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Determination of chlortetracycline residues in swine tissues using high performance liquid chromatography

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Abstract
A study was undertaken to detect residues of Chlortetracycline in swine tissues using High Performance Liquid Chromatography (HPLC). About 300 nos. of representative swine tissue samples were collected from pork markets and roadside meat 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. Chlortetracycline residues were extracted with EDTA-McIlvaine buffer. Solid phase extraction clean up was done with SPE C_{18} cartridge. Recovery ranged from 94-98 %. About 8 nos. of the screened samples were detected to be positive for trace residues of chlortetracycline below the Maximum Residue Limit (MRL). It may be concluded that the screened samples were detected for residues well below the MRL.

Keywords: Chlortetracycline, residue, HPLC, MRL, Assam

1. Introduction
Antibiotics are freely used as growth promoters and for treatment of animal diseases without adequate knowledge. 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. For these reasons, the control of antibiotic residues in edible animal tissues is mandatory. To protect the health of consumers, many countries have established Maximum Residue Limits (MRLs) for different antibiotics in food-producing animals. The indiscriminate use of this antibiotic 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 per livestock census, total meat production in Assam is 30.69 thousand tonnes in the year 2008-09, out of which pig meat (pork) contributes the highest share i.e.; 39%. More than 90% of the entire people in Assam are reported to be meat eaters as against 67% of the entire country (Hazarika and Bora, 1986) [4]. The North-Eastern States of India is characterized by a high proportion of tribal people for whom pig keeping is integral to their way of life. Pigs have been an integral component of farming system and support a large rural population of Assam. Also, pork is considered as an important food item in Assam.
Chlortetracycline antibiotic is routinely used in farm animals for the treatment, prevention and control of infectious diseases. Traces of chlortetracycline may be present as residue after slaughter in trace amount. Chlortetracycline residues in meat may cause allergic reactions in individuals and may produce antibiotic resistance. Kidney, Muscle and Liver are the main sites for the deposition of Chlortetracycline residue. The recommended MRL for Chlortetracycline in swine kidney, muscle and liver are 1.2, 0.2 and 0.6 µg/g respectively (FAO/WHO, 2002) [3]. Thus, the people of Assam may risk the chance of intake of Chlortetracycline left as residues in pork. Thus the present study was undertaken to determine Chlortetracycline residues in swine kidney, muscle and liver using a High Performance liquid Chromatographic technique.

2. Materials and Methods
About 300 samples of swine kidney, muscle and liver (100 nos. each) were collected from meat stalls in and around Guwahati city (Table 1). Screening and analysis of samples for the presence of Chlortetracycline residues was performed with High Performance Liquid Chromatography (HPLC).

Table 1: Pork samples collected from market and roadside stalls in and around Guwahati city

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

2.1 Chemical and reagents
Chlortetracycline standard (Dr. Ehrenstofer, Germany); HPLC grade Acetonitrile, Methanol (Qualigens); other chemicals and solvents of analytical grade and HPLC grade water were used for the study.

2.2 Preparation of sample
The fascia and fat of pork were removed and then cut into small pieces.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 C_{18} polymeric cartridge after which it was further filtered using 0.22 µm membrane filter.

2.3 Chromatographic analysis
Residue in samples were detected and quantified using HPLC system equipped with UV-Vis Detector and RP C_{18} 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 Sep-pak C_{18} polymeric cartridge. Chlortetracycline hydrochloride of pure technical grade (Dr. Ehrenstorfer) was used as standard.

3. Results and Discussion
Linear calibration curve of Chlortetracycline having correlation coefficient (R^2) of 0.999 was obtained. Recoveries of chlortetracycline ranged from 94-98%. Similar recoveries were reported by Cinquina *et al*. (2003) [2], Biswas *et al*. (2007) [1] and Shahid *et al*. (2007) [7]

Overall, 300 samples of swine kidney, muscle and liver were collected and analyzed for the presence of Chlortetracycline residues. After HPLC screening, 8 nos. of swine tissue samples were detected to be positive of chlortetracycline residues. 3 kidney, 1 muscle and 4 liver samples were detected with trace residues of Chlortetracycline which were well below the Maximum Residue Limit (MRL) as shown in Table 2.

Chlortetracycline residues were detected in 4 samples from Beltola, 1 from Sixmile, 2 from Ganeshguri and 1 from Noonmati. Samples collected from Nine mile, Khanapara, Dispur - Supermarket, Zoo-Narengi and Ulubari were found to be negative for the residues (Table 3).

The detectable residue levels of Chlortetracycline were found in 3 kidney samples. All samples showed detectable residue for Chlortetracycline well below the MRL (1.2 µg/g). The level of residues detected for Chlortetracycline in kidney samples was 0.004–0.033 µg/g. The detectable residue levels of Chlortetracycline were found in 1 muscle sample. The sample showed detectable residue for Chlortetracycline well below the MRL (0.2 µg/g). The level of residues detected for Chlortetracycline in muscle sample was 0.079 µg/g. The detectable residue levels of Chlortetracycline were found in 4 liver samples. All samples showed detectable residue for Chlortetracycline well below the MRL (0.6 µg/g). The level of residues detected for Chlortetracycline in liver samples was 0.009–0.032 µg/g. The result was similar with the findings of Muriuki *et al.* (2001) [6] where Chlortetracycline residue was detected in liver samples.

Table 2: Tissue distribution of Chlortetracycline Residues in screened pork samples

Pig tissue samples	No. of samples collected	No. of Residue detected	Concentration range(µg/g)	Residue above MRL
Kidney	100	3	0.004-0.033	ND
Liver	100	4	0.009-0.032	ND
Muscle	100	1	0.079	ND
Total	300	8	-------	ND

ND- Not Detected

Table 3: Location wise distribution of Chlortetracycline Residues in screened pork samples

Place	Total samples collected	No. of Residue detected
Nine mile	18	ND
Khanapara	30	ND
Beltola	105	4
Six mile	30	1
Dispur	21	ND
Ganeshguri	30	2
Zoo road	21	ND
Noonmati	21	1
Ulubari	24	ND
Total	300	8

4. Conclusion
A total of 300 samples of swine tissues (100 each of kidney, muscle and liver) 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 only 3, 1 and 4 nos. of the kidney, muscle and liver samples were positive for chlortetracycline residues. All the detected samples were below the MRL.

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