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Effect of boiling on oxytetracycline and Amprolium drug residues in chicken meat

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Abstract

The indiscriminate use of antibiotics in livestock sector including poultry production has increased many folds in India. The improper use of the drug may accumulate in the edible foods of animal origin, which may results the potential health risks to the consumers. In the present study, the evaluation of boiling effect on experimentally spiked chicken meat samples with known concentrations for two drug viz. Tetracycline and Amprolium. These samples were analysed for their residual concentration by using High Performance Liquid Chromatography (HPLC). The results showed the significant reduction in residual concentration on boiling for both drug, oxytetracycline by 75.46–81.47% and amprolium by 84.43–85.49%, respectively. The present findings demonstrate that boiling is an effective household processing method for reducing antibiotic residues. Proper cooking conditions, including adequate temperature and duration, play a crucial role in minimizing residue levels and enhancing the safety of poultry meat for consumers.

Keywords: HPLC, Oxytetracycline, Amprolium, MRL

1. Introduction

Chicken meat is one of the most widely consumed animal protein sources globally, and its consumption has risen considerably over recent decades in many countries. In poultry production systems, veterinary drugs are routinely used for therapeutic purposes, disease prevention, and growth promotion (Dong *et al.*, 2009) ^[1]. Consequently, the presence of antibiotic residues in food products exceeding maximum permissible limits has been reported worldwide by various regulatory and public health agencies (Singh *et al.*, 2014) ^[2].

Since, foods of animal origin are generally consumed after cooking, it is essential to evaluate the impact of cooking on the stability and degradation of antibiotic residues. Such information is necessary to accurately estimate consumer exposure to both the parent compounds and their degradation products. Despite its importance, limited research has been conducted on the occurrence and behaviour of antibiotic residues in cooked foods (Lolo *et al.*, 2006; Javadi *et al.*, 2011) ^[3, 4].

2. Materials and Methods

2.1. Sample Collection

A total number of 120 chicken meat samples were collected from poultry farms and retail market from Jabalpur district of Madhya Pradesh.

2.2 Chemicals and reagent

Standards of Oxytetracycline dihydrate and Amprolium were obtained from Sigma Aldrich. HPLC grade methanol, acetonitrile and oxalic acid were obtained from Merck. Analytical grade Na₂HPO₄, EDTA, heptansulphonic acid and citric acid obtained from Merck, Germany.

2.3 Standard preparation

Standard solution was prepared by dissolving 1mg of each antibiotic standard powder in 1ml

of mobile phase. Stock standard solutions were filtered and stored at 4 °C. Working standards were prepared by serial dilution.

2.4 Sample preparation for oxytetracycline

Chicken meat samples (2 g) were fortified ($n = 10$) with known concentrations of oxytetracycline drug in five samples each as 100 µg/g and 50 µg/g. After fortification, the samples were allowed to stand for 30 minutes to facilitate proper absorption of the antibiotic and were subsequently boiled at 100 °C for 30 minutes in a water bath. After boiling the, samples were homogenized, and residues were extracted using McIlvaine buffer–EDTA, followed by centrifugation at 2500 rpm. The extraction was repeated, and supernatants were pooled and re-centrifuged. The clear extract was purified using solid-phase extraction after conditioning the cartridge with HPLC-grade water. Oxytetracycline was eluted with methanolic oxalic acid, diluted with water, vortexed, and 10 µL of the final extract was injected into the HPLC system for analysis (Salehzadeh *et al.*, 2006) [5].

2.5 Sample preparation for amprolium

Chicken meat samples (2 g) were fortified ($n = 10$) with known concentrations of amprolium drug in five samples each as 100 µg/g and 50 µg/g. After fortification, the samples were allowed to stand for 30 minutes to facilitate proper absorption of the antibiotic and were subsequently boiled at 100°C for 30 minutes in a water bath. After boiling, the samples was homogenized, extracted with 5 mL acetonitrile, and centrifuged at 3500 rpm for 15 minutes. The supernatant was diluted with water and applied to a preconditioned C18 SPE cartridge. Amprolium was eluted with methanol, evaporated to dryness, reconstituted in methanol, filtered (0.45 µm), and 10 µL was injected into the HPLC system for analysis (Kimera *et al.*, 2015) [6].

2.6 Chromatographic Conditions of oxytetracycline

The analysis was performed using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) equipped

with a solvent delivery pump and a UV detector. Separation was achieved on a reversed-phase C18 column. The mobile phase consisted of 0.03 M oxalic acid, methanol, and acetonitrile in the ratio of 60:20:20 (v/v/v), and was delivered under isocratic conditions. The flow rate was maintained at 1 mL/min. Detection of the analyte was carried out at a wavelength of 360 nm using a UV detector. A sample injection volume of 10 µL was used, and the total run time for each analysis was 5 minutes (Gupta *et al.*, 2014; Ibrahim *et al.*, 2016) [7, 8].

2.7 Chromatographic Conditions of amprolium

The HPLC system was equipped with photodiode array UV-vis detector. Chromatography conditions were maintained as with slight modification. Particle separation was done using hypersil column with C18 selectivity (Supelco, USA, column dimensions: 15 cm x 4.6 mm, particle size: 1.9 µm) and the temperature of column was set at 25°C. The mobile phase comprised of methanol and water containing 0.5% of heptanes sulfonic acid sodium respectively in 60:40(v/v) ratio with flow rate adjusted at 0.8ml/min 10 µL treated samples was required for injection (Ali *et al.*, 2017) [9].

3. Results and Discussion

3.1 Effect of boiling on residual concentration of oxytetracycline in chicken meat samples

The thermal stability of oxytetracycline was evaluated using experimentally fortified chicken meat samples. The results showed the significant reduction of oxytetracycline residues in the fortified samples. Residue level was decreased by 81.87% (Table 01) in samples fortified at 100 µg/g and by 75.46% in samples fortified at 50 µg/g. However, both fortified concentrations were reduced significantly ($p < 0.01$). The mean residual concentrations after boiling were 18.13 ± 0.62 µg/g and 12.27 ± 0.94 µg/g, respectively. The high calculated t-values further confirmed the significant effect of boiling in reducing oxytetracycline residues in chicken meat samples.

Table 1: Effect of boiling on residual concentration of oxytetracycline in experimental chicken meat samples

Samples	No. of samples (n)	Initial concentration of fortified samples (µg/g)	Mean residual concentration after boiling (µg/g± SE)	Percent reduction	t-values
Chicken meat	5	100	18.13 ± 0.62	81.87	131.9**
	5	50	12.27 ± 0.94	75.46	40.14**

** Significant ($p < 0.01$)

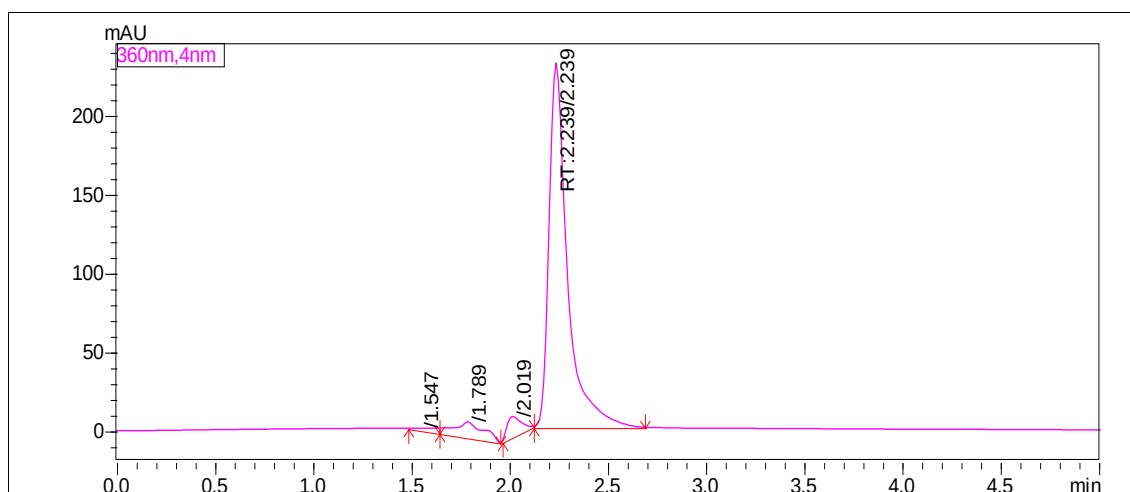


Fig 1: Standard chromatogram of Oxytetracycline

The present findings were consistent with earlier reports demonstrating the effect of heat treatment on oxytetracycline residues, reported significant reductions in oxytetracycline

residues in beef after boiling, with reductions reaching up to 90.9%.^[10]

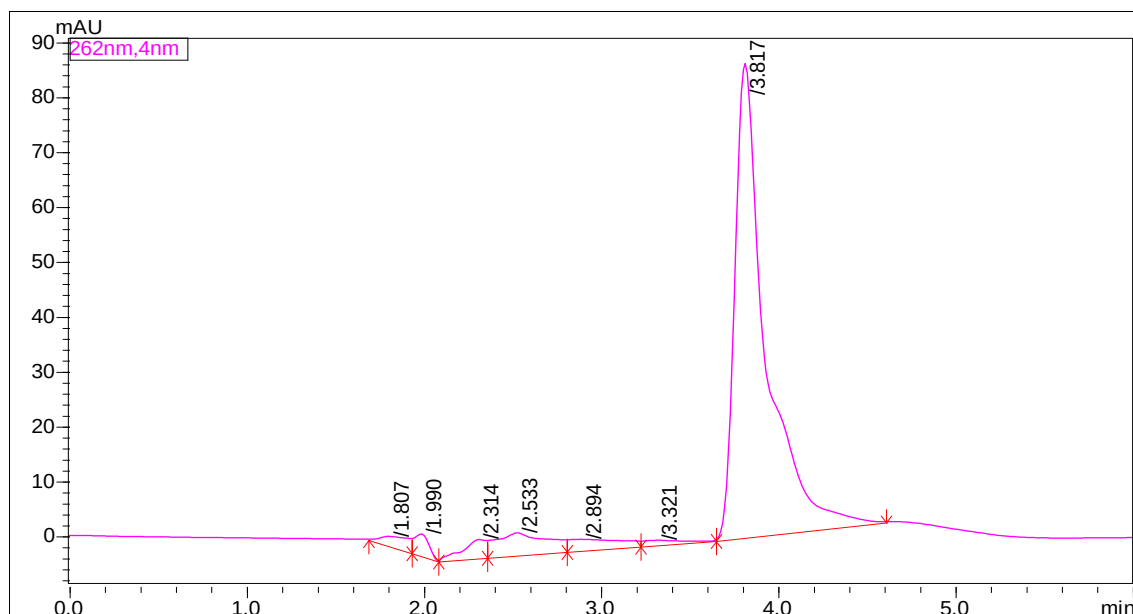


Fig 2: Standard chromatogram of Amprolium

Table 2: Effect of boiling on residual concentration of Amprolium in experimental chicken meat sample

Samples	No. of samples (n)	Initial concentration of fortified samples (µg/g)	Mean residual concentration after boiling (µg/g± SE)	Percent reduction	t-values
Chicken meat	5	100	14.51 ± 0.78	85.49	110.02 **
	5	50	7.58 ± 0.34	84.83	124.33 **

** Significant ($p < 0.01$)

3.2 Effect of boiling on residual concentration of amprolium in chicken meat samples

The thermal stability of amprolium was also evaluated using experimentally fortified chicken meat samples. The results showed the significant reduction of amprolium residues in the samples, with 85.49% and 84.83% (Table 02) reduction observed at initial concentrations of 100µg/g and 50µg/g, respectively. The reductions were statistically significant ($p < 0.01$), indicating that boiling is an effective household processing method for lowering amprolium residues in chicken meat. The decrease in residue concentration may be attributed to thermal degradation of the drug and partial diffusion into the cooking medium.

4. Conclusion

Boiling treatment was found to be highly effective in reducing both oxytetracycline and amprolium residues in chicken meat samples. A significant decline in oxytetracycline levels was observed, with reductions of 81.87% and 75.46% at initial concentrations of 100 µg/g and 50 µg/g, respectively. Similarly, amprolium residues showed even greater reductions of 85.49% and 84.83% at the same fortification levels. In all cases, the reductions were statistically highly significant ($p < 0.01$), confirming the strong impact of thermal processing. The decrease in residue concentrations can be attributed to heat-induced degradation of the drugs as well as their partial transfer into the cooking medium. Overall, the findings demonstrate that boiling, as a common household cooking method, plays a crucial role in lowering antibiotic residues in chicken meat, thereby enhancing food safety and reducing potential health risks to consumers

Conflict of Interest

Not available

Financial Support

Not available

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