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# Quantification of antibiotic residues in raw and layers hen eggs by rp-hplc

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**Abstract**---Antibiotics (Oxytetracycline & Enrofloxacin) residual levels in raw and layered samples are to be quantified using RP-HPLC. The antibiotic (oxytetracycline and enrofloxacin) residual levels in raw and layers hen egg samples were evaluated qualitatively by high performance liquid chromatography in this investigation, which used one hundred randomly selected samples (HPLC). C18 (Hypersil ODS-BPS, 250 4.6mm; 5) was used for the separation at a flow rate of 1 ml/min in a mobile phase of 0.1 percent formic acid: acetonitrile (50:50, v/v). At a detection wavelength of 350 nm, the residues were

measured. Found oxytetracycline residues, which indicate widespread usage of antibiotics on farms and a lack of application of required withdrawal durations, were detected in samples. Because of this, the antibiotic residues found in broiler hen eggs have been reduced. Restrictive methods and stricter restrictions should be implemented to prevent the presence of abuse residues prior to marketing, according to these findings.

**Keywords**--Antibiotic, oxytetracycline, enrofloxacin, Maximum residue limit, HPLC.

## Introduction

The use of antibiotics in chicken production has made it possible for consumers to acquire high-quality meat and eggs at an affordable price. Despite the fact that these applications are beneficial to all parties involved, the edible tissues of fowl contained dangerous quantities of medication residues.<sup>1-6</sup> For the treatment of a variety of ailments, as well as to increase feed efficiency and stimulate growth, they were widely employed in the chicken business. As a result of this widespread usage of antibiotic, there may be residues and allergic responses in meals. The fungus *Streptomyces rimosus* produces oxytetracycline, a naturally occurring tetracycline chemical.<sup>7-11</sup> Due to its great water solubility, it is excreted almost entirely in its parent form by the target species. Bactericidal fluoroquinolone of second generation, enrofloxacin. Ciprofloxacin, the primary active metabolite formed in the liver after oral administration, is a well-absorbed and widely dispersed drug in the body's tissues.

Oxytetracycline and Enrofloxacin can be detected in hen eggs using a variety of chromatographic approaches, including diode array and fluorescence detection and liquid chromatography–mass spectrometry (LC–MS).<sup>12-15</sup> It is currently unknown how to simultaneously analyse OXY and ENR in eggs by RP-HPLC.<sup>[16-18]</sup> A specific and sensitive approach for the simultaneous detection and confirmation of OXY and ENR residues in hen eggs is required. The study's goal is to use RP-HPLC to measure antibiotic residual levels in raw and broiler meat (Oxytetracycline and Enrofloxacin).

## Instrumentation

Hypersil ODS-BPS (250 × 4.6 mm, 3.5) stainless steel column was used for method development and validation. There is a UV detector in the HPLC system (Agilent 1200 Infinity series) as well as an HPLC pump (Agilent 1200 Infinity series). Ezichrome Elite Compact was the programme of choice.

## Chemicals

Oxytetracycline & Enrofloxacin (Hetero labs, Hyderabad), Formic acid AR (Qualigens), HPLC grade acetonitrile and grade water (Merck India), Hydrochloric acid (Merck India), (Merck India).

### **Chromatographic conditions**

One millilitre per minute of C18 (Hypersil ODS-BPS; 250 × 4.6mm; 3.5) in acetonitrile with 0.1 percent formic acid was used in the mobile phase. At 350 nm, the residues were analysed for their concentration. It took 10 minutes to complete the analysis at a flow rate of 1 mL/min. It was set to the ambient temperature in the column oven. The injection volume of the sample into the HPLC was increased to 20 L in order to improve the chromatography. OXY and ENR had retention times of 2.7 0.02 min and 5.2 0.02 min, respectively.

### **Standard Solutions**

The individual standard solutions of the pure chemicals were prepared by dissolving accurately 10 mg OXY and ENR in a 10 mL volumetric flask using acetonitrile. Intermediate and calibration solutions were prepared by with mobile phase water prior to use. The solutions were stored in refrigerator for further use.

### **Sample extraction**

The hen eggs samples (20 of each) were purchased from supermarket and samples were finely sliced and homogenized for 2 min. To each 5g of sample 20 ml of 1M HCl: acetonitrile (1:1) was added and the mixture sonicated followed by vortexed for 10 min. The tube was centrifuged for 5 min at 4000 rpm and supernatant was filtered through a 0.45 µm nylon filter. After filtering supernatant was evaporated at 120° C. The residue was dissolved in mobile phase and 20 µl of solution injected into HPLC for analysis.

### **Method Validation**

The method was validated according to the Decision 2002/657/EC under Council Directive 96/23/EC and the analytical parameters were specificity, linearity, LOD, LOQ, accuracy and precision.

### **Specificity**

Ten eggs samples from various sources were analysed to examine probable endogenous interferences in samples to test the specificity of the proposed HPLC approach. To ensure that no interferences occurred around the retention time of the investigated residues, the sample preparation and clean-up procedures as well as the chromatographic settings were optimised.

### **Linearity**

The method's linearity was verified using a calibration curve constructed from triplicate analyses of the matrix's five concentration levels. Oxy and ENR were spiked at 0.05, 0.1, 0.2, 0.4, and 0.6 g/ml. Peak areas were used to quantify the reactions.

### **LOD and LOQ**

Analytical extracts of blank eggs samples were used to compute the LOD and the LOQ, respectively, based on a threefold change in the signal-to-noise ratio (S/N=3:1).

### **Accuracy and precision**

Spiked analytes were spiked into eggs, and their average recoveries were used to measure the method's accuracy. Over a period of five days, the average recoveries were measured in eggs that had been spiked at three different levels. By comparing the peak regions of measured and spiked concentrations, the recoveries were computed. The RSD (relative standard deviation) was used to define the precision and was evaluated in terms of intra-day and inter-day accuracy. Analysis of spiked samples in five repetitions on the same day yielded intra-day precision at three fortification levels. For the same fortification levels in five replicates on five consecutive days, inter-day precision was measured.

### **Results and Discussion**

An evaluation of the accuracy, precision, and linearity of the high-performance liquid chromatographic method has been completed, and it has been found to be more convenient and effective for determining the identification of ENR and OXY in hen eggs.

With an ENR/OXY correlation coefficient of 0.998 and 0.996, this new approach was shown to be easy while also being exact, accurate, specific and selective throughout a concentration range of 0.05-0.9g/ml. A chromatographic technique that can rapidly analyse a large number of samples while using less solvent (1mL/min) and a shorter analytical run time (10 minutes) has been developed. Figures 1-2 and Table-1.0 show the findings.

Table 1  
Precision and accuracy data

Level of % Recovery	Mean % Recovery	Intra day % RSD	Inter day % RSD
80	99.99	0.82	0.827
100	100.91	1.77	1.75
120	99.69	0.44	0.44

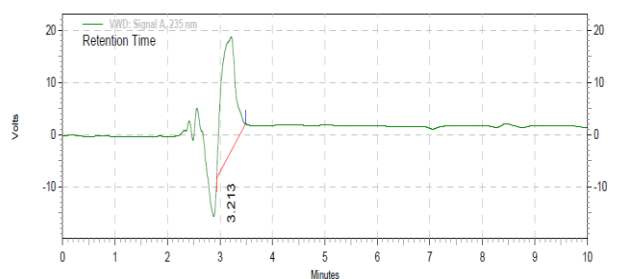


Figure 1: Chromatogram blank sample of hen egg sample

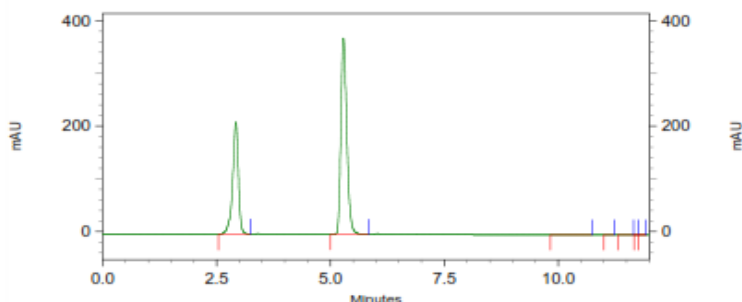


Figure 2: Chromatogram OXY and ENR residues in hen egg sample

A HPLC assay was used to measure oxytetracycline and enrofloxacin residues, and the positive samples were the outcome. Each case yielded a total of twenty egg samples for analysis. Layers hen egg samples have mean residual levels of enrofloxacin of 0.635  $\mu\text{g/g}$  and oxytetracycline of 0.526  $\mu\text{g/g}$ . In domestic egg samples, the residual enrofloxacin concentration is 0.089  $\mu\text{g/g}$  and the oxytetracycline concentration is 0.062  $\mu\text{g/g}$ . A visual representation of the findings may be found in Figures 3-4 and Table 2.0.

Table 2  
Antibiotic residue levels in raw and layers hen eggs

Antibiotic	Sample number	Mean residue level ( $\mu\text{g/g}$ )				MRLs ( $\mu\text{g/g}$ ) according to EC (2010)
		Un-boiled domestic hen egg ( $\mu\text{g/g}$ )	Un-boiled layers hen egg ( $\mu\text{g/g}$ )	Boiled domestic hen egg ( $\mu\text{g/g}$ )	Boiled layers hen egg ( $\mu\text{g/g}$ )	
Enrofloxacin	20	0.089	0.635	0.064	0.178	0.1
Oxytetracycline	20	0.062	0.526	0.055	0.159	

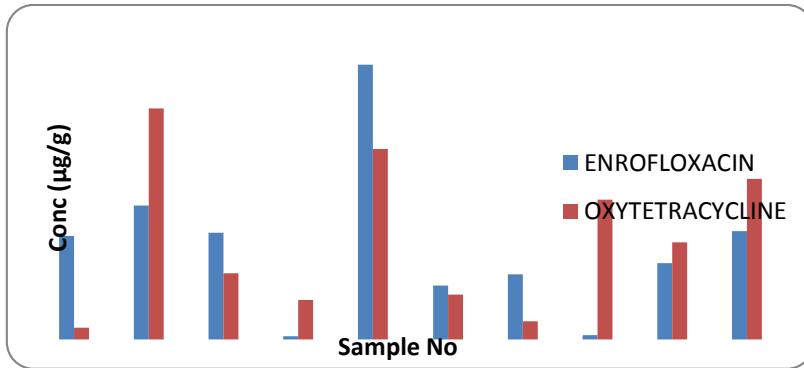


Fig. 3: Detectable residue levels (µg/g) of Enrofloxacin and Oxyteracycline in layers eggs

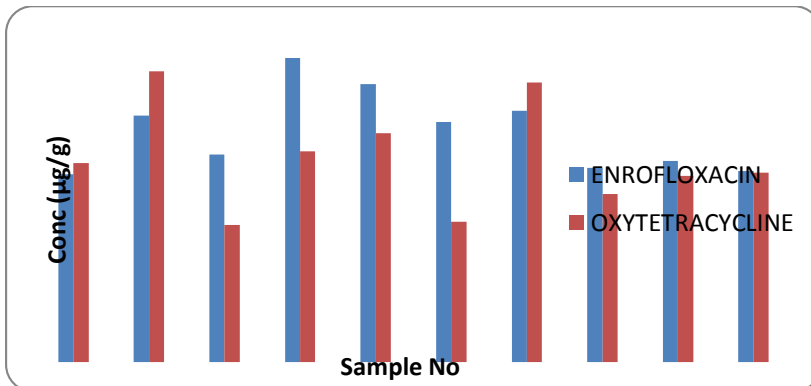


Fig. 4: Detectable residue levels (µg/g) of Enrofloxacin and Oxyteracycline in domestic eggs

For oxytetracycline and enrofloxacin, the concentration of antibiotic residues in layers hen eggs decreased by 0.178 and 0.152 micrograms/gram, respectively, when the eggs were boiled. Figure-5 shows the results.

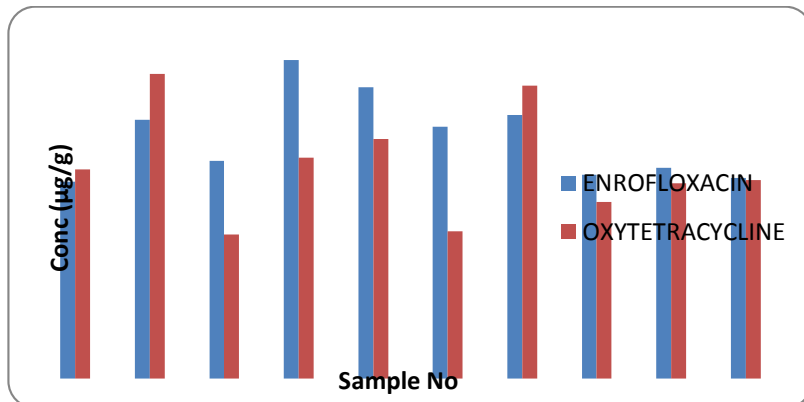


Fig. 5: Detectable residue levels (µg/g) of Enrofloxacin and Oxyteracycline in boiled layers eggs

## Conclusion

MRLs for oxytetracycline and enrofloxacin in the edible tissues of food-producing animals were set at 0.1 g/g, according to the European Union commission (EC, 2010). This may be due to a lack of application of the appropriate withdrawal time and a lack of attention given to the withdrawal period of eggs. Animal products that contain substantial levels of antibiotic residues may also cause intestinal flora disorders in humans. There has to be a greater emphasis on the control of the use of pharmaceuticals in the poultry business and the inspection of broilers prior to marketing, as outlined in this report.

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