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ORIGINAL PAPER

Development and validation of a method for quantification of residual florfenicol in various tissues of broiler chicken by UPLC-MS/MS

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Abstract

Florfenicol is a chloramphenicol antibiotic that plays an essential role on bacteriostasis. Long-term use of florfenicol in livestock and poultry can cause immunetoxicity and reproductive toxicity and the consumption of animal-derived products with excessive residues of florfenicol will pose a certain threat to human health. To study residue depletion of oral florfenicol in chickens, 48 healthy 30 -day -old AA broilers received continuous administration of 150 mg/kg/d florfenicol for five days. Muscle, liver, kidney and sebum were collected at 0.16, 1, 3, 5, 7 and 9 days after discontinuation of the drug and detected by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Most previous studies have shown that acid hydrolysis is necessary for tissue sample extraction. However, the steps of acid hydrolysis are tedious and time-consuming. In this experiment, we tried to improve the solvent extraction method to simplify the pretreatment process and the effect of acid hydrolysis was further analyzed and compared. Using the WT1.4 software the withdrawal time in muscle, liver, kidney and sebum obtained by F-QuEChERS-AOAC 3202 method was 7.82, 0.06, 4.38, 13.71 days respectively while acid hydrolysis method were 8.89, 5.84, 5.39 and 9.65 days, respectively. To reduce the residues of florfenicol and ensure the safety of chicken products, it is recommended that chickens taking oral florfenicol should be subjected to a 14 -days withdrawal time.

Keyword Florfenicol · Florfenicol amine · Chicken · Residue · Extraction · Acid hydrolysis

Introduction

Florfenicol (FF) is a broad-spectrum antibiotic developed by Schering-Plough in the United States. It was first used for aquatic animals in Japan in 1990 and achieved the desired objectives. FF is a type of chloramphenicol antibiotic that can inhibit transpeptidase and block the growth of peptide enzymes, which hinders the formation of the peptide chain, prevents protein synthesis, and plays an essential role in inhibiting and killing bacteria. FF has a strong antibacterial effect on gram-positive bacteria (G+), gram-negative bacteria (G-), rickettsia, amoebae, and other microbes. It also has distinct advantages in safety and high efficacy compared with chlorampenicol and thiamphenicol [1, 2] At present, more than 20 countries have approved this drug for the prevention and treatment of bacterial diseas.

However, FF is not absolutely safe, e.g., Bretzlaf et al. [3] found that FF could inhibit the phagocytosis of neutrophils in bovine blood, Sieroslawska et al. [4] discovered that FF (40 mg/g) could reduce the proliferation of B and T cells of

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carp head kidney and spleen in vitro, and can also inhibit the phagocytic ability of polymorphonuclear neutrophils and monocytes. Yang et al. [5] found that florfenicol (100 mg/kg) could depress the activity of marrow hemogenesis. Therefore, people should be alert to the immunotoxicity, reproductive toxicity, and hematological toxicity caused by the abuse of florfenicol [6, 7].

In recent years, antibiotic resistance genes had attracted wide attention. Jang [8], Ping [9], Wu et al. [10] discovered that long-term use of a drug would increase the drug resistance of the pathogenic bacteria. Till date, the reported resistance genes to FF are CFR [11], estDL136 [12], fexA [13], fexB [14], floR [15, 16], and pexA [17]. Activation of efflux pumps [18–20], targeted mutations or modifications of antibacterial drugs [11, 21], and enzymatic hydrolysis [12] may be responsible for drug resistance in pathogenic bacteria. If people eat 'florfenicol-containing eggs' and 'florfenicol-containing meat' for a long time, pathogenic factors such as drug resistance may be transferred to the human body [22–26].

To protect consumers from the harm caused by drug residues in animal-derived products, The European Commission has formulated the maximum residue limits (MRLs) for FF (the sum of FF and its metabolites measured as florfenicol amine (FFA)) in animal products [27]. In many countries, FF is prohibited during egg production, because it has the potential to induce early embryonal death in chickens [28]. The route of FF administration is a most commonly oral or intramuscular injection. Among different tissues, the concentrations and distribution of FF are varied. Some studies have found that the detection rate of FF in the liver, muscle tissue and digestive system is relatively prominent. The digestive system may be due to the 'medicated' food consumed by livestock and poultry. FF remains in the gastrointestinal tract, which further suggests that the main intake of FF maybe through the farmer's incorporation of drugs or 'medicated' feed-in livestock and poultry food. The plasma protein binding rate of FF is relatively low, which is consistent with its large steady-state distribution volume and wide distribution. The concentration of FF in livestock and poultry tissues is similar to or higher than the corresponding plasma concentration, indicating that FF has good permeability in these tissues. Its high distribution and low protein binding rate in animals make it last for a long time in tissues [29].

Therefore, understanding the reduction of oral FF residues in poultry is of great significance to guide clinical treatment and ensure the safety of food obtained from poultry. FF metabolizes quickly in animals and can get a variety of metabolites, among which FFA is the long lasting metabolite in animal liver, and all metabolites of FF can be converted into FFA after hydrolysis in 6 mol/L hydrochloric acid solution for 2 h at 100 °C. Therefore, FFA is generally used as one of the indicative residues of FF in foodstuffs of animal origin [30, 31]. Most of the published FF analytical methods involved acid hydrolysis [32–34]. However, the steps of acid hydrolysis are tedious and time-consuming, which is not conducive to the detection of large quantities of samples. Moreover FF and all its metabolites are converted to FFA by acid hydrolysis, so it is impossible to determine the specific residues of FF and FFA in each tissue.

In this study, we tried to improve the solvent extraction method to simplify the pretreatment process to extract FF and FFA in the sample. Further the effect of acid hydrolysis was analyzed and compared to explore the metabolic reduction of FF in chickens. The extraction process of acid hydrolysis referred to the method published by Muhammad Imran et al. [33]. Considering the differences in the metabolic function of white feather chickens of different genders, we made a brief comparative analysis of the residual metabolism of FF and FFA in various tissues of roosters and hens.

Materials and methods

Chemicals and reagents

FF (99%), FFA (99.8%), florfenicol-d_3 (99.6%, internal standard of FF), and florfenicol-d_3 amine (95%, internal standard of FFA) were obtained from A Chemtek, Inc. (Worcester, MA, USA). FF powder (20%) was bought from Guangdong Dahuanong Animal Health Products Co., Ltd. (Guangdong, China). UPLC-grade acetonitrile (ACN) and formic acid (FA) were purchased from Merk (Darmstadt, Germany). Sodium chloride (NaCl ≥ 99.5%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water was purified via a Milli -Q treatment system (Millipore, Bedford, MA). The F-QuEChERS-AOAC 3202 multi-function needle filter (containing $MgSO_4$ (300 mg), PSA (100 mg), C18 (100 mg), and with a particle size of 40 μm) was from the Institute of Quality Standard and Testing Technology for Agro-Products, CAAS (Beijing, China).

Preparation of standard solutions

The standard solutions of FF, FFA, florfenicol-d_3, and florfenicol-d_3 amine were diluted to 10 μg/mL as the stock solution, respectively. Then the standard mixture of them with a concentration of 1 μg/mL was prepared from the individual stock standard solutions. The standard mixture was stored at − 18 ℃ and updated every 3 months, which was used for preparing a series of matrix standard products.

Experimental chicken and sample collection

Animal experiments were performed as per the animal management regulations [35]. Fifty-six healthy AA broiler chickens at the age of 30 days were selected from the farm for the pharmacokinetic test, including 48 in the administration group and 8 in the control group (half roosters, half hens), and each chicken weighs about 1.5 kg. The feeding conditions were strictly controlled before the experiment to ensure that the animals did not ingest FF.

FF powder was added to the drinking water of the experimental group at the dose of 150 mg/kg/d (recommended therapeutic dose) for 5 days, while FF was not added to the drinking water of the control group. Eight (half roosters, half hens) chickens in the drug administration group were randomly slaughtered at 0.16, 1, 3, 5, 7, and 9 days after stopping drug administration and 8 controls were slaughtered on the last day. Muscle, liver, sebum, and kidney tissues were collected and marked. Freeze-dried chicken breast, whole liver and double kidney were ground and used for analysis. The weights of samples before and after drying were recorded. After the sebum was chopped, it was also homogenized as another trial material. All of the trial materials were stored at -20 ºC.

Sample processing

An aliquot of sample (muscle and liver: 0.5 g, kidney: 0.2 g, sebum: 2.0 g) were weighed into 50 mL polypropylene centrifuge tubes and spiked with 50 µL of 1.0 µg/mL internal standard solution (The mixture of florfenicol-d3 and florfenicol-d3 amine), then 5 mL water and 0.5 mL ammonium hydroxide (25.0—28.0%) were added. The sample was given 1 min on a high-speed shock (2500 r/min) which was on a vortex mixer; then 10.0 mL of acetonitrile was added, the tube was shaken vigorously for 1 min, sonicated for 20 min. Next 3 g NaCl was added, and again, the tube was shaken vigorously for 1 min, sonicated for 10 min. Followed by centrifugation for 5 min with 4000 r/min at 4 ºC. The above supernatant of 1 mL was taken and passed through the F-QuEChERS-AOAC 3202 multi-function needle filter (making the liquid flow out drop by drop) into sample feeding vials for UPLC-MS/MS analysis.

The blank sample from the controlled group after homogenization was taken as negative control and 0.05 mL of mixed standard working solution of FF and FFA working fluid (1 mg/kg) was added as the positive control.

Chromatographic conditions

UPLC was performed on a Waters Acquity (Milford, MA, USA) system. Chromatographic separation was achieved on an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µm) (Waters, USA) at 30 ºC column temperature. The mobile phases were water containing 0.1% FA (phase A) and ACN (phase B), with gradient elution at a flow rate of 0.3 mL/min. The gradient elution program was as follows: initial, 5% B; 1.5 min, 90% B and hold for 1 min; return to 5% B at 3.0 min, followed by a re-equilibration time of 2 min, to give a total run time of 5 min. The injection volume was 2.0 µL.

Mass spectrometry analysis was carried out using an AB 5500 triple-quadrupole tandem mass spectrometer (ABSciex, Framingham, MA, USA). The parameters were set as follows: 4.5 kV ion spray voltage, 35 psi curtain gas pressure, 55 psi pressure for the nebulizer (gas 1) and turbo (gas 2) gases, and 600 ºC turbo heater temperature. The declustering potentials and collision energies of FF and FFA were optimized using an automatic function in Analyst software 1.6.2 (ABSciex, Shanghai, China). MRM parameters and retention times of the four target compounds were shown in Table 1.

Method validation

To quantify more accurately, the internal standard is used to correct the final results. Calibration curves for FF and FFA in the blank matrixes at concentrations of 1, 2, 5, 10,

Table 1 Multiple reaction monitoring parameters for detection of the four target compounds by the mass spectrometer

Antibiotics	Quantitative ions	Qualitative ions	Fragmentor (V)	Collision energy (eV)	Retention (min)
Florfenicol	355.900/184.900	355.900/184.900	− 106	− 26	2.32
		355.900/119.100	− 103	− 40	
Florfenicol-d_3	359.000/339.000	359.000/339.000	− 110	− 13	2.39
		359.000/188.000	− 110	− 24	
Florfenicol Amine	247.800/230.100	247.800/230.100	120	19	0.96
		247.800/130.200	66	30	
Florfenicol-d_3 Amine	250.900/233.100	250.900/233.100	75	17	0.87
		250.900/130.200	75	30	

25, 50, 100 and 200 ng/mL (mixed with 50 µg/kg internal standard) were constructed by drawing the relationship between the peak areas (y-axis) and concentration (x-axis). To determine the accuracy of the method, an additional recovery experiment was designed. Three enhancement levels of FF (low: 20 µg/kg, medium: 50 µg/kg, high: 100 µg/kg) were added to the blank samples (n = 6), respectively. The sensitivity of the UPLC-MS/MS analysis method was determined by the LOD and LOQ, which were calculated as S/Ns of 3 and 10, respectively.

Sample solution and 50 µg/kg FF standard solution were selected for single-point calibration, ensure that the response values of FF and FFA in the control solution and sample solution should be within the linear range of the instrument detection. During the sample solution determination process, the control solution should be inserted into every 10 batches of samples for accurate quantification.

Pharmacokinetic and Statistical Analyses

Determination of the pharmacokinetics of FF (sum of the amount of FF and FFA) was carried out by the WT1.4 software which was based on the statistical methods developed by Germany [36] and the Committee for Veterinary Medicinal Products (CVMP) used to calculate the withdrawal time. The abscissa in the figure (Fig. 1) is the time, the ordinate is the concentration, the small circle represents the data point.

Results

Method validation

The chromatograms for the analytical standards revealed retention times of 2.32 min for FF; 2.39 min for florfenicol-d3; 0.96 min for FFA; and 0.87 min for florfenicol-d3 amine. Calibration curves for FF and FFA in the blank matrixes

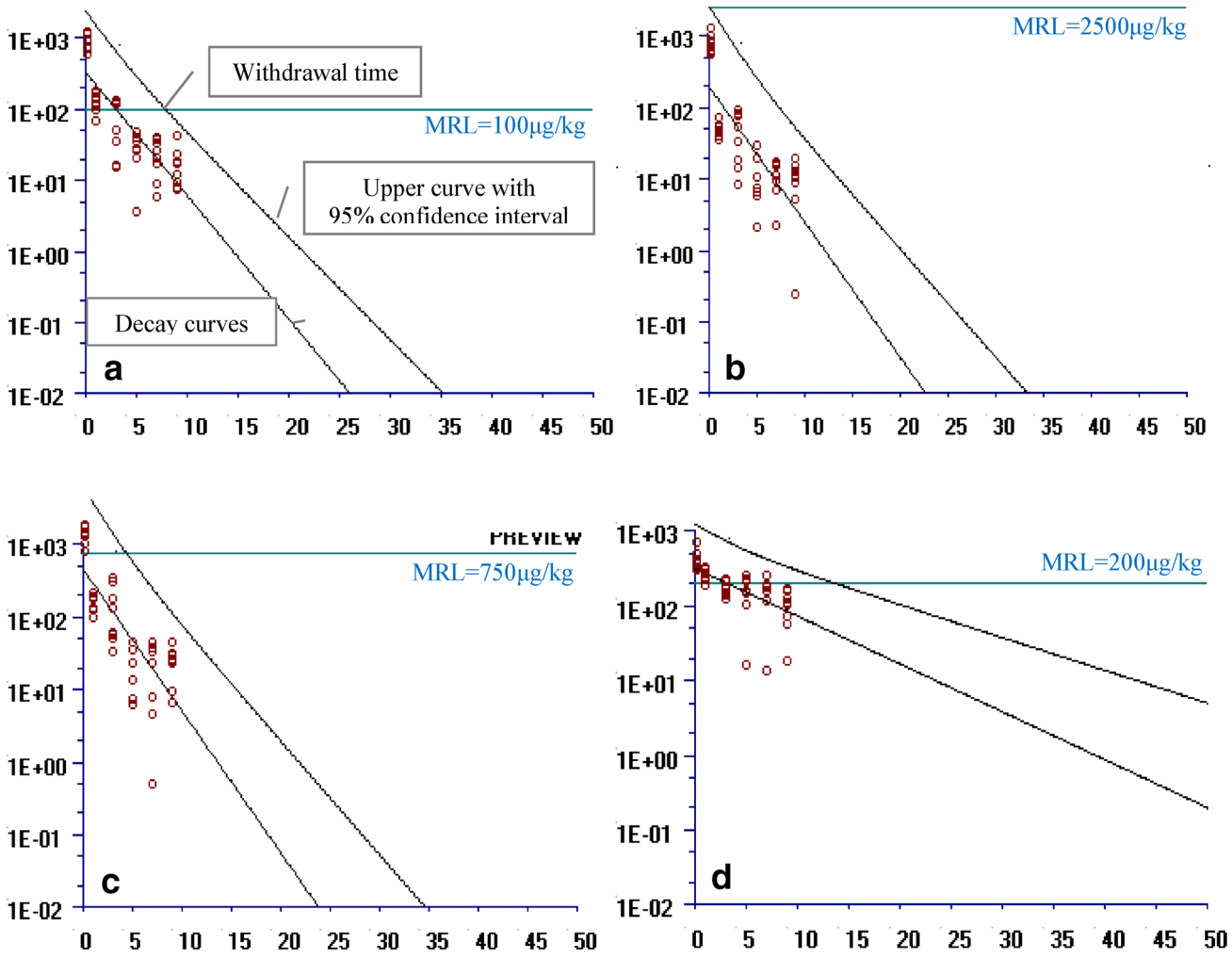

Fig. 1 Fitting diagram of depletion of oral florfenicol residues in tissues (a-muscle, b-liver,c-kidney and d- sebum)

were constructed and good linearity was realized over the experimental concentration ranges, their linear equations were: y = 0.0001x-1E-04, ($r^2 > 0.999$), y = 0.0354x + 0.0233 ($r^2 > 0.994$). The LODs and LOQs of FF and FFA in different tissues are slightly different, they were ranged from 0.3–0.5 μg/kg and 0.8–1.0 μg/kg, respectively. The precision experiments were performed by analyzing spiked samples, which were injected three times to determine intraday of them were 6.54–14.28% and 6.01–9.84%, the recoveries of them were 72–120% and 75–126% indicated good compliance of this method with the requirements given in the European Commission Decision 2002/657/EC [37].

Residual FF and FFA in tissues

A matrix-matched calibration curve and solvent blank were included to ensure the accuracy of the results, for the samples whose detection quantity exceeds the detection limit, the samples were diluted with blank matrix and then re-analyzed and quantified. In short, after taking FF for five consecutive days the rapid absorption of FF led to a high distribution level in the tissues of chickens. Among the four tested tissues, the maximum amount of FF was found in kidney samples, which was consistent with that of Muhammad Imran. et al. [33], and that was related to the kidney is the main route of drug excretion. Whereas in sebum tissues the least amount of FF was detected at the same periods at 0.16 d after discontinuation (Table 2). In this study, we counted the residues of FF and FFA in tissues, as shown in Table 3 and Table 4, it could be seen that the residue of FFA in muscle, liver and kidney of at 0.16 d after stopping drug administration was much higher than that of FF, but there was no significant difference between them in the following 1–9 days. The metabolic rate of FF in sebum is relatively slow, the difference between FF and FFA at 0.16 d after stopping drug administration was smaller than that of the other three tissues, but in the next few days, the difference between them becomes larger. It is not difficult to see that the reduction rate of FFA was higher than that of FF.

Individual differences between roosters and hens

The data in Tables 5 and 6, showed that on the 0.16 d after drug discontinuation, the drug residues in each tissue of the roosters and the hens reached their peaks. The concentrations of FF in the muscle, liver and kidney of the roosters and hens were 1122.49 ± 134.15 μg/kg, 969.87 ± 241.65 μg/kg, 1694.72 ± 147.99 μg/kg and 751.78 ± 137.30 μg/kg, 659.16 ± 111.86 μg/kg, 1130.19 ± 231.83 μg/kg, respectively, the difference between the residual concentrations

Table 2 Residues of florfenicol and florfenicol amine in various tissues during withdrawal for all chickens ($\bar{X} \pm S$, n = 8) (μg/kg)

Tissue	Total florfenicol and f florfenicol amine residues in chicken tissues (μg/kg)					
	0.16 d	1 d	3 d	5 d	7 d	9 d
Muscle	937.14 ± 229.74	130.79 ± 36.42	78.82 ± 50.49	30.25 ± 12.89	23.98 ± 12.00	17.52 ± 11.22
Liver	814.51 ± 244.11	50.18 ± 10.58	47.94 ± 31.75	11.00 ± 8.53	12.03 ± 5.07	10.59 ± 5.69
Kidney	1412.45 ± 342.78	161.92 ± 36.88	145.20 ± 111.93	18.52 ± 14.21	24.70 ± 17.09	24.46 ± 11.57
Sebum	399.54 ± 128.22	251.99 ± 44.96	178.59 ± 38.25	170.20 ± 75.69	158.93 ± 66.91	102.00 ± 47.40

Table 3 Residues of florfenicol in various tissues during withdrawal for all chickens ($\bar{X} \pm S$, n = 8) (μg/kg)

Tissue	Total florfenicol residues in chicken tissues (μg/kg)					
	0.16 d	1 d	3 d	5 d	7 d	9 d
Muscle	327.63 ± 58.98	97.61 ± 27.15	54.88 ± 37.79	25.66 ± 12.20	19.03 ± 9.96	10.91 ± 8.60
Liver	174.42 ± 48.17	15.22 ± 10.80	10.88 ± 5.70	6.08 ± 7.30	3.14 ± 3.06	0.27 ± 0.34
Kidney	302.98 ± 49.03	48.79 ± 16.83	42.24 ± 44.45	11.25 ± 11.53	7.23 ± 5.53	2.16 ± 1.74
Sebum	247.04 ± 59.04	160.55 ± 20.61	150.92 ± 31.24	150.53 ± 67.26	135.45 ± 55.41	76.18 ± 37.01

Table 4 Residues of florfenicol amine in various tissues during withdrawal for all chickens ($\bar{X} \pm S$, n = 8) (μg/kg)

Tissue	Total florfenicol amine residues in chicken tissues (μg/kg)					
	0.16 d	1 d	3 d	5 d	7 d	9 d
Muscle	609.50 ± 205.83	33.18 ± 11.02	23.95 ± 14.35	4.58 ± 1.48	4.96 ± 2.86	6.61 ± 3.09
Liver	640.09 ± 218.54	34.96 ± 12.73	37.06 ± 26.40	4.92 ± 5.31	8.88 ± 4.20	10.29 ± 5.66
Kidney	1109.48 ± 296.26	113.13 ± 27.69	102.95 ± 68.48	7.27 ± 9.25	17.41 ± 13.21	22.29 ± 11.58
Sebum	152.50 ± 78.15	91.44 ± 34.71	27.67 ± 11.13	19.67 ± 9.15	23.49 ± 15.22	25.83 ± 11.47

Table 5 Residues of florfenicol and florfenicol amine in various tissues during withdrawal for roosters ($\bar{X} \pm S$, n = 8) (μg/kg)

Tissue	Total florfenicol and florfenicol amine residues in rooster tissues (μg/kg)					
	0.16 d	1 d	3 d	5 d	7 d	9 d
Muscle	1122.49 ± 134.15	147.10 ± 32.49	50.76 ± 49.55	23.73 ± 12.22	22.75 ± 9.08	14.53 ± 6.53
Liver	969.87 ± 241.65	55.45 ± 10.34	38.10 ± 32.91	9.40 ± 6.43	11.03 ± 5.72	11.06 ± 7.05
Kidney	1694.72 ± 147.99	151.57 ± 43.89	112.77 ± 108.87	12.83 ± 6.87	12.98 ± 15.03	22.09 ± 7.94
Sebum	394.59 ± 61.82	269.49 ± 40.80	184.04 ± 41.92	124.85 ± 75.26	185.15 ± 52.11	113.61 ± 49.79

Table 6 Residues of florfenicol and florfenicol amine in various tissues during withdrawal for hens ($\bar{X} \pm S$, n = 8) (μg/kg)

Tissue	Total florfenicol and florfenicol amine residues in hen tissues (μg/kg)					
	0.16 d	1 d	3 d	5 d	7 d	9 d
Muscle	751.78 ± 137.30	114.48 ± 32.64	106.89 ± 32.68	36.77 ± 9.89	25.22 ± 14.23	20.51 ± 13.84
Liver	659.16 ± 111.86	44.91 ± 7.82	57.78 ± 27.19	12.59 ± 9.96	13.03 ± 4.09	10.12 ± 3.82
Kidney	1130.19 ± 231.83	172.27 ± 24.06	177.62 ± 106.06	24.21 ± 17.09	36.42 ± 9.14	26.82 ± 13.91
Sebum	404.49 ± 170.33	234.50 ± 42. 02	173.14 ± 33.32	215.55 ± 40.98	132.72 ± 69.75	90.39 ± 41.75

of roosters and hens tissues in all three tissues reached more than 300 μg/kg. On 9 d they were 14.53 ± 6.53 μg/kg, 11.06 ± 7.05 μg/kg, 22.09 ± 7.94 μg/kg, and 20.51 ± 13.84 μg/kg, 10.12 ± 3.82 μg/kg, 26.82 ± 13.91 μg/kg, respectively. The residues in most tissues of roosters are higher than those of hens. The T-test showed that there was no significant difference in FF residues in tissues between roosters and hens from 1–9 d after stopping drug administration, while the difference is significant in muscle and kidney on 0.16 days ($p < 0.05$).

Metabolism of FF in tissues

From 0.16 d to 1 d, it could be seen from Fig. 2 the clearance rates of FF by the tissues were kidney > muscle > liver > sebum. On 1 d after discontinuation, the residual concentration in the kidney was 161.92 ± 36.88 μg/kg and it was lower than the MRL (750 μg/kg), and it could be seen that FF was metabolized faster in the kidneys. On 5 d after discontinuation, the residual concentrations in the muscle (30.25 ± 12.89 μg/kg) was lower than the MRL (100 μg/kg), and the residual concentrations in sebum (102.00 ± 47.40 μg/kg) with the relatively slow metabolic rate for florfenicol was lower than the MRL (200 μg/kg) on 9 d, whereas in liver tissues it never exceeds the MRLs during the withdrawal period.

At 0.16–1 d after discontinuation, the concentration of FF in muscle, liver and kidney of chickens decreased significantly. In the following days, the concentration of FF also showed a decreasing trend. It was worth noting that FF has two distinct elimination stages in each tissue. Comparatively speaking, the metabolism of sebum was slower, there was no significant large-scale reduction in the entire metabolic process, and the concentration was higher than the other three tissues during 1–9 d.

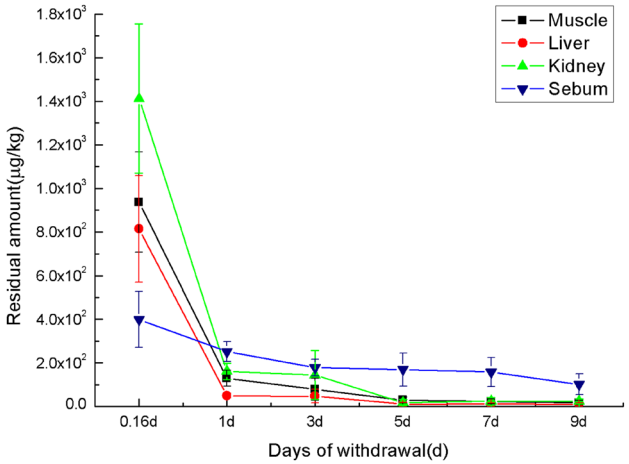

Fig. 2 Residual elimination curves of total florfenicol and florfenicol amine in muscle, liver, kidney and sebum for all chickens by F-QuEChERS-AOAC3202 method

Comparison of the current method with acid hydrolysis method

We compared the extraction effects of FF and FFA by F-QuEChERS-AOAC 3202 and acid hydrolysis method [33], respectively. The results were as shown in the Fig. 3. It was not difficult to see that in muscle and sebum, the extraction efficiency of the two extraction methods was almost the same, the results showed that this method could completely extract the target substances from the tissue. In the kidney, the extraction result of the acid hydrolysis method

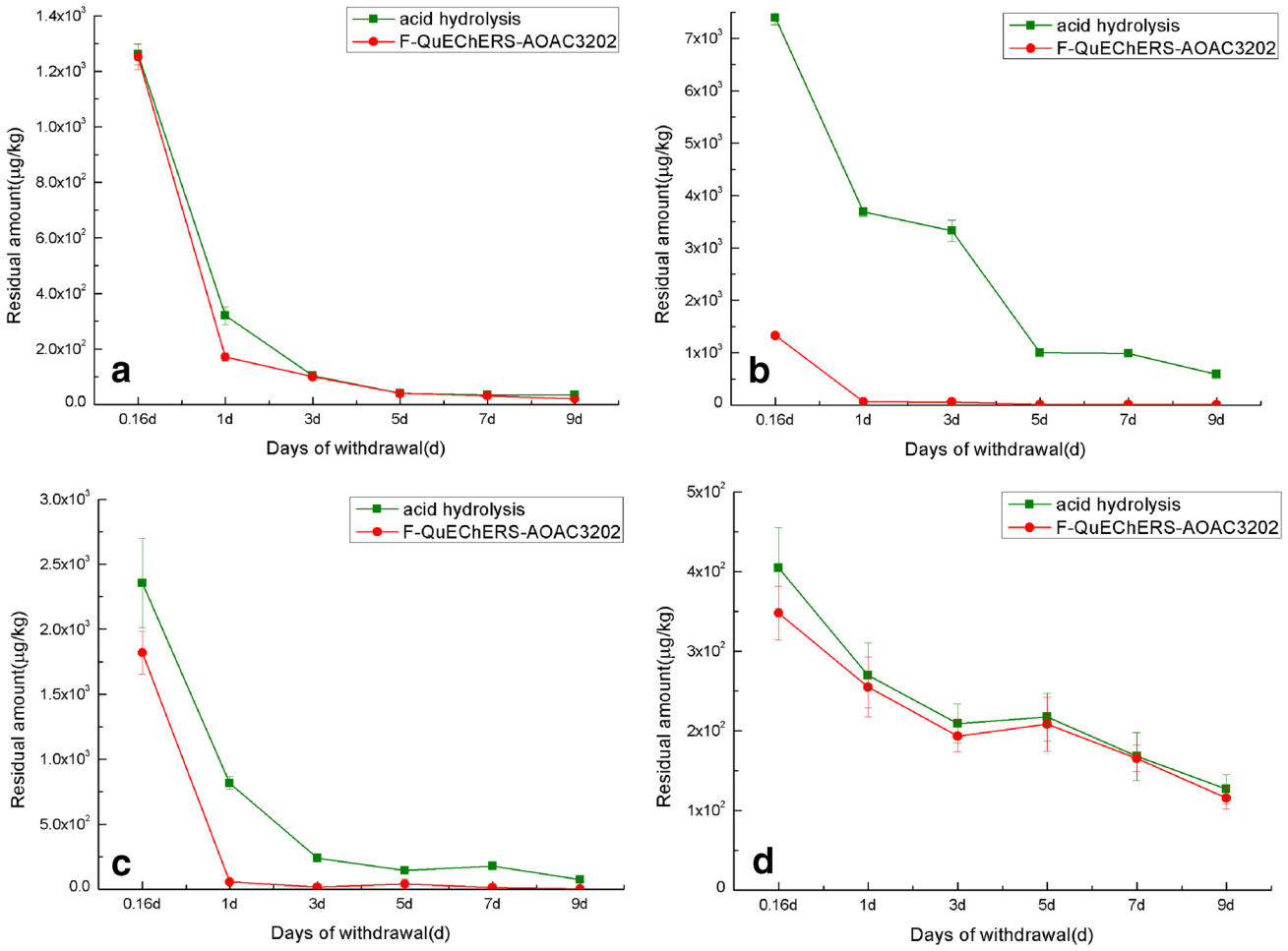

Fig. 1 The extraction effects of florfenicol and florfenicol amine by F-QuEChERS-AOAC 3202 and acid hydrolysis (a-muscle, b-liver,c-kidney and d- sebum) (n = 3)

was slightly higher than that of F-QuEChERS-AOAC 3202 method, and with the extension of stopping drug administration, the gap between them gradually decreases. However, in the liver, the extraction amount of acid hydrolysis method was about four times that of F-QuEChERS-AOAC 3202 method, and similarly, the gap between the two methods gradually decreased on the fifth day after stopping drug administration.

Calculating the withdrawal time

According to the relevant regulations of the Ministry of Agriculture and Rural Affairs on the MRL of FF residue markers and the calculation of the withdrawal time from the European Union, the remaining FF in the muscle, liver, kidney, and sebum of AA broiler chickens was calculated using the WT1.4 software. The relevant time in muscle, liver, kidney and sebum obtained by F-QuEChERS-AOAC 3202 method were 7.82, 0.06, 4.38, and 13.71 days (Table 7, Fig. 1), respectively while for acid hydrolysis method they were 8.89, 5.84, 5.39 and 9.65 days, respectively.

Table 7 Maximum residue limits (MRL) of florfenicol and the withdrawal periods for florfenicol in different tissues for all chickens

Tissues	Muscle	Liver	Kidney	Sebum
MRL (μg/kg)	100	2500	750	200
Withdrawal period (d)	7.82	0.06	4.38	13.71

Discussion and conclusions

On the 0.16 d after stopping the feeding, the residual amount of the four tissues reached the maximum value. On the 1 d, the residual amount in the muscle, liver and kidney was significantly reduced, showing that it was easily absorbed and rapidly metabolized. FF is a time-dependent

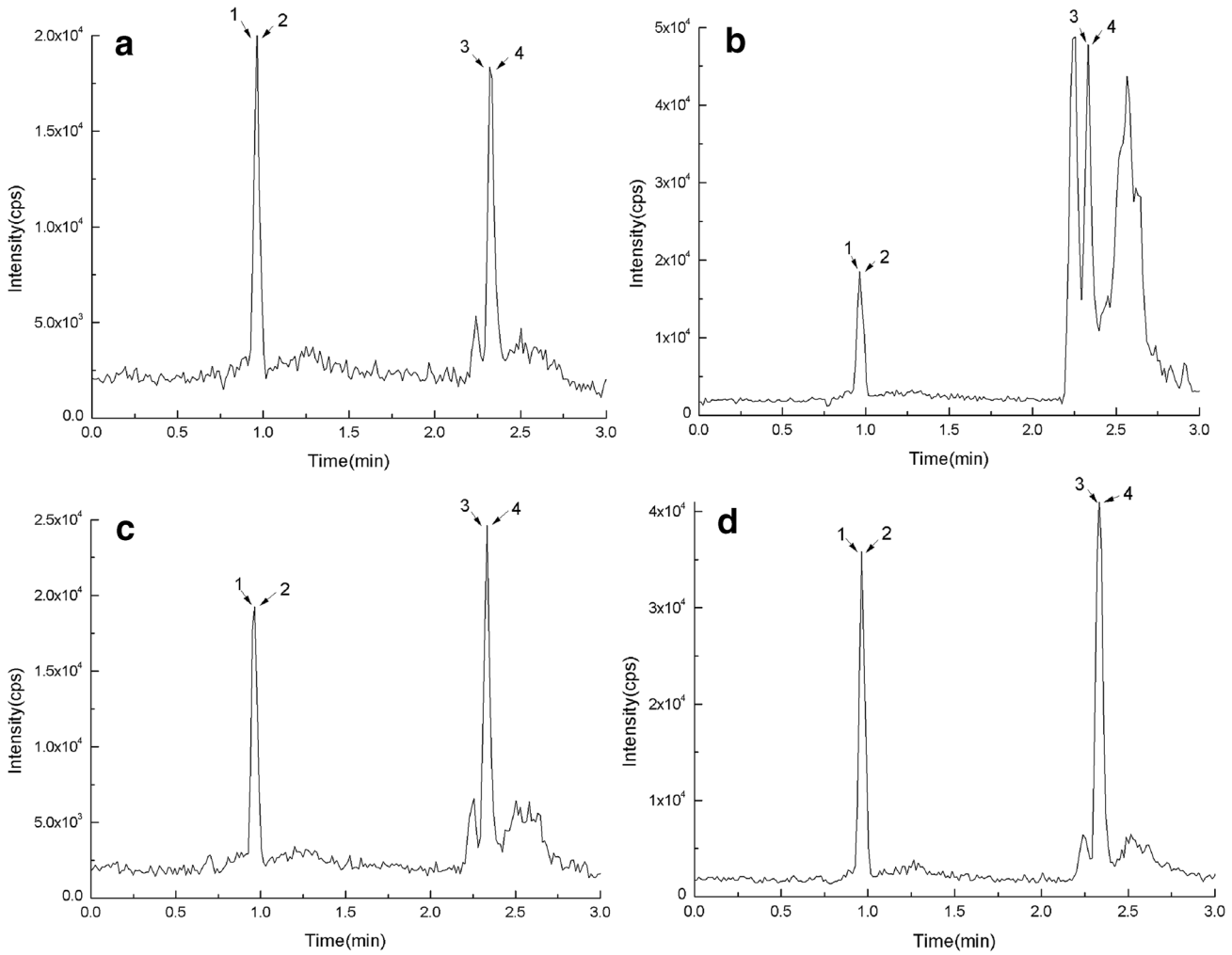

Fig. 3 Total ion chromatograms of florfenicol and florfenicol amine in sample (The concentration of each standard target was 50 μg/kg) (a- muscle, b- liver, c- kidney, cxd- sebum). Peak identification: 1-florfenicol-d_3 amine; 2-florfenicol amine; 3-florfenicol; 4-florfenicol-d_3

antimicrobial drug; thus, its effective therapeutic concentration in tissues of the chicken must exceed the minimal inhibitory concentration (MIC) of the pathogen during the medication time [38]. The MIC of FF to 9 standard strains such as chicken *E. coli* is 0.2 to 1.6 μg/g [39, 40]. Results from the present study showed that the drug concentrations in tissues were 0.81–1.41 μg/g, which were within the valid range of effective bacteriostasis.

The differences in metabolic capacity between roosters and hens were because that the digestive ability and the anti-stress ability of roosters were better than those of hens. Further the individual liver drug metabolism and gastrointestinal content differences in the number of substances may affect the absorption of the drug [41–43]. It was worth noting that FF has two distinct elimination stages in each tissue, Feng et al. [44] found the same effect when studying the pharmacokinetics of FF in orange-spotted grouper after oral administration in warm seawater. This phenomenon was generally thought to be caused by the enterohepatic circulation, gastric emptying, and an 'absorption window' along the intestine [45, 46]. In this work, the calculated withdrawal time was slightly different from Lu et al. [47] (Oral administration at a dose of 200 mg/kg for three consecutive days). The differences could be attributed to differences in atrioventricular pharmacokinetics, the mechanism of the enterohepatic circulation, different feeding doses, bioavailability and other factors.

Some people had suggested that after oral treatment, antibiotics could disposition into the rachis of feathers through the blood [48], concurrently, the antibiotics could exit the body by excretion through the uropygial gland and were dispositioned on the feathers through grooming behaviour [49], and Jansen et al. [50] had confirmed this view in the

analysis of tetracyclines, quinolones, macrolides, lincosamides, pleuromutilins, and sulfonamides. At present, it was unknown whether the drug deposition on feathers will affect the drug metabolism of other tissues and how they relate to each other in the chicken, perhaps this could also be used as a method to study the metabolic pathways of florfenicol and its residual monitoring.

Figure 3-a and d showed that F-QuEChERS-AOAC 3202 method and acid hydrolysis method could completely extract FF and FFA from muscle and sebum. As the largest digestive gland of an organism, the liver had as many as 500 chemical reactions. As shown by Fig. 4-b, its matrix was particularly complex, while the kidney is the main excretory organ, which contains more metabolites than muscle and sebum. F-QuEChERS-AOAC 3202 method mainly extracts FF and FFA from tissues, while the acid hydrolysis method converts all the metabolites of FF into FFA, so this may be one of the reasons for the difference between the final results of the two extraction methods.

In brief, after taking FF continuously for 5 consecutive days, it had the largest residual amount and the fastest metabolic rate in the kidney. The residual amount of FF in the sebum was the lowest, and the metabolic rate was the slowest, too.

To sum up, compared with the acid hydrolysis method, the F-QuEChERS-AOAC 3202 method had certain credibility and reference value in the detection of FF residues and the calculation of drug withdrawal period in white feather chicken tissues. The FF pharmacokinetics showed variability due to physical, chemical, physiological and individual difference. To reduce the residues of FF and ensure the safety of chicken products, it is recommended that chickens taking oral FF should be subjected to a 14-days withdrawal time. Drug absorption after oral administration is a very complicated process, the clinical application and residue depletion of FF still need further indepth and comprehensive research.

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Compliance with ethical standards

Conflict of interest The authors declare to have no potential conflict of interest.

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