HPLC AND SPECTROPHOTOMETRIC ANALYSIS OF TETRACYCLINE RESIDUES IN MARKETED PORK OF ASSAM

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ABSTRACT

The present study was undertaken to detect residues of Tetracycline in marketed pork using a High Performance Liquid Chromatography (HPLC) System and a UV-Vis Spectrophotometer. 300 samples of marketed pork were collected for the study. The samples after collection were preserved at -20°C. Analyses of the samples using High Performance Liquid Chromatography with UV-Vis Detector were done as per the method of Oka et al., 1985 while analyses of the same samples using UV-Vis Spectrophotometer were done as per the method of Yongrianian et al, 2010. Recovery ranged from 85-93% (HPLC) and 68-83% (Spectrophotometer). Out of the tested samples, 4 samples were detected to be positive for trace residues of tetracycline using Spectrophotometer while 6 samples were detected for tetracycline residues using HPLC method which were well below the Maximum Residue Limit (MRL) value. The method of HPLC is more sensitive than the Spectrophotometric one. Thus, the HPLC method is useful for monitoring of tetracycline residues in pork.

Key words: HPLC, Spectrophotometric, Tetracycline and marketed pork

INTRODUCTION

Tetracycline antibiotic is routinely used in farm animals for the treatment, prevention and control of infectious diseases. Tetracycline residues in meat may cause allergic reactions in individuals and may produce antibiotic resistance. To ensure human food safety, the FAO/WHO have recommended Maximum Residue Limits (MRL) for tetracycline in animal products. The recommended MRL for tetracycline in pig muscle, kidney and liver is 0.2, 1.2 and 0.6 µg/g (FAO/WHO, 2002).

The total meat production in Assam as per livestock census is 30.69 thousand tonnes in the year 2008-09, out of which pork contributes the highest share i.e.; 39% (Anonymous, 2008-09). 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 and pig meat is considered as an important food item.

Screening of pork samples for tetracycline residues using both Spectrophotometric and HPLC method has not been reported till date in Assam. Thus, the present study was undertaken for determination of tetracycline residues in pork samples from various areas of Assam by using both Spectrophotometric and HPLC method.

**MATERIALS AND METHODS**

300 nos. of muscle, kidney and liver samples from slaughtered pig carcasses were collected from meat stalls and markets of Assam (Table 1). Representative samples weighing 30 g each belonging to same carcass were wrapped in polythene bags and transported in thermo-cooled containers jacketed with ice. Screening and analysis of samples for the presence of tetracycline residues was performed with the High Performance Liquid Chromatography (HPLC) method of Oka et al., 1985 while analyses of the same samples using UV-Vis Spectrophotometer were done as per the method of Yongnianian et al., 2010.

Residue in samples were detected and quantified using Waters® HPLC system equipped with 515 Binary pump system, 2487 Dual e Absorbance detector, a manual injector and RP C_{18} column (particle size of 5 µm; 4.6mm × 250mm). The mobile phase used was a mixture of acetonitrile, methanol and water in the ratio of 20:30:50 v/v (pH 2.0). The flow rate was maintained in an isocratic mode at 1 ml/min. The extraction of the samples was done by Solid Phase Extraction cleanup with a Sep-pak C_{18} polymeric cartridge. The levels of Tetracycline were determined using a UV-Vis Spectrophotometer at 526 nm and a HPLC system with UV-Vis detector operated at 350 nm.

**Chemical and reagents:**

Tetracycline 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.

**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. Ten grams 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 0.22mµ filter paper.

**RESULTS AND DISCUSSION**

Linear calibration curve of tetracycline having correlation coefficient (r²) of 0.999 was obtained. Recovery of tetracycline ranged from 85-93% in HPLC method and 68-83% in Spectrophotometeric method. Overall, 300 numbers of muscle, kidney and liver sample of pigs were collected and analyzed for the presence of tetracycline residues. Out of 300 samples, only 4 (2 kidney, 1 liver and 1 muscle)
HPLC and Spectrophotometric analysis of tetracycline samples showed detectable tetracycline residues using Spectrophotometer. 3 samples from Guwahati and 1 from Sivsagar were detected to be positive for trace residues of tetracycline. While samples from Dibrugarh, Jorhat, Kokrajhar, Karbi Anglong and Dhemaji were not detected with residues of tetracycline. Not a single sample of pork tissues were found to be above the MRL value as listed in Table 2.

As listed in Table 3, only 6 (3 kidney, 2 liver and 1 muscle) samples showed detectable tetracycline residues using HPLC. 4 samples from Guwahati, 1 each from Sivsagar and Karbi Anglong were detected to be positive for trace residues of tetracycline. While samples from Dibrugarh, Jorhat, Kokrajhar and Dhemaji were not detected with residues of tetracycline. All the samples were below the permissible limit.

Residue level of tetracycline detected using HPLC in kidney, liver and muscle were 0.015-0.833 µg.g⁻¹, 0.350-0.448 µg.g⁻¹ and 0.165 µg.g⁻¹ respectively whereas residue level of tetracycline using Spectrophotometer were 1.008-1.060 µg.g⁻¹, 0.430 µg.g⁻¹ and 0.183 µg.g⁻¹ respectively in kidney, liver and muscle samples as listed in Table 4. The finding were similar with that reported by Biswas et al. (2007) and Shahid et al. (2007) where tetracycline residue were detected in trace levels.

**CONCLUSION**

300 samples of pork were collected from different pork markets of Assam. Out of the screened samples, 6 samples were detected to be positive for trace residues of tetracycline using HPLC while 4 samples were detected for tetracycline residues using Spectrophotometer which were well below the MRL value. It can be concluded that HPLC method is more sensitive than spectrophotometric method and hence useful for detection of tetracycline residues in pork.

Table -1 : Pork samples collected from market and roadside stalls in and around Assam

<table>
<thead>
<tr>
<th>Place</th>
<th>Kidney</th>
<th>Liver</th>
<th>Muscle</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guwahati</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>135</td>
</tr>
<tr>
<td>Dibrugarh</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>48</td>
</tr>
<tr>
<td>Jorhat</td>
<td>07</td>
<td>07</td>
<td>07</td>
<td>21</td>
</tr>
<tr>
<td>Sivsagar</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Kokrajhar</td>
<td>07</td>
<td>07</td>
<td>07</td>
<td>21</td>
</tr>
<tr>
<td>Karbi Anglong</td>
<td>07</td>
<td>07</td>
<td>07</td>
<td>21</td>
</tr>
<tr>
<td>Dhemaji</td>
<td>08</td>
<td>08</td>
<td>08</td>
<td>24</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>300</strong></td>
</tr>
</tbody>
</table>
### Table 2: Tabular representation of location wise distribution of tetracycline (TC) residues using Spectrophotometer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Locations</th>
<th>Samples Screened</th>
<th>Residues Detected</th>
<th>Detected percentage (%)</th>
<th>Residue above MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guwahati</td>
<td>135</td>
<td>3(K-1,L-1,M-1)</td>
<td>2.22</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Dibrugarh</td>
<td>48</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Jorhat</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Sivasagar</td>
<td>30</td>
<td>1(K-1,L-0,M-0)</td>
<td>3.33</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Kokrajhar</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Karbi Anglong</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>Dhemaji</td>
<td>24</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TOTAL</td>
<td>300</td>
<td>4(K-2,L-1,M-1)</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Table-3 : Tabular representation of location wise distribution of tetracycline (TC) residues using HPLC

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Locations</th>
<th>Samples Screened</th>
<th>Residues Detected</th>
<th>Detected percentage (%)</th>
<th>Residue above MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guwahati</td>
<td>135</td>
<td>4(K-2,L-1,M-1)</td>
<td>2.96</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Dibrugarh</td>
<td>48</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Jorhat</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Sivsagar</td>
<td>30</td>
<td>1(K-1,L-0,M-0)</td>
<td>3.33</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Kokrajhar</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Karbi Anglong</td>
<td>21</td>
<td>1(K-0,L-1,M-0)</td>
<td>4.76</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>Dhemaji</td>
<td>24</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TOTAL</td>
<td>300</td>
<td>6(K-3,L-2,M-1)</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
### Table -4

Tissue distribution of Tetracycline(TC) residue in pork

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Total Samples collected</th>
<th>Using HPLC</th>
<th></th>
<th>Using UV-Vis Spectrophotometer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of samples detected residue (concn. in µg g-1)</td>
<td></td>
<td>No. of samples detected residue (concn. in µg g-1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of samples detected residue above MRL</td>
<td></td>
<td>No. of samples detected residue above MRL</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>100</td>
<td>3 (0.015-0.833) ND</td>
<td>2(1.008-1.060) ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>2(0.350-0.448) ND</td>
<td>1(0.430) ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>100</td>
<td>1(0.165) ND</td>
<td>1(0.183) ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>6 ND</td>
<td>4 ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ACKNOWLEDGEMENT**

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**REFERENCES**

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