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Liquid chromatographic assay of edible tissues of pig (*Sus scrofa*) for chlortetracycline residue

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Abstract

The present study was conducted for liquid chromatographic assay of edible tissues of Pig (*Sus Scrofa*) for Chlortetracycline (CTC) residue. Representative edible pig tissue samples comprising of kidney, liver and muscle were collected from markets and roadside pork stalls located in and around Guwahati city of Assam. The samples after collection were preserved at -20 °C and then screened by High Performance Liquid Chromatography with UV-Vis Detector. CTC residue was extracted with EDTA-McIlvaine buffer. Solid phase extraction clean up was done with SPE C₁₈ cartridge. Recovery was more than 90%. About 2.67% of the samples were detected to be positive for trace residues of CTC. The detected residue levels of CTC in the edible tissues of Pig were below the Maximum Residue Limit (MRL). It may be concluded from the present study that using liquid chromatographic technique the samples were precisely detected with CTC residues.

Keywords: Chlortetracycline, Guwahati, HPLC, MRL, Pig

1. Introduction

Antibiotics are freely used as growth promoters and for treatment of animal diseases without adequate knowledge. The indiscriminate use of antibiotics pose a considerable risk due to the presence of residues in meat meant for human consumption. Thus, awareness and need for regular screening of marketed meat samples is imperative in the interest of trade and consumers. As a result of which the presence of drug residues becomes quite apparent in meat samples meant for human consumption. Undesirable levels may lead to many health hazards in human. The possible effects include toxic or allergic reactions, development of bacterial resistance and disturbance of normal intestinal microflora composition [1]. For these reasons, the control of antibiotic residues in edible animal tissues is mandatory. To protect humans from harmful effects of veterinary drug residues in animal-derived food sources, the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have set standards for maximum residue limits in foods. These limits apply to the parent drug or chemical and its metabolites that may accumulate and be deposited or stored within the cells, tissues or organs following administration of the compound. The acceptable Maximum Residue Limits (MRL) for tetracycline-based compounds, including chlortetracycline in swine kidney, muscle and liver are set at 1.2, 0.2 and 0.6 µg/g respectively [2].

Pigs (*Sus scrofa*) have been an integral component of farming system and support a large rural population of Assam. The North-Eastern States of India is characterized by a high proportion of tribal people for whom pig rearing is integral to their way of life. Pig meat (pork) is considered as an important food item in Assam.

Chlortetracycline (CTC) is routinely used in farm animals for the treatment, prevention and control of infectious diseases. Traces of CTC may be present as residue after slaughter in trace amount. CTC residues in meat may cause allergic reactions in individuals and may produce antibiotic resistance [3]. Kidney, Muscle and Liver are the main sites for the deposition of CTC residue. Thus, the people of Assam may risk the chance of intake of CTC left as residues in pork. Thus the present study was undertaken to determine CTC residues in edible tissue (pork) samples of Pig (*Sus scrofa*) using a High Performance liquid Chromatographic technique.

2. Materials and Methods

The present study was part of postgraduate research work conducted at the Department of Pharmacology and Toxicology during the year 2010-11.

2.1 Sample collection

Representative edible pig tissue samples comprising of kidney, liver and muscle were collected from markets and roadside pork stalls located in and around Guwahati city of Assam (Table 1).

Table 1: Collection of edible tissue samples of Pig

Place	Kidney	Muscle	Liver	Total
Nine mile	06	06	06	18
Khanapara	10	10	10	30
Beltola	35	35	35	105
Six mile	10	10	10	30
Dispur	07	07	07	21
Ganeshguri	10	10	10	30
Zoo road	07	07	07	21
Noonmati	07	07	07	21
Ulubari	08	08	08	24
TOTAL	100	100	100	300

2.2 Preparation of sample

The fascia and fat of pork were removed and then cut into small pieces. About 10 g of the sample was taken in a blender and to it added equal volume of distilled water. 10 g of each blended sample was transferred to centrifuge tube. After few minutes 10 ml of acetonitrile was added. The sample was ultrasonicated and left undisturbed for 10 min. The samples were centrifuged and the collected supernatant was filtered. The filtrate then was passed through C₁₈ polymeric cartridge after which it was further filtered using 0.22 µm membrane filter.

2.3 Chromatographic analysis:

The chromatographic analysis was conducted as per the method of Gogoi *et al.* [4] Residue in samples was detected and quantified using HPLC system equipped with UV-Vis Detector and RP C₁₈ column. The mobile phase used was a mixture of acetonitrile, methanol and 0.01 M oxalic acid in the ratio of 1:1.5:2.5 v/v/v (pH 2.0). Wavelength of the detector was set at 350 nm and the flow rate was maintained in an isocratic mode at 0.6 ml/min. The extraction of the samples was done by Solid Phase Extraction cleanup with a SPE C₁₈ polymeric cartridge.

3. Results and Discussion

Calibration curve of Chlortetracycline having Coefficient of determination of 99.9% was obtained (Figure 1). Recoveries of CTC in the tissue were more than 90%. Similar recoveries

were reported by Cinquina *et al.* [5], Biswas *et al.* [6] and Shahid *et al.* [7]. Overall, 300 samples of pig kidney, muscle and liver were collected and analyzed for the presence of CTC residues. After HPLC screening, 2.67% of the samples were detected to be positive of CTC residues. About 3.0% kidney, 1.0% muscle and 4.0% liver samples were detected with trace residues of CTC which were well below the Maximum Residue Limit (MRL) as shown in Table 2. As shown in Figure 2, CTC residues were detected in 3.80% samples from Beltola, 3.33% from Sixmile, 6.67% from Ganeshguri and 4.76% from Noonmati. Samples collected from Nine mile, Khanapara, Dispur - Supermarket, Zoo-Narengi and Ulubari were not detected with residues. The detectable residue levels of CTC were found in 3.0% kidney samples. All samples showed detectable residue for CTC well below the MRL (1.2 µg/g). The level of residues detected for CTC in kidney samples was 0.004–0.033 µg/g. The detectable residue level of CTC was found in 1.0% muscle sample. The sample showed detectable residue for CTC well below the MRL (0.2 µg/g). The level of residues detected for CTC in muscle sample was 0.079 µg/g. The detectable residue levels of CTC were found in 4.0% liver samples. All samples showed detectable residue for CTC well below the MRL (0.6 µg/g). The level of residues detected for CTC in liver samples was 0.009–0.032 µg/g. The result was similar with the findings of Muriuki *et al.* [8] where CTC residue was detected in liver samples.

Higher incidence of chlortetracycline residue was found in liver and kidney tissue than in muscle, which can be attributed to their being organs of metabolism and excretion and therefore they are at greater risk of exposure to residues [9]. After administration, chlortetracycline enters all tissues and body fluids, but higher concentrations are found in the kidney, liver, bile, lungs and bones [10]. Chlortetracycline is excreted mainly via urine and bile, which explains the high concentrations of residue observed in kidney and liver tissues in this study.

Nisha [1] reported that indiscriminate use of antibiotics to treat pyrexia, inflammation, wounds and viral diseases is associated with levels of residues in edible tissues of food-producing animals. The incidence of chlortetracycline residue level in the present study probably reflects pig being sold for slaughter whilst under a therapeutic or prophylactic regimen of chlortetracycline or animals being slaughtered before the end of the withdrawal period.

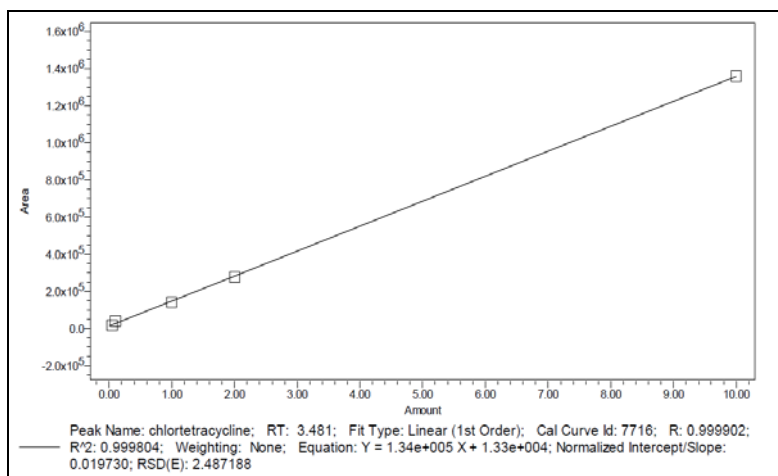


Fig 1: Standard curve of Chlortetracycline

Table 2: Tissue distribution of CTC Residues in edible pig tissues

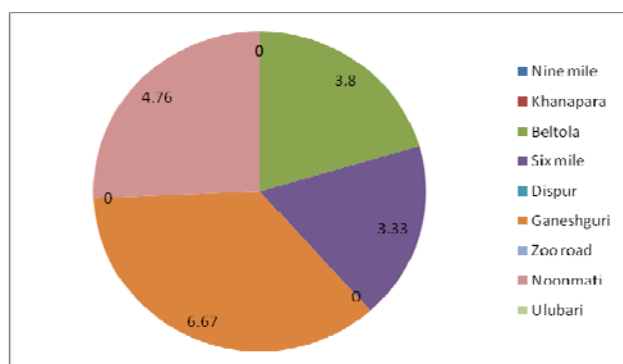
Pig tissue samples	No. of samples collected	% CTC Residue detected	Residue above MRL
Kidney	100	3.0	ND
Liver	100	4.0	ND
Muscle	100	1.0	ND
TOTAL	300	2.67	ND

ND- Not Detected

Table 3: Location wise distribution of CTC Residues in edible pig tissues

Location	Total samples collected	%of Residue detected
Nine mile	18	ND
Khanapara	30	ND
Beltola	105	3.80
Six mile	30	3.33
Dispur	21	ND
Ganeshguri	30	6.67
Zoo road	21	ND
Noonmati	21	4.76
Ulubari	24	ND

ND-Not Detected

**Fig 2:** Graphical representation of Location wise percent positive chlortetracycline residue in edible pig tissue

4. Conclusion

About 100 each of kidney, muscle and liver tissue samples of pig were collected randomly from market and meat stalls of Guwahati city of Assam and analyzed for the presence of Chlortetracycline residues. The result showed that about 2.67% of the samples were detected to be positive for trace residues of CTC. The detected residue levels of CTC in the edible tissues of Pig were below the Maximum Residue Limit (MRL). It may be concluded from the present study that using liquid chromatographic technique the samples were precisely detected with CTC residues.

5. Acknowledgement

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