit illegal use of clenbuterol in animals. "Food animal veterinary drugs and other compounds disabled list" in March 2002 issued by Chinese Ministry

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of Agriculture has been clearly defined β-doping ban all uses, banned from all animals, but there are still many farms illegal use of clenbuterol a serious threat to people's health (Ni *et al*., 2006). Therefore, the detection of clenbuterol in animal tissues is very important class of residues.

Turberg such as the use of high performance liquid chromatography coupled with electrochemical determination of ractopamine in serum levels of monkeys and pigs, the authors first serum diluted with phosphate buffer, ractopamine is separated from the serum matrix by ion exchange solid phase extraction technology, ethyl acetate and then further purified by liquid-liquid separation and finally the use of electrochemical characteristics of high sensitivity quantitative determination in monkey serum detection limit of 2 ng/mL, Qian and other side with capillary zone electrophoresis, in 20.0 mmolL-Na_2HPO_4-NaOH buffer solution 1, to 60.5 cm×75.0 um (effective length 50 cm) is a capillary separation channel, voltage 25 kV, salbutamol and ractopamine realized separated and the target analyte can be completed in 4.5 min testing (Wang *et al*., 2010). Sirichai reported using capillary electrophoresis; eliminate interference by optimizing the experimental conditions, rapid detection of drugs and human urine clenbuterol, salbutamol, procaterol and fenoterol detection limit in 0.5-2.0 mg/L. Goyal like nanoparticles modified with indium tin oxide electrodes, using the content of salbutamol Oersted Yangfang Bo voltammetry measurement and with indium tin oxide unmodified bare gold electrode and the comparison electrode, the discovery of new proposed method improves the sensitivity. Simultaneous determination of the detection limit of clenbuterol, salbutamol and ractopamine, a method for 0.5 and 20 ug/Kg Song such as Yan by gas chromatography mass spectrometry method by pre-treatment improvements. Zhoujieyu already established methods such as laser-induced fluorescence capillary electrophoresis competitive immunoassay rapid and accurate detection of clenbuterol, increased sensitivity.

Substitutes for the detection of clenbuterol content in pharmaceutical preparations, biological fluids and animal tissues have different methods of Liquid Chromatography (HPLC), Gas Chromatography (GC), gas chromatography-mass spectrometry, Enzymatic Linked Immunosorbent (ELIA), chemical analysis methods such as acid method (such as acid method) and spectrophotometry. Chemical analysis method does not require expensive equipment and reagents, operation, but because of an impurity interference, low sensitivity, poor reproducibility, only as a screening (Zou and Mo, 1997). GB/T 5009.192 specify the standard detection methods for clenbuterol liquid chromatography, gas chromatography and enzyme-linked immunosorbent assay of. However, liquid chromatography, gas chromatography, the instrument to be expensive, difficult to operate, the detection time is long, complex pre-processing step, wherein the cation exchange SCX cartridge is expensive, complicated steps and the recovery is relatively low. ELISA Kit testing time is too long, the high cost of testing. Capillary electrophoresis requires sample pre-treatment is simple, the separation time is short and no complex sample pre-treatment, simple.

MATERIALS AND METHODS

Materials: Electrical sensitivity and accuracy of analytical chemistry methods are high, with good selectivity, easy operation, fast analysis, features a wide range of applications, especially in the modern instrumental analysis and computer combined with automated analytical work. Currently, in the fields of industry, agriculture, medicine and health, food inspection, environmental protection and medical tests such as access to a wide range of applications. However, in actual measurement, the drug voltammetric analysis and polarographic analysis systems often have complex background current, electrode oxidation or reduction process is often irreversible and their peak potential will change with changes in the drug concentration (Ren and Gao, 2009), the Determination of nonlinear adduct system will cause serious impact. And for more complex drug system, often overlapping spectrum of serious and cannot be alone component analysis. The traditional method for determination of the drug to be pre-treated sample purification and separation, on the interference components in the sample to reduce the measurement error is removed. In recent years, the rapid development of biology, life science, pharmaceutical science and other disciplines, requiring the analysis of a complex mixture of chemical workers in the drug system, including in particular the complexity of the drug in blood mixed system gives rapid qualitative and quantitative analysis, so the chemical electrical metrology methods used in drug chemistry has been widespread concern (Hong *et al*., 2013).

Table 1 clenbuterol, salbutamol and ractopamine hydrochloride on the molecular structure of these three β-an agonist, on Single-Wall carbon Nanotubes modified Glassy Carbon Electrode (SWNTs/GCE) oxidation spectrum have some overlap, the optimal conditions still cannot be separated, so the paper, chemical multivariate calibration methods metrology Partial Least Squares (PLS) for spectral overlap parsing clenbuterol class of hybrid systems and with the Principal Component Regression (PCR) and High Performance Liquid Chromatography (HPLC) were

Table 1: Agonist class of drugs to make three kinds of formula

Name	Formula
Clenbuterol hydrochloride	$C_{12}H_{18}C_{12}N_2O{\cdot}HCl$
Ractopamine hydrochloride	$C_{18}H_{23}NO_3{\cdot}HCl$
Salbutamol	$C_{13}H_{21}NO_3$

compared and used for meat drug residues in tissues actual measurement.

Reagents and instruments: Standard solution 0.1 mg/mL: Weigh accurately clenbuterol, ractopamine hydrochloride and salbutamol amount, with the dissolution of a small amount of ethanol transferred to 1000 mL volumetric flask to volume with double-distilled water, stored in the refrigerator within 5°C.

pH = 4.56 in BR buffer solution: Take 2.71 mL 85%, respectively orthophosphate, 2.36 mL boric acid in glacial acetic acid and 2.47 g 1000 mL flask, dubbed 0.04 mol/mL mixed acid, to take the mixed acid 100 and 30 mL 0.2 mol/L NaOH mixed.

Experimental procedure: Standard solution electrolysis cup Pipette the appropriate amount of the three drugs, adding BR buffer solution (pH 4.56) and diluted to 10 mL, shake. After electrode placement, enrichment 60 sec, standing 2 sec, then within 0-1.2 V potential range, using differential pulse voltammetry scans, potential interval ΔE = 4 mV, experiments performed at room temperature about 25°C.

RESULTS AND DISCUSSION

Supporting electrolyte and buffer solution value selection: This experiment investigated the effects of acidity on drug voltammetry curves. Configure a set pH (1.98-7.96) of Britton-Robinson (BR) buffers are widely respectively voltammograms of three drugs in this series of electrochemical buffer solution.

It was found that, with the increase of pH value, the peak current of the three drugs in general decreasing trend and the peak shape is gradually widened. Peak potential shifted negatively with increasing pH and showed a linear relationship. The linear equations were: Clenbuterol Ep = -1.2761-0.0653 pH (r = 0.9981), salbutamol Ep = 1.0048-0.0443 pH (r = 0.9985), ractopamine hydrochloride Ep = 1.0185-0.0598 pH (r = 0.9992). Taking into account the sensitivity and peak shape, as the acidity of the experiment select pH 4.56 buffer solution. The relationship is shown in Fig. 1.

With the increase in scanning speed, the peak current of the first three substances increased, then decreased. Finally, the peak current is reduced, which may deteriorate the peak symmetry, lead to changes in the peak current. The peak potential of three substances remained basically unchanged in Fig. 2.

Select the experimental conditions: This experiment investigated the accumulation time, pulse width and pulse amplitude influence on the determination. With increasing concentration of time, clenbuterol, ractopamine hydrochloride and salbutamol sulphate peak current increases, indicating that the three with adsorption. The peak current in 60 sec at the three basic is stable, indicating that the adsorption has reached equilibrium, so I chose 600 sec for pre-concentration time. With the increase of pulse width, peak current decreases linearly; peak potential shift is not obvious. When the pulse width is small, a high sensitivity is obtained, but also broadens the peak width, the peak current of the measurement and therefore the selection pulse width of 40 msec. With the pulse amplitude increases, the peak current increases linearly, the peak potential slightly positive shift. However, due to the half width also increases with pulse amplitude widened, making poor peak shape, so this was chosen pulse amplitude 50 mV.

Cyclic voltammetry study adsorption electrode reaction: Experimental results show that with the increase of the peak current of each component of the scanning speed increases and the variation of the peak current and scan speed of the linear relationship of the components (Fig. 3 and 4), the square root of the scan rate relationship curve upwardly curved illustrating these two drugs are adsorbed on the electrode during the oxidation peak potential by nature of the scanning speed is not affected.

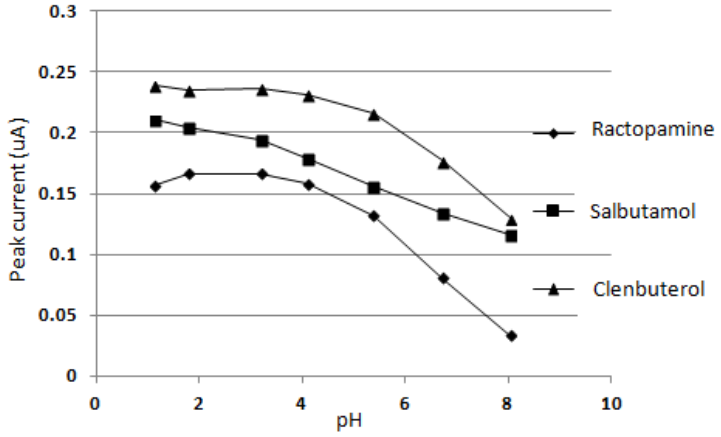

Fig. 1: pH influence on peak current, clenbuterol, ractopamine and salbutamol concentrations were 0.05, 0.1 and 0.2 ug/mL, respectively

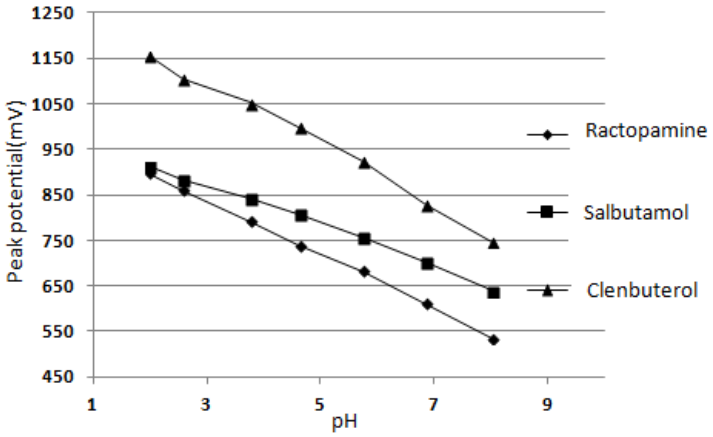

Fig. 2: pH influences on the peak potential, clenbuterol, ractopamine and salbutamol concentrations were 0.05, 0.1 and 0.2 ug/mL, respectively

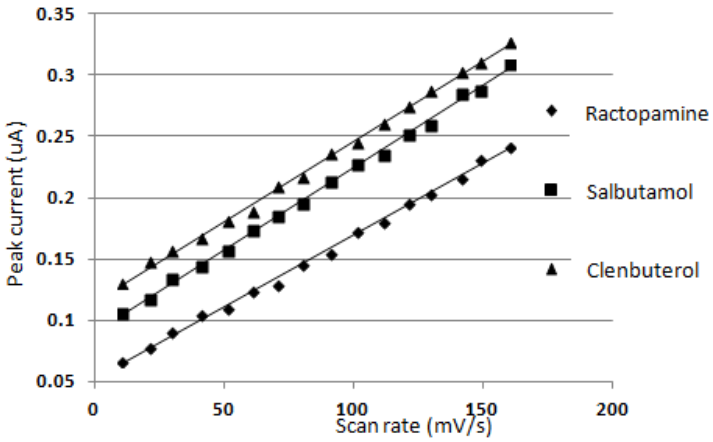

Fig. 3: Linear sweep voltammetry diagram of each component peak current vs. scan speed-clenbuterol, ractopamine and salbutamol concentrations are 0.06 ug/mL

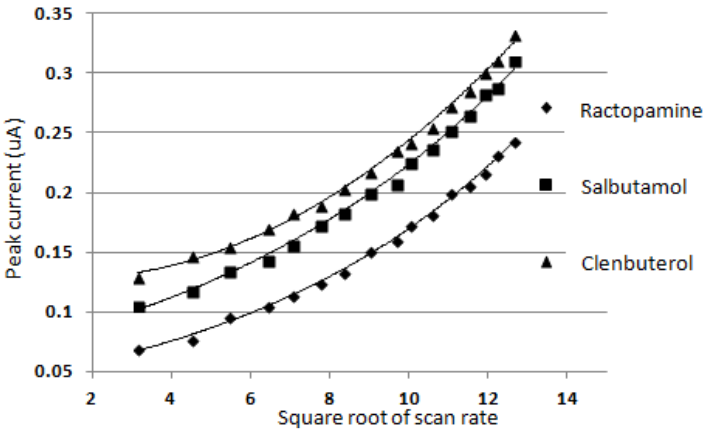

Fig. 4: Linear sweep voltammetry diagram of each component peak current changes with the square root of the scanning speed-clenbuterol, ractopamine and salbutamol concentrations are 0.06 ug/mL

Three kinds of antibiotics increases the peak current enrichment time increases, but the last time is too long may lead to substance decomposes because the peak current decreased, so chose 60 sec for pre-concentration time.

Determination of the linear range and the detection limit: Under the optimal experimental conditions, the drug concentration and the electrochemical signal, linear ranges were: Clenbuterol 0.05~0.55 ug/mL, low detection limit of 0.023 ug/mL, salbutamol 0.05-0.45

Table 2: Regression coefficients of each component

Items	Clenbuterol hydrochloride	Albuterol	Ractopamine hydrochloride
Samples	10	10	10
Line range (g/mL)	0.0500-0.5500	0.1500-1.200	0.0500-0.4500
Working curve intercept (nA)	0.4400	8.7300	0.1000
Working curve slope (uA mL/ug)	0.2050	0.0592	0.1130
Related parameters	0.9949	0.9994	0.9989
The standard deviation of intercept	2.3500	0.7700	0.5930
The standard deviation of the slope	6.9200	0.9530	2.1100
Regression coefficient	3.6278	0.7562	0.8168
Detection limit (ug/mL)	0.0230	0.0383	0.0216

Table 3: Clenbuterol, ractopamine and salbutamol sulphate concentration of three-component solution consisting correction group (unit: ug/mL)

No.	Clenbuterol hydrochloride	Ractopamine hydrochloride	Albuterol
1	0.15	0.05	0.15
2	0.15	0.08	0.30
3	0.15	0.12	0.45
4	0.15	0.15	0.60
5	0.10	0.05	0.30
6	0.10	0.08	0.15
7	0.10	0.12	0.60
8	0.10	0.15	0.45
9	0.20	0.05	0.45
10	0.20	0.08	0.60
11	0.20	0.12	0.15
12	0.20	0.15	0.30
13	0.05	0.05	0.60
14	0.05	0.08	0.45
15	0.05	0.12	0.30
16	0.05	0.15	0.15

Table 4: Clenbuterol, ractopamine and salbutamol sulphate concentration of three-component solution forecasting group consisting of (unit: ug/mL)

Clenbuterol hydrochloride	Ractopamine hydrochloride	Albuterol
0.08	0.06	0.20
0.08	0.09	0.25
0.08	0.10	0.35
0.13	0.06	0.25
0.13	0.09	0.20
0.13	0.10	0.50
0.13	0.12	0.35
0.16	0.09	0.50
0.16	0.10	0.20
0.16	0.12	0.25
0.18	0.06	0.50
0.18	0.09	0.35
0.18	0.10	0.25
0.18	0.12	0.20

ug/mL, the lowest. The detection limit was 0.0216 ug/mL, ractopamine hydrochloride 0.15-1.2 ug/mL, the lowest detection limit of 0.0383 ug/mL. Regression parameters are shown in Table 2.

Determination of synthetic samples: Before using chemometric methods of measuring an unknown mixture, should first create a set of calibration solution, i.e., the mathematical model, in order to forecast the unknown concentration of the drug in the mixing of the components. In general, taking orthogonal design table to arrange the composition of calibration solution, because it can be used to extract fewer experiments as much information. In this experiment, in order to minimize the error of prediction results, the proposed use of orthogonal table L16 (45), i.e., 16 sets of measurement data as a calibration model calibration solution, as shown in Table 3, the ratio of the concentration of any forecasting group (Table 4) determined by multivariate calibration methods of Classical Least Squares (CLS), Principal Component Regression (PCR) and Partial Least Squares (PLS) analysis and compared their prediction errors.

From the measurement results shown in Table 5, the use of partial least squares method can get the best forecasting results, which is due in the differential pulse voltammetry scans, there is often background current, there are oxidative peaks overlay, classical least squares method to resolve this kind of system, there are some difficulties and principal component regression and partial least squares method is based on the correction factor analysis method can better solve such nonlinear increase and problems. In principal component regression and partial least squares method, you must select a certain number of the number of factors, structural decomposition of the matrix, so that within the experimental error can reproduce the response matrix, selection of the number of factors have a significant impact on the results, in general, By taking the number of factors to or slightly larger than the fraction, the relative standard deviation of the results less.

Table 5: Three-component synthetic sample analysis results

	RPEs (%)			
Chemometrics methods	Clenbuterol hydrochloride	Ractopamine hydrochloride	Albuterol	RPET (%)
PLS (3)	3.34 (101)	6.33 (99)	5.12 (99)	6.61
PCR (3)	3.53 (101)	6.94 (100)	5.69 (99)	6.67
DPLS (4)	9.22 (107)	9.43 (108)	16.13 (111)	14.92
DPCR (4)	9.74 (106)	10.07 (110)	19.57 (113)	17.93

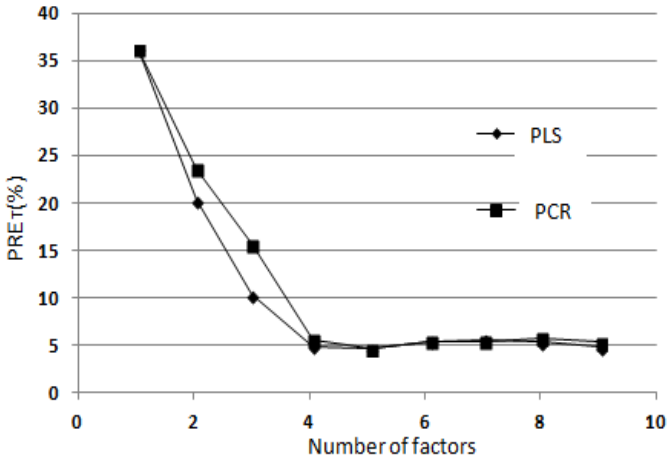

Fig. 5: The number of factors affects the Relative Prediction Error (RPEr)

Before using chemometric methods for measuring an unknown mixture can first establish a mathematical model calibration solution, in order to forecast the unknown mixing the drug concentration of each component. In general, this experiment takes orthogonal design table to arrange the composition of calibration solution, because it can use fewer experiments to extract as much information.

Figure 5 is a graph select number of different factors and the relative error. The system, when calculated using principal component regression method, the number of factors to take four, the relative error resulting smaller when calculated using partial least squares method, the number of factors to take four, the relative error made minimum.

Sample extraction: Weigh 5.0 g of liver placed in a centrifuge tube, was added 15 mL of ethyl acetate, 3 mL 10% sodium carbonate solution, vortexed for 5 min at a rate of 1 min 4000 r/min of. Draw the upper organic phase to another centrifuge tube below 60°C, rotary evaporation nearly dry and then dried by nitrogen, dissolved in methanol and finally with 1 mL residue weighed 5.0 g pork processing method as above.

Determination of sample recoveries: Weigh blank liver and pork are 5.0 g, quantitative adding clenbuterol, salbutamol and ractopamine hydrochloride standard solution amount, 15 min later, the sample processing method according to the above process, test.

The prepared sample solution Pipette 0.1 mL to electrolysis cup, add 2 mL of BR buffer solution, diluted with distilled water twice to 10 mL, shake, placed electrodes, according to the above optimum conditions combined with standard addition method were determined, record relevant data were treated with the same set of mathematical correction system, with PLS method to interpret the results in Table 6 seen from the results measured in real samples, clenbuterol, salbutamol and ractopamine hydrochloride has different levels of residues, as measured spiked better recovery.

Modern drug analysis, both in analytics or analysis techniques have greatly expanded evolved from static analysis to dynamic analysis of the development of in vivo analysis of the in vitro analysis of the development to the quality of the analysis in vivo analysis from a single technology to hyphenated techniques from small sample analysis developed to high-throughput analysis from manual analysis of the development of computer-aided analysis to make drug analysis from a century of expertise in the development of early maturity to become a science-pharmaceutical analysis (Kokot and Li, 2010). With the rapid development of pharmaceutical sciences, drug analysis of relevant disciplines has made new demands. Such as drug residues were identified and ultra trace analysis of drugs and their metabolites in vivo stimulants banned substances in complex system analysis and detection of drug separation and purification of the drug in the determination of trace impurities in pharmaceutical processes and structure analysis and quality control.

Table 6: Partial least squares analytical measurement results with actual samples and HPLC results were compared (unit: ug/mL)

	Concentration in the sample			The amount added			The quantity checked			The recovery rate of		
Samples	CL	Sal	Rac	CL	Sal	Rac	CL	Sal	Rac	CL	Sal	Rac
Pork liver PLS												
1	0.30	0.45	\	2.50	2.50	2.50	2.35±0.02	2.34±0.03	1.93±0.02	83	76	77
2	0.43	\	0.21	2.50	2.50	2.50	2.43±0.03	2.01±0.02	2.14±0.04	81	78	78
3	\	\	\	2.50	2.50	2.50	2.13±0.02	2.12±0.04	1.89±0.04	84	79	80
Pork liver HPLC												
1'	0.22	0.32	\	2.50	2.50	2.50	2.23±0.02	2.34±0.04	1.97±0.04	80	81	76
2'	0.36	\	0.25	2.50	2.50	2.50	2.54±0.04	2.23±0.03	2.13±0.03	82	79	79
3'	\	\	\	2.50	2.50	2.50	2.04±0.02	2.10±0.03	1.88±0.04	81	82	83
Pork liver PLS												
4	0.15	\	0.08	5.00	5.00	5.00	4.35±0.03	3.90±0.04	4.12±0.04	84	77	80
5	0.06	0.14	\	5.00	5.00	5.00	4.21±0.04	3.87±0.03	3.98±0.03	82	79	75
6	\	\	\	5.00	5.00	5.00	4.43±0.02	3.79±0.03	4.03±0.04	88	81	78
Pork liver HPLC												
4'	0.17	\	0.08	5.00	5.00	5.00	4.43±0.03	3.92±0.02	3.97±0.03	85	75	73
5'	0.08	0.13	\	5.00	5.00	5.00	4.25±0.04	3.88±0.03	3.89±0.02	83	79	78
6'	\	\	\	5.00	5.00	5.00	4.36±0.02	3.79±0.03	3.87±0.02	87	76	76

Samples: (1, 2, 3) and (1 ', 2', 3 ') from a different supermarket liver, (4, 5, 6) and (4', 5 ', 6') from a different supermarket pork; CL: Clenbuterol; Sal: Salbutamol; Rac: Ractopamine hydrochloride

With the development of bio-engineering, gene drugs also increasing drug analytical methods and quality control of higher demands put forward more, so some of the new methods of drug analysis from various research not only needs to adapt to drug research and the development of the new situation and the pharmaceutical industry, but also to the introduction of new biotechnology research and new biochemical methods to solve new problems of drug research and innovative drug research-based process of scientific arise.

In addition, with the development of computer technology, its use will continue in-depth analysis, which led to the replacement of the instruments will also shorten the time and will be a variety of intelligent, miniaturized automatic analytical instruments, in order to adapt modern analysis needs.

In the electric field of analytical chemistry, due to the presence of non-linear superposition relations, current peak shift and broadening of nonlinear characteristics between the current compositions of different nature, resulting in electrical spectrum of analytical chemistry information analysis sometimes becomes a very challenging problem it also is chemometrics play a role in this area provide useless. The electrochemical methods and means of combining chemometrics applied to the analysis of drugs (Zheng and Mo, 1999), both full play to the power analysis accuracy, high sensitivity saving agents, non-polluting equipment is simple, inexpensive, etc., can be low-cost, short time achieve drug minor or trace analysis and simplifies the experimental procedure, enabling multi-component drugs online without pretreatment while quantitative determination.

CONCLUSION

Clenbuterol, salbutamol and ractopamine hydrochloride, since the body can increase lean rouge, has been widely used. In this study, differential pulse stripping voltammetry meat tissues examined three drug residues salbutamol and ractopamine hydrochloride oxidation peak on the SWNTs/GCE serious overlapping and partially overlapping with clenbuterol, among the three components there is a strong interaction, the mixture is difficult to determine the content of each single component directly. In this study, the optimization of the experimental conditions, based on the introduction of chemometric methods to resolve its blending component, to avoid the tedious separation and purification steps, simplifying the measurement process, to achieve the simultaneous determination of three components in the sample, the results make people satisfied.

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