ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

HPLC Determination of Cloxacillin Residue in Milk and Effect of Pasteurization

Mishra A*,Singh Swatantra K, Sahni Y P, Mandal TK, Chopra S, Gautam VN, Qureshi SR.

*Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata-37.
Department of Pharmacology and Toxicology, College of Veterinary Science and A.H., M.P.P.C.V.V, Jabalpur (M.P.)

ABSTRACT

Antibiotic residues occur in various types of foods of animal origin including milk and milk products due to large-scale application of antibiotics in veterinary practice. Antibiotics used as therapeutic agents or as feed supplement in milk animal lead to secretion of their residues into milk. These residues not only create problems in dairy industry but also have immense public health importance. In this study, determination of cloxacillin residue is done by using high performance liquid chromatography (HPLC). The aim of this study was to observe the effect of pasteurization on cloxacillin residue in milk. Milk samples were collected at day-0 (control), day-1, day-3 and day-5 after single IM administration of cloxacillin in 8 lactating cows. The milk samples were extracted with organic solvent and then separated by C_{18} RP-Column using an isocratic elution and detected with UV detector at 220nm. All 8 cows treated with cloxacillin showed significant level of residues concentration of cloxacillin on day-1. However, similar animals did not show residue concentration of cloxacillin in milk samples collected on day-3 and day-5. The effect of pasteurization (LTLT) at 65 ^{0}c for 30 min on cloxacillin residue in milk was also evaluated. No significant ($p>0.05$) reduction of cloxacillin residue in milk was found on pasteurization.

Key words: HPLC, Cloxacillin, Pasteurization (LTLT), Milk

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Antibiotics are widely used as medicinal drugs to treat in numerous bacterial infections both in human beings and animals. The frequent use of antibiotics in clinical practice cause the occurrence of antibiotic residues in various food products of animal origin. Antibiotics residues occur in various types of foods of animal origin including milk, meat and other food products. Residues of antimicrobial drugs are either the parent compound or their metabolite that accumulate, deposit or store within the cells, tissues, organs and also in edible products of animal origin. Antibiotic residues occur in various types of foods of animal origin including milk, egg and meat due to large-scale application of antibiotics in veterinary practice. The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products from animal origin, such as milk or meat. Presence of drugs or antibiotics residue in food above the maximum level has been recognized worldwide by various public authorities [1]. The antibiotic contamination of milk was reported to be due to intramammary infusions of drugs for mastitis treatment (92%), injections (6%), and other causes (2%) [2]. The presence of even small quantities of antibiotics in milk was found to create problems in dairy industry. The commonly encountered problems include (a) inadequate curdling of milk and improper ripening of cheese during their production, (b) decreased acid and flavor production in cultured products, (c) interference with starter culture resulting in loss of production and (d) difficulties in validation of certain quality control tests [3]. In recent times, the problem of antibiotics residues has increased significantly due to the indiscriminate and frequent use of antibiotics in clinical practice. The potential hazards of antibiotic residues include allergic reactions, interference in the intestinal flora, hepatotoxicity, bone marrow depression, reproductive disorders and transfer of antibiotic resistant bacteria to the human or animal. Milk, since ancient time, is known as an excellent source of protein rich in organoleptic and nutritional supplement essential for human health. Production of safe and quality milk for ensuring better human health is the key aspect.

MATERIALS AND METHODS

Experiment design:

A group of 8 healthy lactating cows (400 kg b.w.) was used. The animals were treated with a single intramuscular administration of Bioclox, a commercial formulation of cloxacillin, corresponding to a dose of 10 mg/kg b.w. of drug, in accordance to good veterinary practice. The milk samples were collected at day-0 (before), day-1, day-3, and day-5 (after) administration of cloxacillin. Milk samples were stored at -20 ^{0}c until HPLC analysis.

Instrument and chromatographic condition:

Milk samples analyses were performed on a HPLC system (SHIMADZU, SPD–M10A, JAPAN) fitted with binary pump (LC-20AT), diode array detector, sampler and data station. A 5µ Luna Phenomenox (250mm×4.6 mm) RP C-18 HPLC column was used. The solvent system was: (A) Acetonitrile (B) Ammonium acetate-20 part + Methanol-10 part. The analysis was carried

ISSN: 0975-8585

out using an isocratic elution 70% (A): 30% (B). Flow rate was 1 ml/min and the UV wavelength was set at 220 nm.

Extraction of Cloxacillin from Milk:

To 1 ml of milk, 3 ml of ice cold methanol (HPLC) grade was added, vortexed for 20 second and filtered the mixture through sodium sulphate (~ 4 gm). The filtrate was centrifuged at 8000 rpm for 20 min. the supernatant was collected and dried with the help of rotary vacuum evaporator (60 ^{0}C). Reconstituted the residue with 1 ml of methanol (HPLC) grade. Filtered the content through filter paper (AS N. 1) and 20 µl injected to HPLC injection port [4].

Recovery:

In order to evaluate the recovery of method, blank milk samples (day-0) were spiked with 25, 50 and 100 ppm of cloxacillin (Three times for each concentration), considering that the Maximum Residue Limits (MRL) set by FDA in milk is 10 µg kg^{-1}. The recoveries were 78.32%, 81.29% and 88.54%.

RESULTS AND DISCUSSION

All animals treated with cloxacillin showed significant level of residues concentration of cloxacillin on day-1 (post-treatment), however, in similar animals the residue concentration of cloxacillin was below detection limit in milk samples collected on day-0 (pre-treatment), day-3 and day-5 (post treatment) Table:1. The reports stated by Maots [4] determine residue concentration of cloxacillin more than MRL in milk samples which is in close conformity to the findings of the present study. The detection of cloxacillin residue concentration in milk samples has been determined by various researcher and elsewhere. The report published by [8] analyzed 18 milk samples for cloxacillin residue concentration and findings revealed that out of 18 milk samples 7 milk samples were found positive for residue concentration of cloxacillin. Chromatogram of standard cloxacillin is shown in figure -1. The effect of pasteurization (LTLT) on cloxacillin residue in milk is shown in table: 2. No significant difference ($p<0.05$) was found in mean residue concentration cloxacillin between non-heated and heated milk.

ISSN: 0975-8585

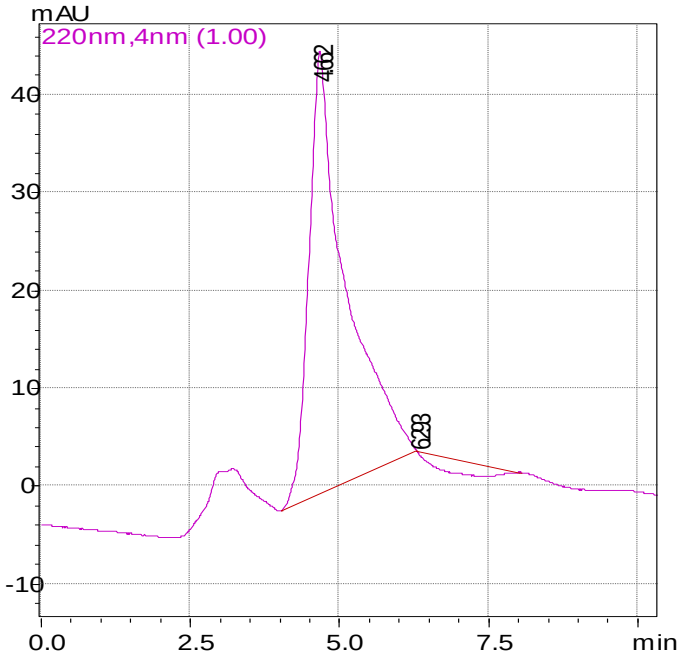

Figure-1. Standard Cloxacillin 50ppm

TABLE-1. Concentration of cloxacillin residue in milk at day-0 (Before), day-1, day-3, day-5(After) single IM administration

S.N.	Animal No.	Concentration of Cloxacillin residue(ppb)			
		Day-0	Day-1	Day-3	Day-5
1	A	BDL	271.23	BDL	BDL
2	B	BDL	292.35	BDL	BDL
3	C	BDL	285.67	BDL	BDL
4	D	BDL	300.83	BDL	BDL
5	E	BDL	278.28	BDL	BDL
6	F	BDL	298.56	BDL	BDL
7	G	BDL	269.87	BDL	BDL
8	H	BDL	280.38	BDL	BDL

TABLE-2. Effect of pasteurization (LTLT) on Cloxacillin residue in milk

Concentration of Cloxacillin residue in milk (ppb)	
Non-heat	Heat
271.23	265.52
292.35	289.12
285.67	278.56
300.83	300.15
278.28	279.86
298.56	295.34
269.87	268.32
280.38	277.79
Mean=284.64±11.78	Mean=281.83±12.25

ISSN: 0975-8585

CONCLUSIONS

In the era of global antibiotic health hazard, securing public health and to generate confidence that we are practicing medicine in their best interests is the prime target area of veterinarians. Both veterinarians and food producers have become extremely conscious of the need for high public confidence in the products they produce. The surveillance study in target areas indicated that a large number of antibiotics were frequently used for treating various infections in dairy animals. The residue concentration of cloxacillin in all milk samples of day-1 was beyond the maximum residual value (10ppb) as stated by Food and Drug Administration and the Japan Food Chemical Research Foundation. However the residue concentration of cloxacillin in milk samples of day-0, day-3, and day-5 were found below detection limit. The findings substantially indicated that a withdrawl period of at least 24 hrs is needed after administration of cloxacillin and thereby milk from such animals should not be used for human consumption for 24 hours post-treatment. The finding was under agreement with many studies relating with heat stability of other antibiotics which showed that pasteurization did not destroy antibiotic residue.

Acknowledgment

The authors are thankful to Indian Council of Agricultural Research (ICAR), New Delhi for funding the study.

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