ORIGINAL ARTICLE

Quantification of residual enrofloxacin and ciprofloxacin in feathers of broiler chickens by high-performance liquid chromatography-fluorescence after oral administration of the drugs

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ABSTRACT

Enrofloxacin (ENR) and ciprofloxacin (CIP) are drugs used in poultry feeding. In general, feathers that are incorporated in the food chain as a protein source for animal feed, have residues of these drugs. In order to study the pharmacokinetic of ENR/CIP residues in feathers of broiler chickens, to calculate the waiting times for these drugs, before human consumption, we developed the present research. Feathers of broiler chickens were enriched with ENR/CIP. After adding acetone, the mix was agitated and centrifuged and supernatant evaporated under nitrogen. The dry residue was suspended in a tetrahydrofuran solution and the supernatant was injected into the chromatographic system for analysis. Animals showed high levels of ENR/CIP in their feathers after administration of 10 mg/kg enrofloxacin dissolved in drinking water for 5 days. Both compounds were detected in feathers during 9 days. The analytical method developed in this paper to determine ENR and CIP in feathers of broiler chicken showed good linearity, selectivity, accuracy and precision in the analysis conditions. This technique could have important applications in the studies on residues of ENR/CIP in feathers, since the effect of this component in animal diets has not been considered yet.

Key words: Enrofloxacin, quantification, quinolones

INTRODUCTION

Antibiotics used in animal feed have become a matter of major public health considering that their residues might be dangerous for both animals and humans due to toxicological effects and development of resistances.[1]

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Enrofloxacin (ENR), a drug of the quinolones group, is an antimicrobial commonly used in the treatment and prevention of poultry diseases.

Feathers are not used as a matrix for detection and quantification of veterinary medication. However, they are frequently incorporated as a protein source in diets of other animals, such as cattle, swine, trout, and salmons.

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Kinetic studies in edible tissues of poultry allow determining the waiting time of ENR and its metabolite ciprofloxacin (CIP) to ensure safe foods for human consumption. In general, the presence of residues in feathers that are incorporated in the food chain as a protein source in animal feed are not controlled and waiting times for ENR and CIP are not determined.

Since the human nutrition incorporates this animal's meat to the diet and considering that the antibiotics could be present in that meat, we initiated the present study with the following goals:

- To develop a method to quantify residues of ENR and its metabolite CIP in feathers of broiler chickens using high-performance liquid chromatography-fluorescence; this method has been specifically validated for feathers
- To study the pharmacokinetic of ENR and CIP residues in feathers of broiler chickens.

MATERIALS AND METHODS

The extraction procedure was a modification of the technique developed by San Martín *et al.*[2] Feathers of broiler chickens were homogenized and enriched with ENR and CIP. After adding acetone, the mix was vigorously agitated and centrifuged. The supernatant obtained was evaporated under nitrogen. The dry residue was resuspended in a tetrahydrofuran solution, and after a second centrifugation, the supernatant was injected into the chromatographic system [Figure 1].

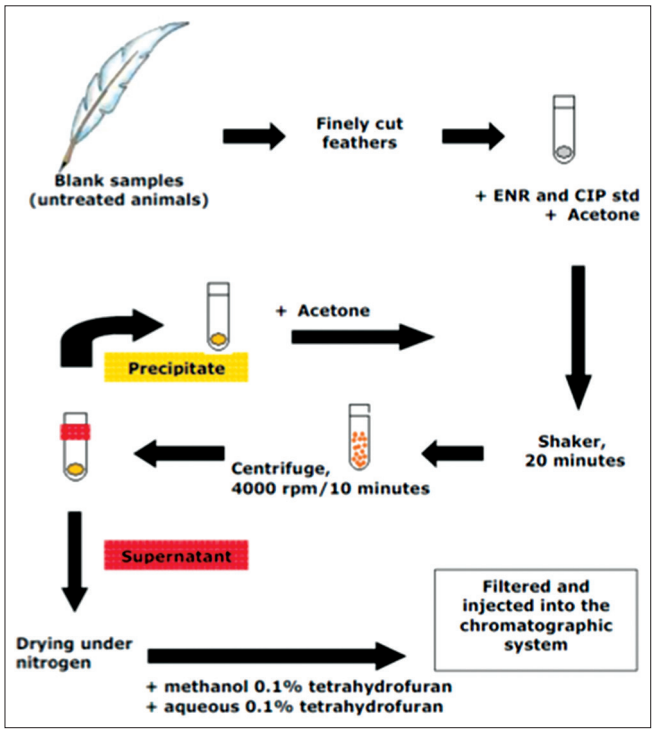

Figure 1: Extraction procedure ENR: Enrofloxacin, CIP: Ciprofloxacin

Chromatography conditions are mobile phase (water, acetonitrile, triethylamine), flow rate at 1,2 ml/min and column C18 (Phenomenex Luna).[3]

The validation procedure was performed following Commission Decision 2002/657/EC of the UE.[4]

Validation parameters included linearity, selectivity, specificity, limit of detection, limit of quantification, accuracy, and precision intra- and inter-day.

Three-week-old broiler chickens were given 10 mg of ENR/kg of body weight for consecutive 5 days. ENR was dissolved in drinking water.

Samples of feathers from 10 chickens were randomly taken on the day 0, 1, 2, 3, 4, 5, 7, and 9, after the last administration of the antimicrobial. The feathers were prepared, labeled, and stored until extraction and chromatographic assay. The extraction procedure and chromatography conditions were described in another summary submitted in the present event.

Calibration standards

The calibration curve was linear in the range of concentrations assessed with a correlation coefficient of 0.9974 for ENR and 0.9986 for CIP. The limits of detection were 0.062 µg/g and 0.040 µg/g and the quantification limits were 0.080 µg/g and 0.050 µg/g for ENR and CIP, respectively [Figures 2 and 3].

The specificity of the method was demonstrated by the analysis of blank samples when compared with standards of ENR and CIP.

Inter- and intra-day accuracy expressed by the variation coefficient was 3.88 at 7.47 in ENR and 7.07 at 2.73 in CIP. Recoveries were 94.57 at 100% and 93.33 at 100% for ENR and its metabolite CIP, respectively [Tables 1 and 2].

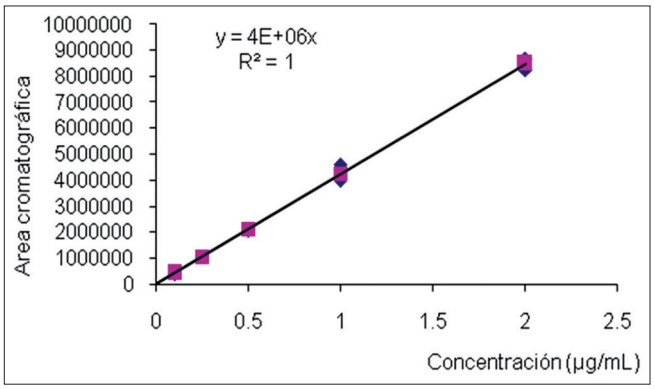

Figure 2: Calibration curve of standard enrofloxacin

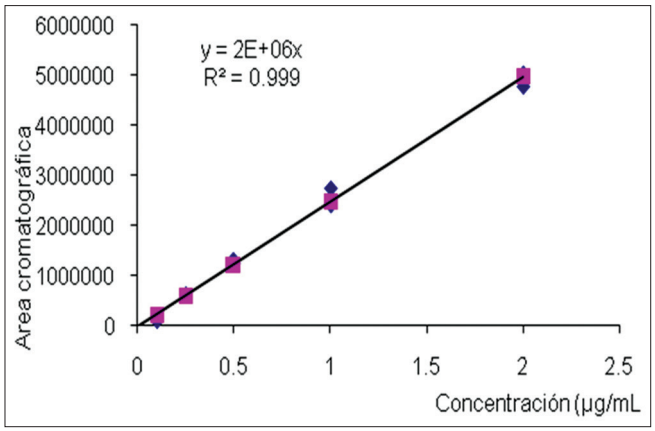

Figure 3: Calibration curve of standard ciprofloxacin

Table 1: Recovery of enrofloxacin in chicken feathers enriched with 0.1, 0.25, and 2 µg/g

Desired	Percentage of recovery		
	Day 1	Day 2	Day 3
0.1	110.00	100.00	90.00
0.25	96.00	96.00	92.00
2	98.00	97.00	97.00
Mean value	101.33	97.67	93.00
SD	7.57	2.08	3.61
CV	7.47	2.13	3.88

SD: Standard deviation, CV: Coefficient of variation

Table 2: Recovery of ciprofloxacin in chicken feathers enriched with 0.1, 0.25, and 2 µg/g

Desired	Percentage of recovery		
	Day 1	Day 2	Day 3
0.1	110.00	100.00	90.00
0.25	96.00	92.00	92.00
2	100.00	95.00	95.00
Mean value	102.00	95.67	92.33
SD	7.21	4.04	2.52
CV	7.07	4.22	2.73

SD: Standard deviation, CV: Coefficient of variation

RESULTS

A group of 10 experimental animals were included in the experience. These specimens showed high levels of ENR and CIP in their feathers after administration of 10 mg/kg ENR dissolved in drinking water for 5 days.

Although the antibiotic residue was similar by the end of the 1st day, CIP has shown slightly higher levels than ENR at the day 3 and 5 after inoculation.

Both compounds were detected in feathers, during 9 days [Figure 4]. After the 10th day, it could be said that antibiotic residue and that after this day samples were clean of drugs residues and suitable for consumption.

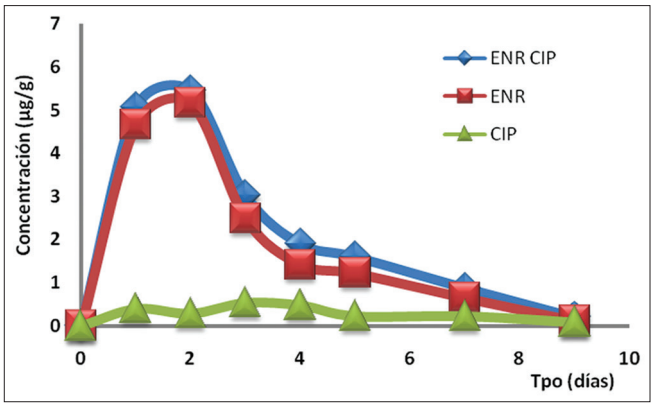

Figure 4: Blood level of drugs according to time administration

DISCUSSION

Feather flour is a potential source of drug residues that can reach humans through the food chain when it is incorporated as a component in the diet of animals for human consumption. The feathers analyzed in the present study showed a high concentration of ENR and CIP. This finding cannot be explained by blood contribution to this tissue since feather vasculature reaches only the lower portion of the calamus. Being penetrating substances with high solubility, it is possible to hypothesize that a source of feather contamination could be the secretion from the uropygial gland that reaches the feathers via grooming behavior.[5]

Therefore, it becomes an extremely important decision to identify antibiotic residues in food destination to human consumption, and also to determinate the removal time period for use as food feathers of other animals, in order to avoid antibiotic resistance in feed chain.

On the other hand, this study exposes a new and effective technique to analyze still not so well-studied tissue in order to give new options for future antibiotics research in birds.

In addition, analyzing withdrawal time for slaughter according to different trademarks, the period would be between 8 and 10 days and this time will be enough to eliminate more than 95% in birds' feathers.

CONCLUSIONS

The novelty of the method is based on the possibility of a real and effective dosage form in the feathers. Besides, it opens the debate on the awareness of the possibility of antibiotic resistance if the time of withdrawal of the antibiotic is not respected even if that food is not destined for human consumption, since it becomes a part of the animal feed chain.

Therefore, the analytical method developed to determine ENR and CIP in pens of broilers showed good linearity, selectivity, accuracy, and precision.

This technique could have important applications in the studies on residues of ENR and CIP in feathers since the effect of this component in animal diets has not been considered yet.

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Conflicts of interest

There are no conflicts of interest.

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