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Liquid chromatographic assay for ciprofloxacin residue in milk

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Abstract

The present study was conducted to investigate residues of Ciprofloxacin in raw milk of cow. A total of 175 raw milk samples of cow were collected from different districts of Assam. Ultra High Performance Liquid Chromatography (UHPLC) with diode array detection system was employed for detection of ciprofloxacin in the milk samples. A mixture of water and acetonitrile (70:30 v/v) was used as the mobile phase. The flow rate was maintained in an isocratic mode at a speed of 1.0 ml.min⁻¹. The wavelength for the detector was set at 277 nm. A standard calibration curve with coefficient of determination of 99.64%. The method yielded good recovery percentage of ciprofloxacin in milk which ranged from 94-99%. Out of the 175 screened samples, 0.57 % sample was detected to be positive for residues of Ciprofloxacin.

Keywords: Assam, ciprofloxacin, milk, residue, UHPLC

1. Introduction

Ciprofloxacin is a broad-spectrum antimicrobial agent which has a wide range of antibacterial activity and so have been in use in veterinary medicine (Nouws *et al.*, 1988) [7]. It is mainly used against urinary, pulmonary and digestive infections (Bauditz, 1990) [1]. An increase in the spectrum of activity and potency of the drugs is seen (Spoo *et al.*, 1995) [8]. Ciprofloxacin acts by inhibiting the bacterial DNA-gyrase (Gilles *et al.*, 1991) [5].

Ciprofloxacin may be found as residues in milk of cow due to indiscriminate use in dairy farms. Due to the rising concern of public health, FAO/WHO has recommended Maximum Residue Limits (MRL) for Ciprofloxacin in milk (FAO/WHO, 2002) [4].

India is the world's largest milk-producing country with an annual production of 132 million tonnes (BAHS, 2014) [2]. The total milk production of the State of Assam for the year 2012-13 was estimated at 838 thousand tonnes (Economy Survey of Assam, 2013-14) [3]. Thus, the present study was conducted to investigate residues of Ciprofloxacin in raw milk of cow.

2. Materials and Methods

2.1 Sample collection

In this study, raw milk samples of cow (175 nos.) of about 100 ml were collected in sterile containers from different districts of Assam (Table 1). The milk samples after collection were transported to the laboratory in thermo-cooled containers jacketed with ice.

Table 1: Milk Samples of Cow collected from different districts of Assam

S. No.	Places of collection	Total Milk samples collected
1	Kamrup	50
2	Nalbari	15
3	Barpeta	20
4	Golaghat	12
5	Jorhat	20
6	Sibsagar	16
7	Dibrugarh	20
8	Dhemaji	12
9	Lakhimpur	10
	Total	175

2.2 Extraction and cleanup

10 ml of the milk sample was taken in a 50 ml centrifuge tube. 10 ml of acetonitrile was added followed by vigorous shaking for 5 mins. The sample was then centrifuged at 6000 rpm for 10 mins. The supernatant was collected 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 C₁₈ cartridge preconditioned with 3 ml of methanol and 3 ml of HPLC grade water. The cartridge containing the sample was washed with 3 ml of water and then finally eluted with 3 ml of methanol.

The extract so obtained was filtered through syringe filter (0.2µm). 20µl of the eluted sample was then injected into the UHPLC system for analysis.

2.3 Chromatographic condition

Ultra High Performance Liquid Chromatography (UHPLC) with diode array detection system was employed for detection of ciprofloxacin in the milk samples. A mobile phase of Water: Acetonitrile (70:30 v/v) was used. The flow rate was kept at 1.0 ml.min⁻¹ keeping mode as isocratic. The wavelength for the detector was set at 277 nm.

2.4 Quantification

10 mg of pure Ciprofloxacin standard (Sigma chemicals) was dissolved in 100 ml of HPLC grade water with drop of HCl until complete dissolution to obtain a concentration of 100µg.ml⁻¹. Further dilutions were made from this solution in the descending concentration of 5.0, 4.0, 3.0, 2.0 and 1.0 µg.ml⁻¹ respectively. An aliquot of 20µl each of these

solutions were injected into the UHPLC system. Peak areas were recorded.

3. Results and Discussion

The presence of residues of antimicrobial agents in food producing animals has received worldwide attention from national and international regulatory agencies due to its public health importance. In the present study, a total of 175 samples of raw milk of cow were collected and screened for residues of ciprofloxacin. The assay of drug residues like ciprofloxacin in biological matrices such as milk is difficult due to presence of many interfering substances like fats, proteins and other components which may interfere with the separation of peak. To solve this, acetonitrile was incorporated in for precipitating protein. After protein precipitation, centrifugation and solid-phase extraction (SPE) the extracts were analyzed by Ultra High Performance Liquid Chromatography (UHPLC) system. Reverse phase chromatography helped in good separation of peaks. Diode array detector at 277 nm detected the residues of ciprofloxacin in the sample. A standard calibration curve with coefficient of determination of 99.64% was obtained by plotting concentration of standard solutions against peak areas obtained (Figure 1). The method yielded good recoveries of ciprofloxacin in milk which ranged from 94-99%. Similar recovery was reported by Navratilova *et al.* (2011) [6]. After screening all the milk samples, Ciprofloxacin residue was detected in only 0.57% of the total samples. In comparison to the findings of Navratilova *et al.* (loc. cit), ciprofloxacin residue was detected in very few samples. This may be due to proper milking of cow after the withdrawal period.

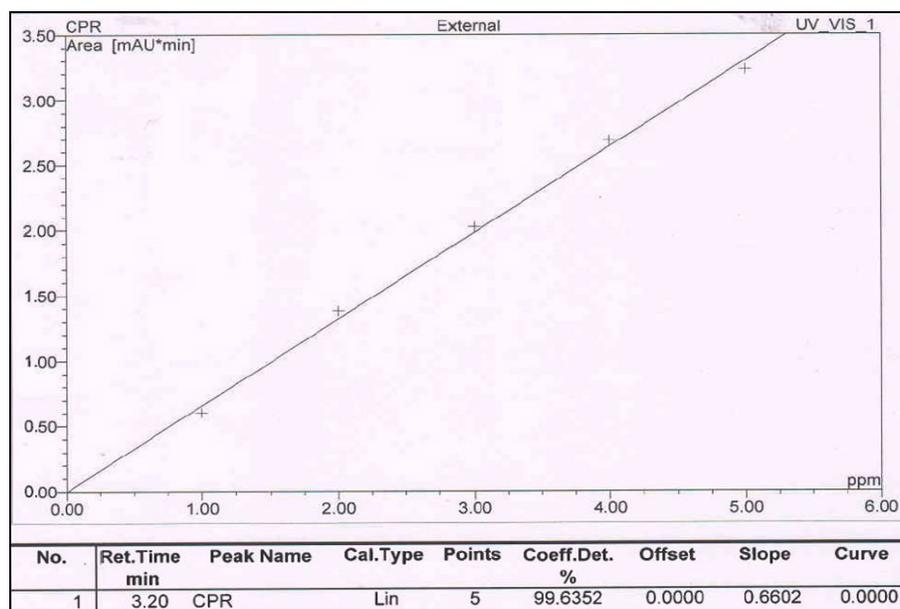


Fig 1: Standard calibration curve of Ciprofloxacin

4. Conclusion

It may be concluded from the present study that Ciprofloxacin is judiciously used in dairy farms of Assam as most of the milk samples were free from residues. So, from public health point of view milk samples screened in the present study were safe for human consumption.

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