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Screening of Marketed Pork for Ceftiofur residues by HPLC And UV-Vis Spectrophotometer

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ABSTRACT: *In the present study pork samples from markets of Assam, India were collected and analyzed to detect the presence of Ceftiofur residues using a High Performance liquid Chromatography (HPLC) System and a UV-Vis Spectrophotometer. 300 nos. of representative pork samples were collected from different pork markets of Assam. 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 Annapurna et al. ,2009. Ceftiofur residues were extracted with Mc-IIvaine buffer. Solid phase extraction clean up was done with Sep-pak C_{18} cartridge. Recovery ranged from 83-95% (HPLC) and 57-80%(Spectrophotometer).Out of the tested samples, 10 nos of the screened samples were detected to be positive for trace residues of Ceftiofur using Spectrophotometer while 14 nos .of same samples were detected for ceftiofur residue using HPLC method which were well below the MRL value.*

Keywords: *Assam, Ceftiofur, High Performance Liquid Chromatography, Pork, Solid Phase Extraction, Spectrophotometer*

I. Introduction

Ceftiofur, a third-generation cephalosporin is a broad spectrum antibacterial agent used for the treatment of digestive and respiratory diseases in livestock [1][2]. Residues of Ceftiofur are reported to be found in animal tissues and milk and their undesirable levels may lead to many health hazards in human [3][4]. For these reasons, the control of ceftiofur residues in edible animal tissues is mandatory. To protect the health of consumers, many countries have established Maximum Residue Limits (MRLs) for different antibiotics including Ceftiofur in food-producing animals. The MRLs of Ceftiofur in swine tissues established by European Union (EU) are 0.5µg g^{-1} in muscle, 3.0µg g^{-1} in liver, and 4.0µg g^{-1} in kidney. Pork is regarded as an important meat in Assam including N.E. States which comprises of about 39% of total meat share in Assam [5]. The present study was undertaken to detect the presence of Ceftiofur residues in marketed pork of Assam by using both Spectrophotometric and HPLC method

II. Materials And Methods

300 nos. of representative pork samples were collected from different pork markets of Assam as listed in TABLE 1. Representative tissue samples of muscle, kidney and liver weighing 30 g each belonging to same carcass were wrapped in polythene bags and transported in thermo-cooled containers jacketed with ice. The samples were stored at -20ºC till the time of processing. The samples after collection were preserved at -20°C.

10 g of each sample was taken in a blender and to it added 10 ml of distilled water and then blended. 5 ml of the mixture was taken and to it added equal volume of 0.1 M Na_2 EDTA - McIIvaine buffer (pH 4.0) and kept for 10 minutes. The mixture was sonicated and left undisturbed for 15 minutes. The mixture was then centrifuged at 0°C at 10,000 rpm for 30 minutes. The collected supernatant was then filtered using Whatman No.42 . Solid phase extraction clean up was done with Sep-Pak C_{18} cartridge. The filtrate was passed through C18 polymeric cartridge, after which it was micro filtered using 0.22µ filter paper and the filtrate was ready for analysis.

Ceftiofur residues in pork were estimated by using a High Performance liquid Chromatography System (Waters HPLC) and a UV-Vis Spectrophotometer (Systronics). Analyses of the samples using High Performance Liquid Chromatography with UV-Vis Detector were done as per the method of Oka *et al*, 1985 [6] while analysis of the same samples using UV-Vis Spectrophotometer were done as per the method of Annapurna *et al.*2009[7].

Table 1: Pork samples collected from different market places of Assam

Place	Kidney	Liver	Muscle	Total
Guwahati	16	18	16	50
Jorhat	12	12	12	36
Nalbari	15	17	15	47
Tezpur	12	12	12	36
Nagoan	15	18	18	51
Goalpara	15	11	12	38
Morigoan	15	12	15	42
TOTAL	**100**	**100**	**100**	**300**

III. Results And Discussion

Over all, 7 number of zones within the state of Assam have been developed and all total 300 number of samples i.e., 100 numbers of sample each of muscle, liver and kidney were collected.

Out of 300 numbers of total samples, only 10 numbers of samples showed detectable ceftiofur residues using Spectrophotometer (4 kidney, 3 liver and 3 muscle samples). 8.33 % of the sample collected from Jorhat were detected to be positive for trace residues of ceftiofur while only 1 sample each from Tezpur, Goalpara and Morigaon was found to be positive for ceftiofur residue as listed in TABLE 2. Not a single sample of pork tissues were found to be above the MRL value.

As listed in TABLE 3, only 14 numbers of samples showed detectable ceftiofur residues using HPLC (5 kidney, 3 liver and 6 muscle samples). All the samples were below the permissible limit.

The detectable levels in kidney were found to be highest as compared to muscle and liver samples. This finding can be correlated with the study of ceftiofur done by Beconi-Berker *et al* (1996) [8] where concentrations were found to be highest in kidney. But in contrast with Payne *et al (loc cited)* who reported higher concentration of ceftiofur in liver of cattle, level of residues in liver in the present study were lower than the kidney samples . Residue level of ceftiofur detected using HPLC in muscle, kidney and liver were 0.015-0.410µg g^{-1}, 0.020-2.540 µg g^{-1} and 0.018-2.229 µg g^{-1} respectively whereas residue level of Ceftiofur using Spectrophotometer were 0.085 - 0.450 µg g^{-1} , 1.380-3.120 µg g^{-1}, 1.570-2.350 µg g^{-1} respectively in muscle, kidney and liver samples as listed in TABLE 4.

Recovery ranged from 83-95% for HPLC and 57-80% for Spectrophotometer. Limit of Detection (LOD) was 0.015 µg g^{-1} for HPLC and 0.085 µg g^{-1} for Spectrophotometer .

Table 2: Tabular representation of location wise distribution of Ceftiofur residues using Spectrophotometer.

Sl. No.	Locations	Samples Screened	Residues Detected	Detected percentage (%)	Residue above MRL
1	Guwahati	50	4(K-2,L-2,M-0)	8.00	ND
2	Jorhat	36	2(K-0,L-1,M-1)	5.56	ND
3	Nalbari	47	ND	0.00	ND
4	Tezpur	36	2(K-1,L-0,M-1)	5.56	ND
5	Nagoan	51	ND	0.00	ND
6	Goalpara	38	2(K-1,L-0,M-1)	5.26	ND
7	Morigoan	42	ND	0.00	ND
	TOTAL	**300**	**10(K- 4,L-3 ,M- 3)**	**3.33**	**ND**

ND- Not detected; K-Kidney; L-Liver; M-Muscle

Table 3: Tabular representation of location wise distribution of Ceftiofur residues using HPLC.

Sl. No.	Locations	Samples Screened	Residue detected	Detected percentage (%)	Residue above MRL
1	Guwahati	50	4(K-1,L-1,M-2)	8.00	ND
2	Jorhat	36	3(K-1,L-1,M-1)	8.33	ND
3	Nalbari	47	2(K-0,L-1,M-1)	4.26	ND
4	Tezpur	36	1(K-1,L-0,M-0)	2.78	ND
5	Nagoan	51	2(K-1,L-0,M-1)	3.92	ND
6	Goalpara	38	1(K-0,L-0,M-1)	2.63	ND
7	Morigoan	42	1(K-1,L-0,M-0)	2.38	ND
	TOTAL	**300**	**14(K-5,L-3,M-6)**	**4.67**	**ND**

Table 4: Tissue distribution of Ceftiofur residue in pork

		Using HPLC		Using UV-Vis Spectrophotometer	
	Total Samples collected	**Residue detected (concn.,µg g^{-1})**	**Residue detected above MRL**	**Residue detected (concn.,µg g^{-1})**	**Residue detected above MRL**
Kidney	100	5 (0.020-2.540)	ND	4 (1.380-3.120)	ND
Liver	100	3 (0.018-2.229)	ND	3 (1.570-2.350)	ND
Muscle	100	6 (0.015-0.410)	ND	3 (0.085 - 0.450)	ND
Total	**300**	**14**	**ND**	**10**	**ND**

IV. Conclusion

300 nos. of representative pork samples were collected from different pork markets of Assam. Out of the tested samples, 14 nos. of the screened samples were detected to be positive for trace residues of Ceftiofur using HPLC while 10 nos. of same samples were detected for Ceftiofur residue using Spectrophotometer which were well below the MRL value. It can be concluded that HPLC method is more sensitive than spectrophotometric method in detection of Ceftiofur residues in pork.

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