MULTI-RESIDUES DETERMINATION OF THREE FLUOROQUINOLONES IN CHICHEN BY POST-COLUMN DERIVED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH TERBIUM-SENSITISED FLUORESCENCE

K.Z. Qi [1*], K.S. Peng [1], L.Q. Zhu[2], G.R. Sun[1], Z.H. Shi [1], J. Tu[1]

[1] (College of Animal Science and Technology, Anhui Agriculture University, Hefei 230036)
[2](Anhui Veterinary Work Station, Hefei 230022)

[*]E-mail: jwcqkz@ahau.edu.cn

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Introduction

Fluoroquinolones (FQs) are widely used in human and food-producing animals for treatment and prevention of diseases. Some FQs (e.g. enrofloxacin (ENR)) have been specifically developed for veterinarian applications , while others like ciprofloxacin (CIP) and norfloxacin (NOR) are restricted to human treatment. However, ENR, CIP and NOR are conventional drugs in poultry industry in China. The misuse of these antibacterial agents may give rise to public health (e.g. Photoallergy, antibiotic resistance, etc.), environmental and commerce problem (Li et al.,2002).

Using of FQs in food-producing animals was prohibited in USA, and the residues of FQs in animal products must be monitor in many countries, e.g. the European Union (EU) countries, China and Japan, etc. Current standard method of FQ analysis in biological matrices is based on high performance liquid chromatography (HPLC), with fluorescence detection. Terbium(III)-sensitised fluorescence with FQs was mainly used to detect the trace terbium in mineral (zhang et al.,1999). However, only a few paper have focused on the determination of FQ residues in foodstuffs of animal origin by HPLC with Tb^{3+}- sensitised fluorescence, especially in multi-residue determination of FQ in chicken. This paper reports the development of a high sensitive HPLC method with Terbium sensitized fluorescence for the simultaneous analysis of three FQ (ENR, CIP and NOR) residues in chicken.

Materials and Methods

Three FQs (＞99.0％) were provided by china institute of veterinary drug control (Beijing, china), other reagents (HPLC-grade or A.R.) were from Fisher or Sigma(USA). The chromatographic system consisted of a HP-1100 series high performance liquid chromatograph from Agilent Technologies (Palo Alto, CA, USA).

The conditions of HPLC and post-column derivation using Tb^{3+} were optimized according to the methods of zhang et al.(1999) and Schneider and Donoghue (2002). Chromatographic separation of the FQs was performed on an Hypersil BDS-C_{18} (Agilent Technologies, USA) maintained at 40□, with the mobile phase of 0.05mol/L acetic acid/sodium acetate (pH6.0)-acetonitrile (89:11,V/V). Isocratic mode at a flow-rate of 0.8 ml/min was used for the separation of analytes. Aliquots of 20 μl were injected into the column. The Post-column derived reagents of 8×10^{-5}mol/L Tb^{3+} onflow Waters 2695(Waters,USA) maintained at 40□ at a flow-rate of 0.5 ml/min. The fluorescence excitation/emission wavelengths were 271/545 nm for the analysis of the FQs.

The Sample preparation was based on the method described in the bulletin (No.236) of China Ministry of Agriculture. SPE clean-up of the sample was automated, using a Supelclean LC-18(Supelco,USA). The evaluation of recovery, RSD, accuracy, and linear range for quantitative analysis were followed the previous

description by Li et al.(2002).

Results and Discussion

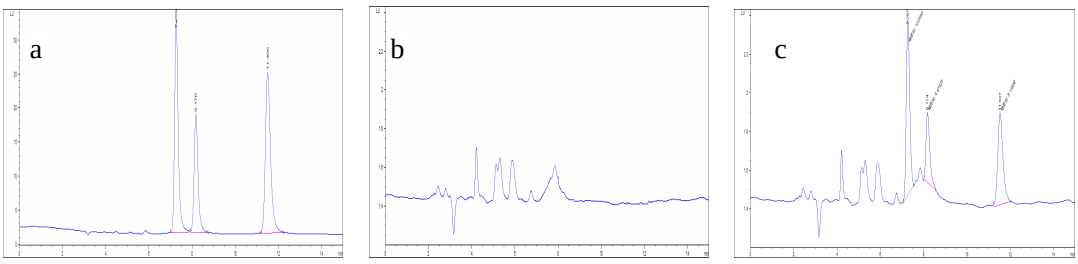

Fig.1 Post-column Derived Chromatograms of 3 FQs （a）the standard solution, （b）the blank muscle, （c）the spiked blank muscle

Three fluoroquinolones were separated on C18 column with the optimized conditions of HPLC and post-column derivation, finally were detected by fluorescence detector (e_x=271nm, e_m=545nm) (**Fig.1**). Three fluoroquinolones were recovered from chicken generally in the range of 66.3～88.0% with added levels of 1.0～100.0 ng/g. The average recovery of ciprofloxacin, norfloxacin and enrofloxacin was 77.65％、79.70％ and 81.40％ in broiler chicken muscle tissues respectively, and all RSDs were less than 3.0% (**Tab.1**). The linear range for quantitative analysis was between 0.1 and 100 ng/mL(g), and the linear regression equation of standard curve for CIP, NOR and ENR were A_{CIP}＝59.81C+6.38 (r=0.9998),A_{NOR}＝87.33C+3.81(r=0.9996), and A_{ENR}＝108.47C+9.39(r=0.9998), respectively. All RSDs of retention time and spike arear were less than 3.0% (**Tab.2**). The detection limit of 0.05 ng/mL(g) for ciprofloxacin and norfloxacin and 0.08ng/mL(g) for enrofloxacin were less than approximate 50 times of the reported lowest detection limit by LC-MS (Schneider & Donoghue,2002).

Tab.1 The recovery of three fluoroquinolones in chicken spiked from 1.0 to 100.0 ng/g

Drug	CIP	NOR	ENR
Average Recovery（%）	77.65±3.90	79.70±10.01	81.40±4.51
RSD（%）	5.02	12.57	5．54

Tab.2 The accuracy of post-column derivation HPLC with terbium sensitized fluorescence

Drug	Concentration added（ng/ml）	Retention time（min）		Area	
		Average retention time	RSD（%）	Average area	RSD（%）
NOR	1.0	7.25±0.142	1.96	8.78±0.1940	2.21
CIP	1.0	8.16±0.084	1.03	12.72± 0.1615	1.27
ENR	1.0	11.49±0.168	1.46	19.63±0.2984	1.52

Conclusions

Our research obtain a novel more sensitive detection method for multi-residues of CIP, NOR and ENR in chicken, based on the effect that Tb^{3+} can sensibilize fluorescence of fluoroquinolones. With the optimal chromatographic condition, three fluoroquinolones were separated well, the detection limits were less than 0.1ng/g, the recoverys were larger than 70％, and RSDs were less than 15％. This novel method satisfied the requiment of the detection of multi-residues of FQs in chicken.

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