Pretreatment of rapid detection of veterinary drug residue in eggs

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Abstract-Eggs in the standard method for rapid detection of veterinary drug residue has not been formed, therefore, it is necessary to explore a rapid, highly effective, the total amount and qualitative method of rapid detection of antibiotic residues in eggs. Many different kinds of veterinary drugs on the market, this study selected: tetracycline, beta lactam, sulfa, fluoroquinolone for veterinary drug residue detection pretreatment research, finally to strip test results prior to determine the best treatment plan.

Keywords-veterinary drug residue; pretreatment; rapid detection

I. INTRODUCTION

Veterinary drug residue is refers to the accumulation of livestock animal medication after or stays in the body or prototype drugs in animal products such as eggs or their metabolites, including related to the veterinary drug residue[1]. These drugs can enter the human food chain through transfer, enrichment of the animal body, eventually leading to human in the process of eating the accumulation of veterinary drugs. Control of veterinary drug residues in eggs plays an important role in human life and health. Veterinary drug residue detection is an important means of eggs food safety, the rapid detection of standard has not been formed and its preparation method, therefore the experiment was carried out to explore, use is made of the existing market the rapid detection of veterinary drug residue detection milk strip for eggs of veterinary drug residues detection, the need for a series of eggs before treatment, including removal of the fat, to protein and so on. In theory, many different kinds of veterinary drug residue in eggs, this study selected: tetracycline, beta lactam, sulfa, fluoroquinolone for veterinary drug residue detection research.

Control of veterinary drug residues in eggs plays an important role in human life and health. Veterinary drug residue detection is an important means of eggs food safety, the existing detection methods at home and abroad for capillary electrophoresis, fluorescence method, uv spectrophotometry, chemiluminescence method, atomic absorption spectrophotometry, microbiological method, high performance liquid chromatography (HPLC) method and liquid mass usage, etc, but has not been a rapid detection of veterinary drug residue in eggs standard method, therefore, it is necessary to explore a rapid, highly effective, the total amount and qualitative method of rapid detection of antibiotic residues in eggs. There are many kinds of veterinary drugs on the market, this study selected: tetracycline, beta lactam, sulfa, fluoroquinolone for veterinary drug residue detection research

II. MATERIALS AND METHODS

Eggs, northern suburb of Beijing changping farmers market to buy; The rapid detection of strip, Beijing often state biological technology co., LTD.; Organic reagents, analytical pure, west long chemical co., LTD.

III. EXPERIMENT

A. Tetracycline class antibiotic residues detection of egg sample pretreatment

Chloramphenicol, oxytetracycline, tetracycline for common three kinds of tetracycline antibiotics. Tetracycline class of antibiotics, tetracyclines, TCs) is yellow crystalline powder, taste bitter, solubility in the alcohol (such as methanol and ethanol), such as ethyl acetate, acetone, acetonitrile dissolved in organic solvent is smaller. This kind of antibiotic produced by Streptomyces, on the chemical structure are hydrogenated pian and four benzene derivatives (pictured) basic structure, near the visible area near (350 nm) with strong ultraviolet absorption. Composition is complicated in biological samples, interference of tetracycline class of antibiotics on material is numerous, high concentration of salt in the sample and the existence of large molecular weight proteins will seriously affect the rapid detection of tetracycline class of antibiotics, so the pretreatment of the sample become one of the most important step in the sample analysis, adopt the method of reasonable and effective will tetracycline antibiotics extracted from the matrix of complex biological samples and to purify is particularly important.

By tetracycline compounds in weak acid solution is stable, so often need to extract the target under the condition of weak acid composition, molecular weight protein precipitation and biological sample cuhk, generally USES the extraction solvents including citric acid salt, phosphate, trichloroacetic acid and perchloric acid, etc [2].

Weigh and homogenizer is homogeneous whole egg sample in 5 g, put in 50 mL with plug polypropylene centrifugal tube, add 5, 8, 10, 15, 20 ml0. 1 mol/L citrate, vortex mixing 1 min, oscillation 5, 10, 15, 20 min, 10000 r/min centrifuge for 10 min, the supernatant fluid to transfer to another 50 mL centrifuge tube, the residue with 5, 8, 10, 15, 20 mL 0.1 mol/L citrate repeat extraction twice, clear liquid on merger, shake well, and let stand for 10 min, after 0.22μmorganic membrane on rapid test.

B. *Beta lactam class residue detection of egg sample pretreatment*

Aminoglycoside antibiotics will act as a veterinary drug used to treat meat and dairy animals, so we need an analysis of the residual effective analysis method in these goods. This class of antibiotics for residue analysis put forward a huge challenge. Unlike most other antibiotics, these compounds could not using acetonitrile, or other organic solvent extracted from the organization or dairy products. In this study, with trichloroacetic acid (TCA) aqueous buffer will aminoglycoside antibiotics extracted from meat or dairy products. Join the TCA can make protein precipitation and inhibit protein with the analyte. Before the LC/MS analysis, the use of effective solid phase extraction (SPE) purification operation to remove residual TCA, to minimize the total extract interference. Through the use of the Oasis HLB (efficient, water can be invasive inverting absorber), in the milk and meat can obtain good solid phase extraction yield and purification effect.Ampicillin, the free acid containing 3 molecular crystal water. White crystalline powder. Taste slightly bitter. Slightly soluble in water, insoluble in ethanol, dissolve in the dilute acid dilute alkali solution. Soluble in water, soluble in ethanol. [3].

Ampicillin and ampicillin. The free acid containing 3 molecular crystal water (for internal use); White crystalline powder. Taste slightly bitter. Slightly soluble in water, insoluble in ethanol, dissolve in the dilute acid dilute alkali solution. PKa of 2.5 and 7.3. 0. 25% aqueous solution of pH 3. 5 ~ 5. 5. The structural formula below. Salt, the sodium for injection is white or kind of white powder or crystal. Odourless or slight odor, taste bitter. Have led to wet. Soluble in water, slightly soluble in ethanol. 10% aqueous solution of pH for 8 ~ 10. Xudong will whole egg, egg white and yolk homogenate respectively, accurately say 5 g and grout, put in 50 mL with centrifugal pipe plug, add 5 mL of acetonitrile solution set spiral mixer blender 1 min, then add 15 mL of acetonitrile solution, the swirling disaster 2 min, oscillation and mixed with 8000 x g centrifuge for 10 min, the temperature is set to 10 °C. Will clear liquid transferred to another 50 mL plug in the centrifuge tube, to add 20 mL with ultrapure water saturated me curse the methylene chloride solution blending 2 min (drug dissolved in the upper water, acetonitrile, soluble in methylene chloride solution

Yan-hong song said samples from 2.0 g homogenate of egg yolk and egg white, accurate to 0.1 g, put in 50 ml with centrifugal pipe plug, add 10 ml of acetonitrile, intense blend for 5 minutes on the vortex mixer, oscillator with low speed oscillation omin, 3 to 5400 r/min, the centrifugal 10 minutes, supernatant fluid in a centrifuge tube, and add 8 ml of acetonitrile to slag, repeat extraction time, clear liquid on merger, 35 °C water bath nitrogen blow dry. In 2 ml (mobile phase of methanol/l % ice acetic acid solution: 23/77, V/V) dissolve yolk residue, add 3 ml n-hexane, oscillation let stand for 5 minutes, 10 minutes at 3000 r/min, the centrifugal, remove n-hexane layer through a straw, add the 3 ml n-hexane repeat to fat, 0.22 mu m organic membrane, for the liquid chromatographic; Egg white without removal of the fat, with LML mobile phase (1% methanol/ice acetic acid solution: 28/72, V/V) dissolved residue, after 0.22 mu m membrane filter, the liquid chromatograph.

According to take in the homogenizer is homogeneous whole egg sample 2 g, put in 50 ml with centrifugal pipe plug, add 5, 10, 15, 20 ml of acetonitrile, intense mixing 5 min on the vortex mixer, oscillator with low speed oscillation, 10, 15, 20, 25 min to 5400 r/min, the centrifugal 10 min, supernatant fluid in a centrifuge tube, and add 8 ml of acetonitrile to slag, repeat extraction time, clear liquid on merger, 35 °C water bath nitrogen blow dry. Add 3 ml n-hexane, oscillation let stand for 5 minutes, 10 minutes at 3000 r/min, the centrifugal, remove n-hexane layer through a straw, add the 3 ml n-hexane repeat to fat, after 0.22μm organic membrane on rapid test.

Sulfa drugs, its molecular weight between 170 ~ 300, soluble in water, soluble in ethanol and acetone, almost insoluble in chloroform and ether. Except for alkaline sulfaguanidine, sulfa drugs because of containing the primary amine and sulfonamide based in acid and alkali sex, soluble in acid and alkali solution. Acid is weak and easy to absorb carbon dioxide from the air and carbonate precipitation. Because of its structure with a benzene ring, all have the ultraviolet absorption [4]. Add egg samples samples from 2 g to 5, 10, 15, 20 ml ethyl acetate oscillating up and down 5, 10, 15, 20 min, at room temperature to 5000 r/min centrifugal 5 min, take the lower liquid, after 0.22 mu m organic membrane on rapid immunochromatographic test. According to the article late rapid test results, it is concluded that under the condition of the following the fastest the most accurate test result. Add egg samples from 2 g to 5 ml 5 min, ethyl acetate and oscillations at room temperature to 5000 r/min centrifugal 5 min, take the lower liquid, after 0.22 μm organic membrane on rapid test.

C. *Fluoroquinolone residues detection of egg sample pretreatment*

Fluoroquinolone drugs (FQs) are white or light yellow crystal powder, usually free acid soluble in dilute alkali and glacial acetic acid, dilute acid solution, the water in the pH6 ~ 8 solubility, minimum in methanol, chloroform, ether and most difficult soluble or insoluble in the solvent. The structure of benzene and heterocyclic or heterocyclic skeleton and carbonyl and carboxyl, heteroatom such as chromophore or help base group of conjugate system, in the ultraviolet region characteristic and strong absorption, the ultraviolet spectrum contains several absorption peak: 240 ~ 300 nm. 330 ~ 350 nm. FQs itself also have fluorescent properties, the Ex = 280 ~ 330 nm, Em =410~430 nm. FQs acid-base amphoteric compounds, ultraviolet absorption spectrum in different pH medium will be slightly different [5].

In fresh egg, after the break with low speed homogenizer homogeneous egg samples, egg white and yellow when fully mixing, as the blank sample. After taking homogenate of blank sample, add the appropriate concentration of standard solution as the blank to add sample. In 2.0 g egg samples to 15 ml polystyrene in centrifuge tube, add 5, 10, 15, 20 ml of acetonitrile, fully use oscillator oscillation 5 min. Learned that 2 ml supernatant fluid to 10 ml of clean glass tube, 50 to 60 °C dry nitrogen flow. Add 2, 5, 8, 10 ml n-hexane, using vortex finder vortex 1 min, centrifugal 5 min at room

temperature, remove the upper organic phase, take 50μl for analysis. According to the article late rapid immunochromatographic test results, it is concluded that under the condition of the following the fastest the most accurate test result. In 2.0 g egg samples to 15 ml polystyrene in centrifuge tube, add 5 ml of acetonitrile, fully use oscillator oscillation 5 min. Learned that 2 ml supernatant fluid to 10 ml of clean glass tube, 50 to 60 °C dry nitrogen flow. Add 5 ml n-hexane, using vortex finder vortex 1 min.

D. Sulfonamides residues detection of egg sample pretreatment

Sulfa drugs, its molecular weight between 170 ~ 300, its chemical structure is as follows. Slightly soluble in water, soluble in ethanol and acetone, almost insoluble in chloroform and ether. Except for alkaline sulfaguanidine, sulfa drugs because of containing the primary amine and sulfonamide based in acid and alkali sex, soluble in acid and alkali solution. Most pKa sulfa drugs within 5 ~ 8, isoelectric point for 3 ~ 5, a few pKa is 8.5 ~ 10.5. Acid is weak and easy to absorb carbon dioxide from the air and carbonate precipitation precipitation. Because of its structure with a benzene ring, so all have the ultraviolet absorption. Zhong Ziqing said in 5 g egg samples, accurate to 0.01 g, 10 copies of put in 50 ml centrifuge tube. Each addition concentration set A, B, C three parallel, each sample set A blank control. Add quantity respectively for 10, 100, 200 ng/g. Add the sample after 30 min. Each centrifugal pipe add 20 g anhydrous sodium sulfate and 20 ml of acetonitrile, homogeneous 2 min, to 3000 r/min, the centrifugal 3 min.

Supernatant fluid in 100 ml bottle heart, residue add 20 ml of acetonitrile, repeat the above for 1 times. Merge extract, to the heart in a bottle and add 10 ml isopropyl alcohol, using rotary evaporator in 50 °C water bath to dry, accurate flow with 1 ml and 1 ml n-hexane soluble residues. Transferred to the 5 ml centrifuge tube, the vortex 1 min, 3000 r/min, the centrifugal 3 min, abandon to upper n-hexane, add 1 ml n-hexane, repeat the above steps. Take the lower solution, after 0.2 mu m membrane filter, using liquid chromatography - tandem mass spectrometry determination. Add egg samples samples from 2 g to 4 ml ethyl acetate oscillation for 3 minutes, up and down at 5000 r/min, the centrifugal 5 minutes, take 300 mu l upper liquid 80 °C water bath top up or down in nitrogen blow dry, with 150 mu l 0.01 mol/l pH7.4 slightly dissolve the residue of phosphate buffer.

IV. RESULTS AND ANALYSIS

A. Ampicillin, tetracycline class antibiotics residue sample detection

Beta lactam and tetracycline class rapid test strip applied the principle of the competitive inhibition of immune chromatography samples in the beta lactam classes and tetracycline drugs in the process of flow and colloidal gold marked specificity monoclonal antibody, suppresses the antibodies and lines of NC membrane (B, T line) on the beta lactam classes and tetracycline drugs - a combination of BSA coupling, leading to lines of depth changes. When the samples have no beta lactam classes and tetracycline drugs or beta lactam and tetracycline drugs concentrations below the detection limit, B, T line color; When the samples in the beta lactam classes and tetracycline drugs concentration equal to or higher than the detection limit, B, T line color; And no matter whether the sample contains beta lactam type and tetracycline drugs, color quality control line C line, to test effectively.

Sample preparation extraction buffer (Na2EDTA NH4OOCH3 10 mM / 0.4 mM / 1% NaCl / 2% TCA) : 0.77 g ammonium acetate (NH4OOCH3) in 1 l of the volumetric flask. Add about 900 mL of reagent water, dissolve. Use 1 N 1 N HCl or NaOH to adjust pH value to 4.0. Add 0.15 g ethylenediamine tetraacetic acid disodium (Na2EDTA. 2 h2o), 5 g of sodium chloride (NaCl) and 20 g trifluoroacetic acid (TCA). Mix, make the solid solution, add reagent water to scale. Preliminary extraction: 2 g even cattle tissues or 10 mL milk in 50 mL centrifuge tube. Add 20 mL extraction buffer, vortex 10 seconds, and then fully shake the 1 minute. Sample of the centrifugal 5 minutes under 4000 RPM, clear fluid collection. According to the need to use dilute HCl or NaOH will clear liquid pH adjustment to 6.5 + / - 0.5.

B. Fluoroquinolone, sulfonamides residue sample detection

Sulfa and fluoroquinolone combined rapid test strip applied the principle of the competitive inhibition of immune chromatography samples of sulfa drugs and fluoroquinolone drugs in the process of flow combined with colloidal gold marked specificity monoclonal antibody, suppresses the antibodies and lines of NC membrane (T) on the sulfa and fluoroquinolone - a combination of BSA coupling, resulting in lines of color depth changes. When there is no sample of sulfa drugs and fluoroquinolone drugs or sulfa drugs and fluoroquinolone drugs concentrations below the detection limit, T1, T2 line color; When sulfa drug concentration in the sample is equal to or higher than the detection limit, a T1 line color, T2 line color; When the sample of fluoroquinolone drugs concentration equal to or higher than the detection limit, T2 line color, not a T1 line color; And no matter whether samples containing sulfa drugs and fluoroquinolone drugs, quality control line (C) all can color, to show detection effectively.

Marry remove all reagent from cold storage environment, balance at room temperature (25 °C) 20-30 min rapidly. The kits and standard sample of the corresponding microporous serial number, each sample and standard substance do two parallel holes. Add to the corresponding standard/sample 50 mu l microporous, soon to join enzyme mark two 50 mu l/hole, oscillation blending, 25 °C avoid light reaction after 60 min in the environment with washing liquid is 250 mu l/hole, full of washing 4-5 times. Join the substrate liquid A liquid 50 mu l/hole, then add the substrate liquid B liquid 50 mu l/hole, oscillation and mixed 25 °C avoid light environment reaction after 30 min. Add terminated liquid 50 mu l/hole, blending, enzyme standard instrument set in each hole OD value at 450 nm. To standard percentage absorbance (samples/zero absorbance value of standard absorbance value) as the ordinate, standard concentration (PPB), the abscissa denotes the semilog, draw standard curve. The percentage of sample absorbance generation into the standard curve, read the concentration of the sample corresponds to the standard curve, multiplied by

the corresponding dilution ratio . SPE purification: this study used the Oasis HLB 96 - well plates (30 mg). If necessary, can use a 1 cc / 30 mg extraction column. Successively used 1.5 mL of methanol, 1.5 mL water, balance board hole or extraction column. The flow velocity is set to 1 mL/min or less. Join the preliminary extraction to get supernatant after pH adjustment, for tissue samples, add 1 mL solution, samples for dairy products, to join a 1.5 mL solution. 1 mL water to rinse. With 0.5 mL 10:5:8 5 formate/isopropanol/water elution. To join (including 1.5 L HFBA, using UPLC/MS/MS analysis[6].

C. Sulfonamides residue sample detection

Li Kui by using immune chromatography rapid determination of sulfonamides residues in eggs. From 4 ℃ strip out of the fridge, return to room temperature. Blank respectively 120 mu l sulfa drug standard samples and waiting for inspection, in turn, add to the MPP hole, insert the strip sample end microporous plate hole, make holes in the liquid by capillary action up swimming, reaction after 10 minutes, judge the results. Sulfa drugs standard strip blank lines appear red stripe; If waiting for the sample strip lines appear similar to the blank lines of standard red stripe, sulfa drugs, not detected in the samples that sentence to negative; Visible light on the blank lines of standard color light red stripe, description of sulfanilamide in oxygen pyrimidine (SMM), sulfanilamide for oxygen pyrimidine (SMD), sulfanilamide dimethyl oxygen pyrimidine (SDM) concentration in the 20 ~ 100 mu g/kg range, or SDZ concentration within 40 ~ 160 mu g/kg, jailed for weakly positive; If the inspected samples without color strip line, SMM, SMD, concentration of SDM in the sample that is more than 100 mu

V. PROSPECT

Laying hens production in our country in the world, but more than 95% of the eggs are produced by farmers and small businesses, as a result of the limitation of capital, technology, management and other aspects of vicious competition and market prices, the price of eggs was far below the normal production cost. Feed producers as the pursuit of profit, and extensive use of cheap inferior raw material; In order to reduce disease risk, abuse of antibiotics; For high yield, and extensive use of synthetic hormones. Individual farmers to buy cheap feed, and the imperfection of the supervision strength, have an opportunity to make illegal activities. Resulting in a decline in egg quality and nutrient unbalance, pathogenic bacteria, antibiotics, hormones and pesticide residues exceeds bid badly. The use of veterinary drugs not science, not specification, led to the occurrence of drug residues. To prevent avian disease, in the case of uncertain etiology abuse of antibiotics, optional increasing dosage, dosage change, do not obey to take medicine.

All in all, First is looking at home and abroad research status, can include physical and chemical properties of the target component, harm, types of existing research methods, and the pros and cons of each method, the current methods of GB and components limited threshold. Research status in a certain extent, have the latest research results. Research focus, and the light spot and so on also can have certain embodiment. And in the veterinary drug detection, drugs, veterinary drugs can be divided into categories of methods summary (similar components of structure, a lot of time there may be a lot of consistency test method).

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