J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (10): 571 - 577, 2012

IMPACT OF SOME TECHNOLOGICAL FACTORS ON THE STABILITY OF CIPROFLOXACIN RESIDUES IN MILK

El-behairy, S. A.
Medicinal Foods Dept., National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

ABSTRACT

The effect of the technological steps used in making and during the storage at 6°C for 15 days of concentrated yoghurt (Labneh) from reconstituted skim milk being spiked with veterinary residues, namely ciprofloxacin at concentration 100 ppm to study the behavior of this residues. In addition, the effect of the technological factors on residues stability during storage period was studied. Samples were analyzed for total solids, pH, organic acids namely formic, pyruvic, lactic and acetic acids and drug residues recovery at time intervals after 3, 7, 10 and 15 days. The obtained results revealed that the presence of the ciprofloxacin residues had no effect on the chemical parameters of concentrated yoghurt (Labenh) namely total solids, pH and the recoveries of organic acids. On the other hand, data indicated that this drug showed high thermal stability by separation using HPLC under the conditions of this study. The recovery of ciprofloxacin residues after 15 days of storage period may indicate that the reaction between drug residues and protein is chemical reaction.

Thus, the application of HACCP system in milk production and processing must be taken into consideration to insure a safe product for the consumer.
Keywords: Labneh, ciprofloxacin, residues, HPLC, organic acids).

INTRODUCTION

Veterinary antibiotics are widely used in dairy cattles management for the treatment of diseases and as dietary supplements (Franks *et al*., 1998 and Dicorcia, *et al*., 2002). Environmental risk of veterinary medicines is assessed according to different regulations depending on whether the applications is therapeutic or non-therapeutic (Jahed, 2007).

This widespread use of antibiotic may cause residues in food stuffs as well as milk and dairy products. Because of the special importance of milk in the diet of infants, children and also adults special attention is focused on the presence of these residues, which may find their way to milk and dairy products (Papai, *et al*., 2009).

Only few drugs are known to undergo active transport, not less than 18 factors that affect the rate and the extent of drug excretion in milk and subsequent consumption. Consumption have been identified (Rossi and Seottwright, 1997). These factors fall into six general areas: (1) The pharmacology of the drug in animals, (2) The physiology of the udder, (3) The milk consumption, (4) The nutritional demands and pharmacology of the consumer, (5), The physical-chemical properties of the drug in the matrix, (6) The effect of the technological processes. Three of the most important biopharmaceutical factors that influence lactil drug excretion are protein binding, ion trapping and lipid solubility (Aniello *et al*., 2002).

In veterinary practice quinolones group (ciprofloxacin) has teen recommended for the treatment of several infections in cattle, i.e. infections of respiratory and alimentary tract. The recommended doses are 2.5-5 mg/kg.bw/daily for 3 to 5 days. Ciprofloxacin is administered in veterinary medicine by subcutaneous injection or orally to cattle and buffalo's for the treatment of mastitis (Hemandez *et al*., 2000). The toxiclogical ADI of ciprofloxacin is 30 ug/kg body weight calculated by applying a safety factor of 100 to the NOEL of 3 mg/kg body weight/day of a dietary 90 day repeated dose study (Boutsoglou and Fletouris, 2001).

However, veterinary residues in dairy products may react with its constituents, and thus suppress its utilization. This assumption will be concerned intensively in the present study which including the effect of ciprofloxacin on the quality of concentrated yoghurt, in the meantime, the stability of these residues upon the technological process of this product and during storage period.

MATERIALS AND METHODS

Skimmed milk: was obtained from Arab cultivator company, Egypt. Ciprofloxacin was obtained from raw materials dept., National Organization for Drug Control and Research (NODCAR).

Reconstituted skim milk was spiked with the drug understudy in concentration of 100 ug/L at 35^{o}C and incubated for 4 hr. as equilibrium time. All experiments for ciprofloxacin were done using skimmed milk as a control. Labneh manufacture was carried out as mentioned by Tamime, *et al*.,(1978). pH was measured according to the method described by the A.O.A.C. (2007) using laboratory pH meter with a glass electrode. Total solids was determined according to the method described by the A.O.A.C. (2007). Organic acids, namely formic, pyruvic, lactic and acetic acids were estimated as described by (Guzel, *et al*., 2000).

The residues of ciprofloxacin was determined according to the method described by (Anadon, *et al*., 2001).

For the ciprofloxacin extraction, ciprofloxacin was added to skimmed milk, equilibrated then mixed with the extraction solution (dichloromethane: phosphate buffer pH 7.0, 0.1 M (3:1) (Anadon, *et al*., 2001). The ciprofloxacin was extracted 3 times and the collected solution was concentrated. The obtained residues were performed by HPLC (Perkin Elemer system quaternary pum). For ciprofloxacin variable wave length detector operated at 277 nm was used and data station software turbochrome with Rheodyne injector of 20 ul-loop were analyzed. The analytical separation was achieved on Lichrosphere RPIS (125 x 5 mm ID). The mobile phase was 0.02 M phosphate buffer pH 25 methanol (85/15 v/v).

Data were statistically analyzed using New State-Multiple Comparison according to the method of described by (Steel and Tenrie, 1980).

RESULTS AND DISCUSSION

Generally, the idea in this work was to study the behaviour of veterinary drug residues understudy, namely ciprofloxacin incorporated with skimmed milk used in labenh during labneh manufacture and storage period. The recovery of ciprofloxacin residues (Figs, 1,2 and 3) , during concentrated yoghurt manufacture and storage periods at the initial time 3,7,10 and 15 days, respectively, proved that these residues were not affected. It was noticed from the plotted curves figures (1, 2 and 3) that no change in the stability of drug residues. A decrease in the absorbance after 15 days was about 40%, compared with initial (0 time) was noticed with constant position on the main peak of ciprofloxacin. One may thus conclude that the decrease in the absorbance might be due to the nature of protein to bind or the possibility of certain interaction between milk protein and drug residues.

Data in table (1) show the effect of ciprofloxacin residues on the recovery of organic acids namely, formic, pyruvic , lactic acid, acetic and citric acids for the tested samples of concentrated yoghurt with negative control (without drug) and in the presence of drug residues (positive control). The tested samples were analysed at the initial time(0 time) and after 3, 7, 10 and15 days of storage period. The obtained results after 15 days of storage period revealed mild increase in organic acids namely lacic, acetic and citric acids in comparison with the initial of spiked samples (positive control). On the other hand, insignificant values were noticed in the developing of formic acid and pyruvic acid values under the same conditions of experiment. On conclusion it could be revealed that the presence of ciprofloxacin residues had no effect on the organic acids content of concentrated yoghurt.

Table (2) shows the results concerning the effect of ciprofloxacin residues on the total solids content of concentrated yoghurt (Labneh). Generally, the obtained data after 15 days show mild increase of total solids values in comparison with the spiked sample (positive control) in the initial time being 11.7 to 11.6 and 10.0 to 10.2 respectively.

Results presented in table (2) show that mild decrease (significant) in pH values between the initial time and by prolongation of storage period up to 15 days in the spiked samples (positive control) being 2.24 to 1.86 against 2.36 to 1.38 for the negative control. It is also obvious that the incidence of drug residues in concentrated yoghurt had no effect on the pH values between the manipulated samples and control. The fluctuation in the pH values of manipulated samples might be due to the demonstrated ability of lactic organisms during the course of experiment.

One may thus conclude that the presence of ciprofloxacin residues in concentrated yoghurt samples had a mild effect on total solids contents, and pH values.

Effect of technological processes and storage period on ciprafloxain residues

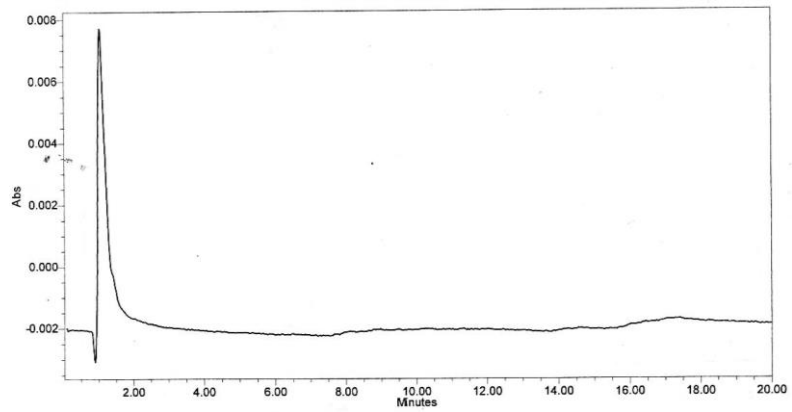

Figure (1): Control drug (100 ppm)

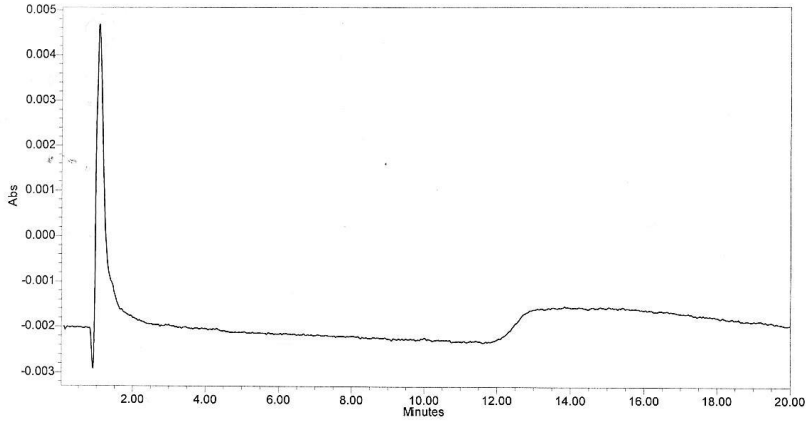

Figure (2): Initial (0) time

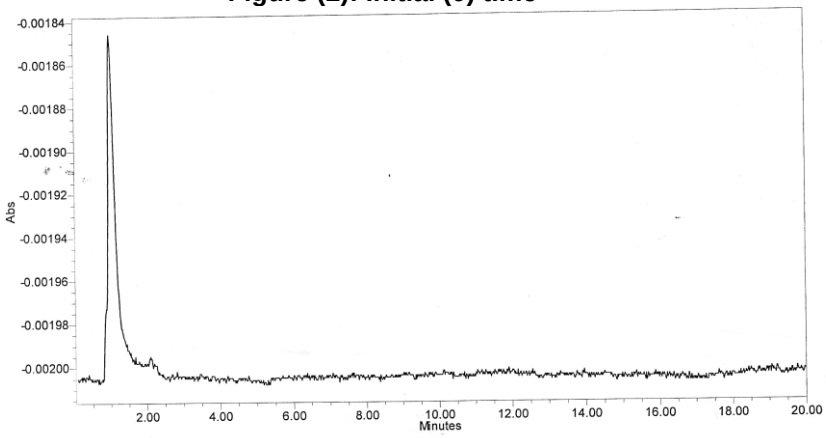

Figure (3): after 15 days

Table (1): Effect of ciprofloxacin residues on organic acids (%) of concentrated yoghurt made from spiked skimmed milk with residues (100 μg/lit) during storage period at 6°C.

Item	Treatment schedule	time Intervals				
		Initial 0 time	3	7	10	15
Formic Acid	Negative control (free drug)	11.7+0.03	9.8+0.29	8.8+0.1	8.76+0.16	11.6+0.38
	Positive Control (with drug)	10.0+0.82	9.38+0.46	8.48+0.46	10.36+0.49	10.2+0.22
Pyruvic Acid	Negative control (free drug)	2.36+0.05	2.30+0.10	2.17+0.06	2.39+0.14	1.38+0.05*
	Positive Control (with drug)	2.24+0.12	2.35+0.18	2.17+0.08	1.47+0.24	1.86+0.14
Lactic Acid	Negative control (free drug)	4.18+0.09	4.4+0.16	4.87+0.21	4.78+0.18	4.06+0.05
	Positive Control (with drug)	5.39+0.14	6.08+0.04	5.59+0.15	6.29+0.11	6.33+0.06*
Acetic Acid	Negative control (free drug)	11.17+0.11	10.99+0.13	8.73+0.08	9.66+0.12	8.98+0.09
	Positive Control (with drug)	10.44+0.13	9.3+0.09	0.22+0.04	9.07+0.24	9.55+0.23*
Citric Acid	Negative control (free drug)	0.74+0.08	1.9+0.02	1.2+0.04	1.73+0.06	0.97+0.06*
	Positive Control (with drug)	1.35+0.06	1.40+0.06	1.44+0.1	0.71+0.05	1.33+0.06*

*** Significant (P < 0.01) with –ve control of each organic acid.**

Table (2): Effect of ciprofloxacin residues on total solid (%) and pH values of concentrated yoghurt made from spiked skimmed milk with residues (100 ug/l) during storage period at 6°C.

Item	Treatment schedule	Interval time				
		Initial 0 time	3	7	10	15
Total Solid (%)	Negative control (free drug)	11.7+0.03	9.8+0.29	8.8+0.1	8.76+0.16	11.6+0.38
	Positive Control (with drug)	10.0+0.82	9.38+0.46	8.48+0.46	10.36+0.49	10.2+0.22*
pH	Negative control (free drug)	2.36+0.05	2.30+0.10	2.17+0.06	2.39+0.14	1.38+0.05
	Positive Control (with drug)	2.24+0.12	2.35+0.18	2.17+0.08	1.47+0.24	1.86+0.14*

*** Significant (P < 01) with –ve control of total solid and pH * Significant with –ve control of each organic acid.**

Generally, from the foregoing result, it could be concluded that our results are in agreement with Papai, *et al*., (2007), who reported that the presence of milk protein decrease the amount of bioavailable ciprofloxacin. In addition, different researchers (Aramayona *et al*., 1996, El-Banna and Abou El-Soaud, 1988 and Malbe *et al*.,1996) found that the ciprofloxacin is bounded to plasma serum protein.

Conclusion:

From the foregoing results, it could be concluded that the presence of ciprofloxacin had no effect on organic acid namely formic acid, pyruvic, lactic , acetic and citric acids. On the other hand the residues of ciprofloxacin had no effect on the content of total solids and pH values. In addition, the obtained data revealed that chemical reaction might occur between drug residues and milk protein. It could also be concluded that the aforementioned residues understudy were not affected during the manufacture concentrated yoghurt steps and stage period. Therefore, we recommend to monitor the veterinary drug residues in the dairy products to achieve a safe food.

REFERENCES

A.O.A.C. (2007). Official methods (18th ED). Helrich, K., and W. Hornitz, (eds). Pub Association of Official Analytical Chemists. Washington, D.C, U.S.A.

Anadon, A.; Martinez, M.R.; Larrangga, Iturbe, J., Diaz, M.J.,Frejo, M.T. (2001). Pharmacokinetics and residues of ciprofloxacin and its metabolities in broiler chickens.Veterinary Science, 71, 101-109.

Aniello Anastasio, Mauro Esposite, Michele Amorena, Paolo Catellani, Luigi Serpe,and Makia Luisa Cortesi, (2002). Residues study in plasma, mil, and mozz-arella cheese following subcutaneous administration to buffo's milk. (*Buballus subalis*). J. Agric. Food Chem., 50, 5241-5245.

Aramayona, J.J.; Mora, J., Fraile, L.J., Garcia, M.A.;Abadia, A.R. and Bregane, M.A. (1996): Penetrating of enrofloxacin and ciprofloxacin into breast milk and pharmacokinetics of the drug in lactating rabbits and neonatal offspring. Am. J. Vet. Rec. 57:547-553.

Boutsoglou, N.A. and Fletouris, D.J.(2001). Drug residues in foods. Marcel Dekker, Inc., New York.

Di Corcia A. and Nazzari M.,(2002). Liquid chromatographic mass spectrometric methods for analyzing antibiotics and antibacterial agents in animals food products. Journal of Chromatography A 974 (2002), p.p. 53-89.

El-Banna,H.A. and Aboel-Sooud, K. (1988): Kinetic of ciprofloxacin in lactating grates. Deutsche Tierarztl. Wschr 105: 35-38.

Frank J. Schenck and Pattrick S. Callery (1998). Chromatographic methods of analysis of antibiotics in milk. Journalof Chromatography A, 812,99-109.

Guzel-Seidim, Z.B., Seidim,A. C., Greene, A.K. and Bodine, A.B.(2000). Determination of organic acids and volatile flavor substances in Kefir during fermentation. J. of Food Composition and Analysis, Vol. 13, Issue 1, February (2000), pages 35-49.

Hemandez Arteseros JA, Compano R., Ferrer R and Part Med (2000), Application of principal components regression to luminescence data for the screening of ciprofloxacin and enrofloxacin in animal tissues. Analyst, June,125 (6):1155-8.

Jahed Khaniki, 2007. Chemical contaminant in mil and public health concerns a review. International Journal of Dairy Science 2:104-115.

Malble, M.; Salonen, M.M.; Fang, W.; Oopik,T.; Jalaks, M.; Kiassen, M. and Sandholm, M. (1996). Disposition of enerofloxacin into the udder after intervenous and infraarterial injections into dairy cows. J. Vet. Med. A. 43:377-380.

Papai K, Ludanyi K, Budai M, Antal L, Klebovich L. In vitro studies on ciprofloxacin-milk interaction using LC-MS methodl. Acta Pharm. Hung., 2007; 77 (1) 59-64.

Papai K.,Ludanyik K.,Budii M, Antal I, Klebovich I, (2009). In vitro food-drug interaction study: which milk component has a decreasing effect in the bioavailability of ciprofloxacin. J. Pharm. Biomed. Anal.May; 52(1): 37-42.

Rossi,T.D. and Scottwright, D. (1997). Analytical considerations for trace determinines of drug in breast mil. J. Pharmaceut. Biomed. Anal. 15:495-504

Steel R.G.D. and Torrie, J.H. (1980): Principles and procedures of Statistics, 2nd ed. McGraw-Hill Book Company, New York, USA.

Tamime, A.Y. and Robinson, R.K. (1978). Some aspects of the production of a concentrated yoghurt (Labneh) popular in the Middle East. Milehwiss-schaft, 33, 209-212 (1978).

تأثير بعض العوامل التكنولوجية على ثبات متبقيات السيبروفلوكساسين فى اللبن

سامى أحمد البحيرى
قسم الأغذية الطبية، الهيئة القومية للرقابة والبحوث الدوائية، الجيزة

تم دراسة الخطوات التكنولوجية المستخدمة فى إنتاج اليوجورت المركز (اللبنة) المصنع من لبن فرز تم حقنه بمتبقيات السبيروفلوكساسين بتركيز ١٠٠ جزء بالمليون وكذا خلال التخزين على درجة ٦°م لمدة ١٥ يوم. وتم تحليل جميع العينات بتقدير المحتوى من الجوامد الصلبة، الpH، والمحتوى من الأحماض الفورميك، البيروفيك، اللاكتيك والخليك، وإستخلاص متبقيات السبيروفلوكساسين بالعينات الطازجة وبعد ٣، ٧، ١٠ و١٥ يوم. وقد أوضحت النتائج عدم وجود تأثير لتلك المتبقيات على الصفات الكيماوية التى تم دراستها خلال الإنتاج والتخزين، فى حين لوحظ بعض التغيرات الطفيفة بالمقارنة بالكونترول. كما إتضح من نتائج التحليل الكروماتوجرافى (HPLC) أن المتبقيات المختبرة قد تميزت بالثبات الحرارى خلال الخطوات التصنيعية والتخزين لفترة إمتدت ١٥ يوما، وأن التفاعل بينها وبين الروتين كيماويا. ويوصى بإستخدام نظام المراقبة (HACCP) خلال إنتاج اللبن ومنتجاته للحصول على غذاء لبنى آمن للمستهلك.

قام بتحكيم البحث

أ.د / طه عبد الحليم نصيب	**كلية الزراعة – جامعة المنصورة**
أ.د / ماهر احمد نور	**كلية الزراعة – جامعة الازهر**