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Solid phase extraction method for detection of ciprofloxacin residue in milk

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Abstract

The present study was conducted for detection of Ciprofloxacin residue in milk using Solid Phase Extraction (SPE) method. Raw milk samples of cow were collected from milk vendors in sterile containers and then preserved until analysis. 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. The standard calibration curve showed good linearity ($r^2 > 0.996$). Accuracy and recovery was in the range of 94-99% for ciprofloxacin in milk indicating that the method can be used as a validated method. The present method very precisely identifies and quantifies low level of Ciprofloxacin in milk which is useful for monitoring purpose.

Keywords: Ciprofloxacin, milk, residue, solid phase extraction, UHPLC

1. Introduction

Food safety is of great importance to all involved in the food chain. The problem of drug residues in food of animal origin remains one of the public health concerns all over the globe [1]. Antibiotics are widely used in food-producing animals for the treatment of disease and as dietary supplements. They may be administered orally as food additives or directly by injection. The use of antibiotics may result in drug residues in the food products of animal origin especially if they are not used according to the labelled directions. Residues of antimicrobials may have toxic effects on both animals and humans [2].

Fluoroquinolones are broad spectrum antibacterial agents often used in livestock in cases of pulmonary, urinary and digestive infections as they act by inhibiting bacterial DNA-gyrase. Fluoroquinolones such as Ciprofloxacin may cause hypersensitivity in humans. Cross-resistance between fluoroquinolones has been reported among a wide range of microorganisms including both gram-negative and gram positive bacilli [3]. 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 (2002) has recommended Maximum Residue Limits (MRL) for Ciprofloxacin in milk (0.1 µg/g) [4].

India is the world's largest milk-producing country with an annual production of 132 million tonnes [5]. The total milk production of the State of Assam for the year 2012-13 was estimated at 838 thousand tones [6]. Screening of milk samples is required for detection of residues of antibacterial agents like ciprofloxacin.

The present study is an attempt to validate a method for detection of residues of Ciprofloxacin in milk. The present method involves simple clean up procedures using solid phase extraction method and detection using Ultra High Performance Liquid Chromatography (UHPLC) coupled to Diode Array Detector (DAD).

2. Materials and Methods

2.1 Sample collection

In this study, raw milk samples of cow of about 100 ml were collected in sterile containers. The milk samples after collection were transported to the laboratory in thermo-cooled containers jacketed with ice.

2.2 Chemical and reagents

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

2.3 Fortification of samples

About 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 $100\mu\text{g}\cdot\text{ml}^{-1}$. Further dilutions were made from this solution in the descending concentration of 5.0, 4.0, 3.0, 2.0 and $1.0\mu\text{g}\cdot\text{ml}^{-1}$ respectively. The milk samples were spiked with these concentrations of Ciprofloxacin. Fortified milk samples were allowed to stand at 4°C for 1 h before analysis.

2.4 Extraction and cleanup

About 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 C_{18} 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 ($0.2\mu\text{m}$). $20\mu\text{l}$ of the eluted sample was then injected into the UHPLC system for analysis.

2.5 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 $1.0\text{ml}\cdot\text{min}^{-1}$ keeping mode as isocratic.

2.6 Detection and Quantification

The separated ciprofloxacin was detected with DAD and quantification by Chromeleon chromatographic software interfaced to a personal computer. The wavelength for the detector was set at 277 nm.

3. Results and Discussion

The present study includes an extraction step with a suitable solvent system along with clean up procedure. Solid phase extraction clean up step is required to isolate Ciprofloxacin from interference of a complex sample matrix prior to the chromatographic analysis. 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 and centrifugation, Solid-phase extraction (SPE) method was applied and 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 good linearity ($r^2 > 0.996$) was obtained by plotting concentration of standard solutions against peak areas obtained. The method yielded good recoveries of ciprofloxacin in milk which ranged from 94-99%. Similar finding was reported in a HPLC method for the determination of fluoroquinolones where recovery for Ciprofloxacin in milk was 97.1% [7]. After screening all the milk samples,

Ciprofloxacin residue was detected in only 0.57% of the total samples.

4. Conclusion

This study demonstrated that the present method reliably identifies and quantifies the selected residue of Ciprofloxacin in the reconstituted milk samples in very low range which can be applied for monitoring purpose.

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