

Residue analysis of ciprofloxacin in milk of dairy animals by LC-FD method

S. K. MODY¹, H. B. PATEL², R. D. SINGH³, H. A. PATEL⁴, D. R. KAMANI⁵,
S.I. BHATIYA⁶, RASHMIKA R. DESAI⁷ AND S. R. VYAS⁸

Department of Pharmacology and Toxicology
College of Veterinary Science & Animal Husbandry
S. D. Agricultural University, Sardarkrushinagar - 385 506 (Gujarat)

Received : 09 June, 2015

ABSTRACT

Increasing use of antimicrobials to combat bacterial diseases among dairy animals has imposed the threat of antimicrobial drug residues in milk of dairy animals. Monitoring of drug residues in foods of livestock animal is essential to establish benchmark for quality of livestock food including milk. The MRL (maximum residue limit) of ciprofloxacin in bovine milk is 0.1 ppm as recommended by European Commission regulations. The present study was executed to establish the residual concentration of ciprofloxacin, an antimicrobial drug of fluoroquinolone group, in milk samples collected from different area of Northern Gujarat. Milk samples were collected from 49 dairy animals from unorganized dairy farms and farmers' door steps located at two districts of North Gujarat namely, Banaskantha and Aravalli. The concentration of ciprofloxacin in milk was analysed using validated high performance liquid chromatography (HPLC) with fluorescent detector (LC-FD). Mobile phase contained mixture of acetonitrile and buffer (50:50) and kept at the flow rate of 2.0 ml/min. Excitation and emission wave length were 295 and 500 nm, respectively. Limit of quantification (LOQ) and limit of detection (LOD) were 0.01 µg/ml and 0.003 µg/ml, respectively. Out of 49 milk samples, none was found to contain the detectable concentrations of ciprofloxacin.

Key words : Ciprofloxacin, milk residue, dairy animals

Dairy production system should be planned and managed in a way that the exposure of milk producing animals to veterinary drugs should not pose a risk to human health through consumption of such milk. Residue level of any veterinary drug above its MRL (Maximum Residue Level), as established by the concerned regulatory authorities of respective countries, is likely to cause adverse effects in consumers. Globally, various regulatory agencies like Codex alimentarius, US-FDA (Food and Drug Administration), European Commission (EC) and FSSAI (Food Safety and Standard Authority of India) recommend legal MRL for veterinary drug residues in foods of animal origin like milk and meat. Indiscriminate use of antimicrobials with overdosing

and non-compliance of withdrawal periods for milking, are the main reasons responsible for causing drug residual concentration in milk above established MRL. Antimicrobial milk residues may exert an adverse effect on consumers in a number of ways, such as toxicological adverse effects (intrinsic nature of antimicrobial), adverse effects on beneficial microflora of the gastrointestinal tract of consumers and drug allergic reactions.

Ciprofloxacin is a second generation fluoroquinolone antimicrobial having broad spectrum activity against many veterinary pathogens. Additionally, it is major metabolite of enrofloxacin, a veterinary fluoroquinolone, and thus, can be present as residues in milk (Choma, 2006). Ciprofloxacin act on pathogens by blocking their DNA synthesis through inhibition of DNA gyrase and topoisomerase IV enzymes. Ciprofloxacin is used commonly for the treatment of pneumonia, metritis, mastitis and other infections

¹Professor and Head, ² & ³Assistant Professor, ⁴Veterinary Officer, LITC, ⁵Veterinary Officer, Dept. of Animal Husbandry, Govt. of Gujarat, Rajkot, ⁶Ph.D. Scholar, Veterinary College, DUVASU, Mathura, ⁷P.G. Student and ⁸Dean, College of Basic Science and Humanities

caused by susceptible bacteria in dairy animals. Such a wide spread use is likely associated with presence of residual concentration in milk of dairy animal at field level. In a pharmacokinetic study, ciprofloxacin was found above therapeutic concentration in milk of lactating goats for more than 10 hours following single intramuscular administration at dose rate of 5 mg/kg body weight and showed a cumulative behaviour in milk after repeated administrations (El-Banna, and Abo, 1998). European Union has set Maximum Residue Limits (MRLs) for quinolones like ciprofloxacin and enrofloxacin in animal products such as milk, with the aim of minimizing the risk to human health associated with their residue consumption. Common toxic reactions of ciprofloxacin in humans involve gastro-intestinal tract, central nervous system and haematological system. It is also reported as cardiotoxic and hepatotoxic (Adikwu and Brambaifa, 2012). For actual risk assessment and regulation of drug residues in milk, it is necessary to determine the prevailing level of different milk residues by monitoring them. Screening for drug residue is necessary to ensure that foods or dairy products containing violative residues are not introduced into the food chain (Mitchell *et al.*, 1998). Thus, the present study was undertaken for the analysis of ciprofloxacin residues in dairy animals' milk in order to monitor the residual concentration of ciprofloxacin in their milk. Limit of quantification (LOQ) and limit of detection (LOD) were 0.01 µg/ml and 0.003 µg/ml, respectively.

MATERIALS AND METHODS

A total of 49 milk samples of cows (29) and buffaloes (20) were collected randomly from unorganized farms and farmer's door steps. The area of collection included two district of North Gujarat namely, Banaskantha and Aravalli. Approximately, 10 ml of milk sample was collected in milk collection bottle from each animal. The samples were labelled properly and transported to the laboratory in thermo-cooled container jacketed with ice and stored in the refrigerator at -40°C till further processing. The concentration of ciprofloxacin in milk was analysed using LC-FD (Liquid Chromatography with Fluorescent Detector) with the excitation and emission wavelengths of 295 and 500 nm, respectively. Mobile phase comprised of mixture of acetonitrile and buffer (50:50) and kept at flow rate of 2.0 ml/min. Buffer was prepared by mixing 560 µl of phosphoric acid and 550 µl of triethylamine in one litre of HPLC water. For sample extraction and preparation, 5 ml acetonitrile and 0.5 ml phosphoric

acid were mixed with 5 ml milk and vortexed for 1 minute. Then mixture was centrifuged at 4000 RPM for 10 minutes and 1 ml supernatant was mixed with equal volume of dichloromethane. It was then vortexed and re-centrifuged at 10000 RPM at 4°C. Finally, aqueous layer was separated and 20 µl processed sample was injected into HPLC machine. The peak area was used for calculation of concentration on the basis of standard curve.

RESULTS AND DISCUSSION

In the present ciprofloxacin residue analysis by LC-FD, the linearity of the analytical method was proved at seven different level of concentration (in duplicates) in milk samples ranging from 0.01 to 10 µg/ml, and value of the mean co-relation co-efficient (R^2) was found to be 0.9591 (Figure 1). Limit of quantification (LOQ) and limit of detection (LOD) were 0.01 µg/ml and 0.003 µg/ml, respectively. Recovery of ciprofloxacin from known milk standards were above 80%. Representative chromatogram for the analysis of standards is shown in Figure 2. Established MRL (Maximum Residue Level) for ciprofloxacin and enrofloxacin in milk of all animal species is 100 µg/kg (*i.e.* 0.1 ppm) set by Regulation No. 99/508 of European Union legislation (European Commission, 1999). Many methods have been developed and validated for the estimation of ciprofloxacin residues in milk *viz.* optical immunobiosensor assay (Mellgren and Sternesjo, 1997), HPLC-DAD (Cinquina *et al.*, 2003), HPLC and TLC with direct bioautography (Choma, 2006), fluorimetric spectrometry method (Hui-Ping *et al.*, 2006), capillary electrophoresis-electrochemiluminescent method (Zhou *et al.*, 2008), LC-UV, LC-FD, LC-MS and LC-MS/MS (Hermo *et al.*, 2008) and indirect competitive ELISA (Huang *et al.*, 2010) but reports on presence of ciprofloxacin residues in milk samples from field is lacking. In the present study, all 49 milk samples were found to be negative for ciprofloxacin residues.

CONCLUSION

A validated LC-FD method was used to monitor the ciprofloxacin residue levels in the milk of milching animals. Out of 49 milk samples collected from dairy animals, none was found to contain the detectable concentrations of ciprofloxacin above its MRL. It is concluded that the milk samples collected in the study do not possess any harmful residual concentration of ciprofloxacin.

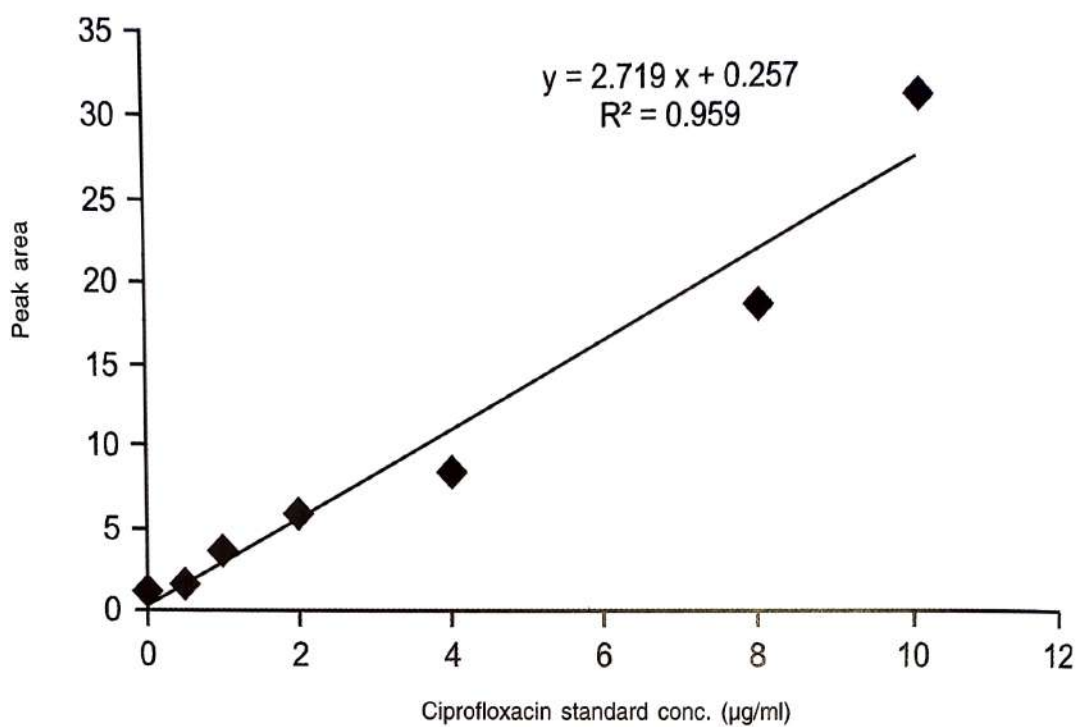


Fig. 1 Standard curve of ciprofloxacin spiked in milk (X-axis shows concentration in µg/ml whereas Y-axis indicates the peak area)

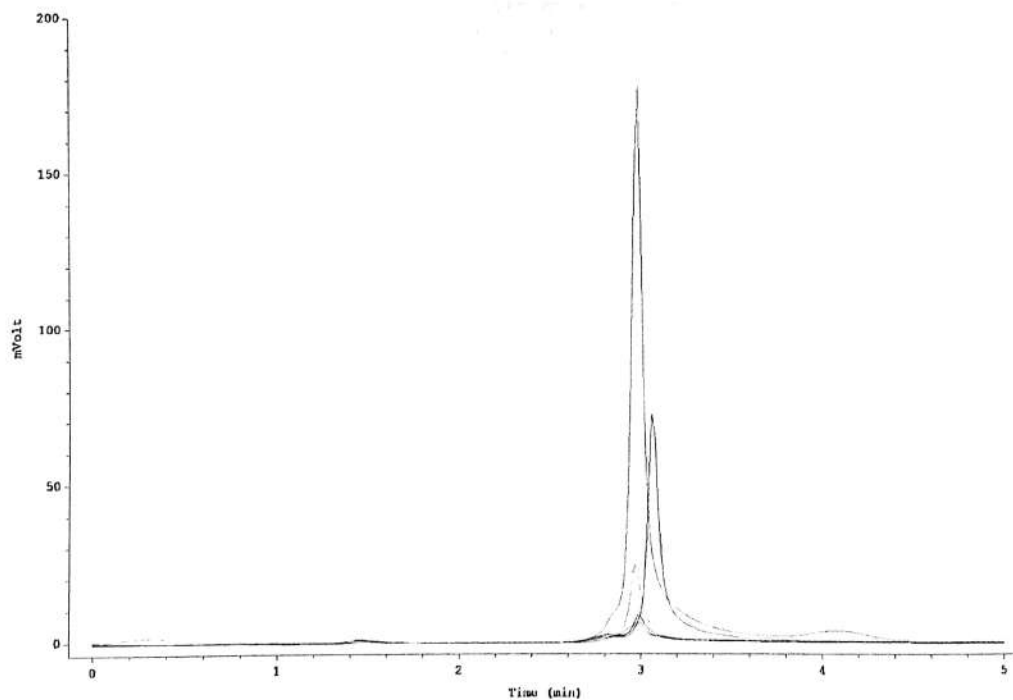


Fig. 2 Chromatogram of ciprofloxacin standards

ACKNOWLEDGEMENT

Authors sincerely thank the Director of Research, S.D. Agricultural University, Sardarkrushinagar, for providing necessary facilities for the research under ICAR project entitled 'Monitoring of Drug Residue and Environmental Pollutant'.

REFERENCES

- Adikwu, E. and Brambaifa, N. 2012. Ciprofloxacin cardiotoxicity and hepatotoxicity in humans and animals. *Pharmacology and Pharmacy*, **3**: 207-213.
- Choma, I. M. 2006. Screening of enrofloxacin and ciprofloxacin residues in milk by HPLC and by TLC with direct bioautography. *Journal of Planar Chromatography*, **19** (108): 104-108.
- Cinquina, A. L., Roberti, P., Giannetti, L., Longo, F., Draisci, R., Fagiolo, A. and Brizioli, N. R. 2003. Determination of enrofloxacin and its metabolite ciprofloxacin in goat milk by high-performance liquid chromatography with diode-array detection: optimization and validation. *Journal of Chromatography A*, **987**(1): 221-226.
- El-Banna, H. A., and Abo, E. S. K. 1998. Disposition kinetics of ciprofloxacin in lactating goats. *Deutsche Tierärztliche Wochenschrift*, **105**(1): 35-38.
- European Commission. 1999. Annex. I . Milk. In: Regulation 99/508 dated 9th March 1999, *Official Journal of the European Communities*, **9.3**. 1999, L60/20.
- Hermo, M. P., Nemutlu, E., Kr, S., Barrón, D. and Barbosa, J. 2008. Improved determination of quinolones in milk at their MRL levels using LC-UV, LC-FD, LC-MS and LC-MS/MS and validation in line with regulation 2002/657/EC. *Analytica Chimica Acta*, **613**(1): 98-107.
- Huang, B., Yin, Y., Lu, L., Ding, H., Wang, L., Yu, T. and Zhang, Y. Z. 2010. Preparation of high-affinity rabbit monoclonal antibodies for ciprofloxacin and development of an indirect competitive ELISA for residues in milk. *Journal of Zhejiang University Science B*, **11**(10): 812-818.
- Hui-ping, X., Xue-zhi, D., Wei-ping, H., Jian-hao, X. and Yan-yan, L. 2006. Surfactant sensitized fluorimetric determination of ciprofloxacin residue in milk. *Chinese Journal of Antibiotics*, **31**(5): 285.
- Mellgren, C. and Sternesjo, A. 1997. Optical immunobiosensor assay for determining enrofloxacin and ciprofloxacin in bovine milk. *Journal of AOAC International*, **81**(2): 394-397.
- Mitchell, J.M., Griffiths, M.W., McEwen, S.A., McNab, W.B. and Yee, A.J. 1998. Antimicrobial drug residues in milk and meat: cause, concerns, prevalence, regulations, tests, and test performance. *Journal of Food Protection*, **61**: 742-756.
- Zhou, X., Xing, D., Zhu, D., Tang, Y., and Jia, L. 2008. Development and application of a capillary electrophoresis-electrochemiluminescent method for the analysis of enrofloxacin and its metabolite ciprofloxacin in milk. *Talanta*, **75**(5): 1300-1306.