



ISSN: 2277- 7695

TPI 2017; 6(2): 97-99

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www.thepharmajournal.com

Received: 15-12-2016

Accepted: 16-01-2017

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## UHPLC detection of OTC residues in swine tissues

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### Abstract

A study was undertaken to detect residues of Oxytetracycline (OTC) in swine tissues using Ultra High Performance Liquid Chromatography (UHPLC) system. A total of 216 nos. of representative swine tissue samples were collected from pork markets and roadside meat stalls of Assam. The samples after collection were preserved at -20 °C and then screened by UHPLC with Diode Array Detector (DAD). OTC residues were extracted with EDTA-McIlvaine buffer. Solid phase extraction clean-up was done with SPE C<sub>18</sub> cartridge. Recovery ranged from 94-98%. A total of 5 nos. of the screened samples were detected to be positive for trace residues of OTC, 2 above the Maximum Residue Limit (MRL). It may be concluded that the present method reliably and precisely detects OTC residue in swine tissues.

**Keywords:** Assam, MRL, Oxytetracycline, residue, swine, UHPLC

### 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.

Oxytetracycline (OTC) is routinely used in farm animals for the treatment, prevention and control of infectious diseases. Traces of OTC may be present as residue after slaughter in trace amount. OTC 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 this residue. The recommended MRL for OTC 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 OTC left as residues in pork. Since few records are available regarding residue study in pig of Assam, thus the present study was undertaken to detect OTC residues in swine kidney, muscle and liver using Ultra High Performance liquid Chromatographic (UHPLC) technique.

### 2. Materials and Methods

#### 2.1 Collection of samples

A total of 216 samples of swine kidney, muscle and liver (72 nos. each) were collected from meat stalls of Assam (Table 1). Screening and analysis of samples for the presence of OTC residues was performed with UHPLC.

#### 2.2 Chemical and reagents

OTC standard (Sigma), HPLC grade Acetonitrile, Methanol, Water, chemicals and solvents of analytical grade were used for the study.

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### 2.3 Preparation of meat samples

The stored frozen meat samples are thawed and then 10g of sample was cut. The fat and fascia were removed. To the 10 g of sample, 10 ml of HPLC grade water was added and then blended in a tissue blender.

### 2.4 Extraction, cleanup and filtration of meat samples

About 5 g of the sample was transferred to a glass test tube. Then, 3 ml of 0.1 M EDTA-McIlvaine buffer (pH 4.0) was added and mixed. The mixture was then sonicated by setting at 15 micron amplitude for 20 cycles with a stop time of 30 sec interval at low temperature which was maintained with crushed ice. The sonicated sample was then left undisturbed for 15 mins for allowing the extract to dissolve in the solvent. The sample was then centrifuged at 10,000 rpm for 15 mins at 0° centigrade in a refrigerated centrifuge machine. The supernatant was separated and filtered through a Whatman filter paper No. 42.

Cleanup of the extract was done by using Solid Phase Extraction (SPE) method. The filtrate was loaded on a C<sub>18</sub> cartridge preconditioned with 3 ml of methanol and 2 ml of water. The cartridge containing the sample was washed with 5 ml of water and then tetracyclines were eluted with 4.5 ml of 0.01M Methanolic oxalic acid.

The extract so obtained was filtered through a Millipore filter paper (0.22µm). 20µl of the eluted sample was then injected into the UHPLC system for analysis.

### 2.5 Chromatographic analysis

Residue in samples were detected and quantified using Dionex® UHPLC system equipped with Quarternary pump system, Diode Array Detector, Autosampler and RP C<sub>18</sub> column. A mobile phase of 0.01 M oxalic acid: Acetonitrile: Methanol (77: 18: 5, v/v/v) was used. The flow rate was kept at 1.0 ml/min keeping mode as isocratic. The wavelength for the detector was set at 350 nm.

### 2.6 Preparation of standard calibration curve of Oxytetracycline

About 10 mg of pure Oxytetracycline hydrochloride standard was dissolved in 100 ml of methanol to obtain a concentration of 100µg/ml. Further dilutions were made from this solution in methanol in the descending concentration of 5.0, 4.0, 3.0, 2.0 and 1.0 µg/ml respectively. An aliquot of 20µl each of these solutions were injected into the UHPLC system.

### 3. Results and Discussion

Linear calibration curve of OTC having correlation coefficient (R<sup>2</sup>) of 0.995 was obtained by plotting concentration against the peak areas (Figure 1). Recoveries of OTC 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]. The separation of the analytes was achieved in less than 5 mins. Acetonitrile was effective in the deproteinization of pork samples and in the isolation of analytes from spiked samples. This method allows the determination of residues of OTC in different matrices with higher sensitivity.

Overall, 216 samples of swine kidney, muscle and liver were collected and analyzed for the presence of OTC residues. After UHPLC screening, 5 nos. of swine tissue samples were detected to be positive of OTC residues. All the positive samples were from Kamrup-Metro (Guwahati). A total of 3 kidney, 1 muscle and 1 liver samples were detected with residues of OTC as shown in Table 2. The muscle and liver

sample were below the MRL with residue level of 0.150 µg/g and 0.545 µg/g respectively. About 2 samples of Pig kidney were above the MRL with level of 1.245 µg/g and 2.708 µg/g. The result was similar with the findings of Muriuki *et al.* (2001) [6] where OTC residue was detected in kidney samples. Wasch *et al.* (1998) [8] also reported similar results where OTC was detected in pork samples.

### 4. Conclusion

A total of 216 samples of pork were collected from different pork markets of Assam. A total of 5 samples were detected to be positive of OTC residue. Out of the screened samples, 2 kidney samples were positive for residues of OTC which was above the permissible limit. Thus, it can be concluded from the present study that the method reliably and precisely detects OTC residue in swine tissues.

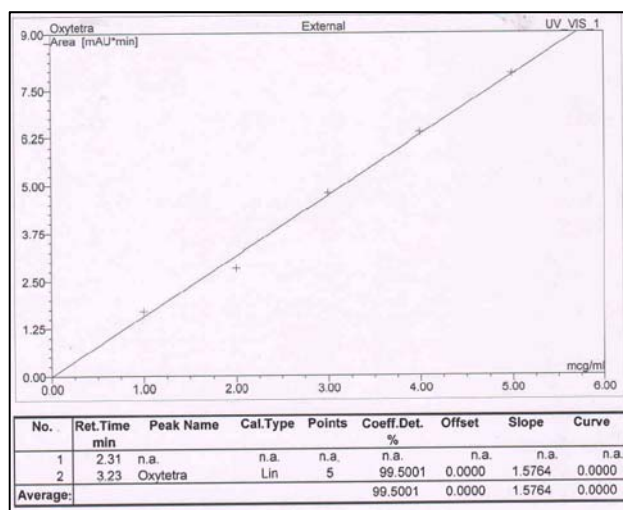
**Table 1:** Swine tissue samples collected from market and roadside stalls of Assam

Place	Kidney	Liver	Muscle	Total
Guwahati (Kamrup Metro)	20	20	20	60
Nalbari	7	7	7	21
Barpeta	8	8	8	24
Golaghat	5	5	5	15
Jorhat	6	6	6	18
Sibsagar	8	8	8	24
Dibrugarh	7	7	7	21
Dhemaji	6	6	6	18
Lakhimpur	5	5	5	15
TOTAL	72	72	72	216

**Table 2:** Distribution of OTC residues in swine tissues

Swine tissue samples	No. of samples collected	No. of Residue detected	Residue above MRL
Kidney	72	3	2
Liver	72	1	ND
Muscle	72	1	ND
TOTAL	216	5	2

ND- Not Detected



**Fig 1:** Standard curve of Oxytetracycline (OTC)

### 5. Acknowledgement

The authors acknowledge the financial assistance and help received from ICAR and AAU, Jorhat.

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