QUANTITATIVE DETERMINATION OF NORFLOXACIN RESIDUES AND EFFECT OF BIOL AND FREEZING IN CHICKEN TISSUES

M. O. Aboul El-Nile and F. E. Gaber

Department of Food Hygiene, Animals Health Institute, Alexandria.

Animal Health Institute, Zagazig, Egypt

ABSTRACT

Fifty tow apparently healthy one day old. At fourth week of age chicks were used in this study injected intramuscularly with norfloxacin at a dose of 5 mg/Kg body weight for 5 successive days for detection of norfloxacin residues, eight broilers were slaughtered on (1st, 3rd, 5th, 7th and 9th day) after last dose. Muscles (breast, thigh) fat, liver and gizzard were examined before and after boiling (100°C for 30 minutes) for determination of norfloxacin residues and studying effect of heat treatment on its persistence, and samples from muscles (breast, thigh) fat liver and gizzard of 12 broilers were examined before and after freezing at -18°C weekly for quantitative determination of norfloxacin residues using HPLC. The results showed that norfloxacin highest concentration level was detected in breast muscle at 1st day from last dose (39.21 ± 3.012 ug/g) and decreased till not detected at 9th day followed by liver (27.23 ± 2.321 ug/g) and fat 25.15 ± 1.951 ug/g) and its level was showed significant decrease at (P < 0.05) till not detected at 9th day. Lowest concentration level showed in Gizzard and thigh (17.32 ± 1.615 and 12.5 ± 1.033 ug/g) and not detected at 7th day.

Regarding to the effect of boiling of the drug residues in breast muscle at 1st day, it is evident that norfloxacin concentration were decreased from (39.21 ± 3.012 to 10.57 ± 0.89 ug/g). Such decrease was detected in the samples examined at the following days until disappeared at 7th day of treatment.

Meanwhile frozen breast muscle samples at -18°C showed significant decrease after 1st week and decreased till not detected at 5th week. (6.55 ± 0.764, 4.833 ± 0.828, 1.486 ± 0.982, 0.3 ± 0.051ug/g) at 1st, 2nd, 3rd, 4th week respectively followed by fat, liver (4.607 ± 0.561, 4.230 ± 0.42 ug/g) decreased till not detected at 5th week. Gizzard, thigh level were (4.230 ± 0.42, 3.897 ± 0.484 ug/g) and decreased weekly till not detected at 4th week.

INTRODUCTION

The modern poultry industry uses much antimicrobial medications to control poultry diseases or to improve their performance, some antibiotics are given to give better weight gain, feed conversion and egg production. However, much medication are used without adequate consideration and much are probably in effeetive or even wasted. Quinolones are a series of synthetic antibacterial agents. The first of which was nalidixic acid introduced in 1964. The first generation quinolones includes nalidixic aeid, oxolinic acid and flumequine. They are active against gram negative organisms and their use is limited to the treatment of urinary and enteric infection. Further synthesis and investigation have given rise to second generation quinolones (fluoroquinoloncs) with extend antibacterial activity and includes enrofloxacin, ciprofloxacin, danofloxacin and norfloxacin **(Brander et al., 1991)**.

Norfloxacin is a new synthetic antimicrobial agent of fluoroquinolone class. It acts principally by inhibiting bacterial DNA gyrase which is necessary for supercoiling of DNA to provide a suitable spatial arrangement of DNA within the bacterial cell **Palumobo et al., 1993 and Shem & Ziv 1993)**.

The extensive use of these drugs during the whole life time of birds give rise to the problem of drug residues. The "residue problem" is the focus of public concern which introduces a serious and novel hazard to the human beings. Some of meat hygienists dealt with some meat treatment for withdrawal of antibiotic residue such as freezing and heating.

Cooking or cold storage may alter the chemotherapeutic residues in the edible tissues **(Sarkisov & Eshov, 1972 and Ionova & Zhecheva, 1997)**.

The present study was conducted to detect the possible residues of norfloxacin in chicken tissues and the effect of heating and freezing storage on norfloxacin residues.

MATERIAL AND METHODS

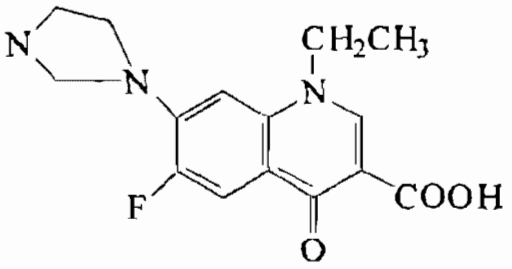
Material

1- Drug

Norfloxacin (floxatril 10%) (R)

Norfloxacin (floxatril 10% injeetable solution 100 ml-pantex Holland B.V.).

It is active against a wide range of Gram positive, Gram negative and Mycoplasma. The chemical name of norfloxacin is (3-quinolone carboxylic acid, 1-ethyl-6-fluoro-1, 4-dihydro-4.oco.7,1-piperozinyl).



Structural formula (After She n and Ziv, 1993)

- Chemical formula: $C_{22}H_{23}FN_4O_5$
- Dose: Norfloxacin is available in an injectable solution to be given subeutaneously or intramuscularly at a dose of 2.5-5 mg/kg body weight. Eaeh ml solution eontains 100 mg norflaxaein.

II- Experimental chicks

(1) Fifty-two apparently healthy, one day old chicks were obtained from El.Khahera farm in 10th of Ramadan city.

They were fed on a balaneed coinmercial ration free from any medications and water was provided adlibitum. All hygienic measures were adopted as reeonimended. Temperature was adjusted aecording to the age (star at 32°C and decreased 2°C each week).

Methods

(1) All chicks were vaccinated against Newcastle disease on 6th day of age and against Gumboro disease on 15th day of age.

(2) At the 4th week chicks were injected intramuscularly with norfloxacin at dose of 5 mg/kg body weight for 5 successive days for detection of drug residue.

(3) Eight broilers were slaughtered on (1st, 3rd, 5th, 7th and 9th day) after the last dose. Muscles (breast, thigh) fat, liver and gizzard and samples were examined before and after boiling (100°C for 30 minutes) for determination of norfloxacin residues and studying effect of heating on its persistence.

(4) Samples from muscles (breast, thigh) fat, liver and gizzard of twelve broilers chicks (each 3 chicks represent one pooling sample). Samples from breast, thigh, fat, liver and gizzard were examined before freezing and kept at -18°C then examined weekly for presence of norfloxaein residues. The time elapsed from the onset of freezing till complete disappearance of the residues was recorded.

Detection of norfloxacin residues

Norfloxacin residues were determined using high performance liquid chromatography (HPLC) knourc, Inc Germany, according to the method described by **Groeneveld and Brouwers (1986)**.

Norfloxacin was extracted from samples with dichloromethane and 0.1 M sodium phosphate buffer at pH 7.4 Chromatography was performed on an amino-exchange column with the mobile phase and tested using UV detector, UV absorbance was monitored at 278 nm.

Into a 10 ml extraction tube of 1 gm of homogenized tissue (Breast, thigh, fat, liver and gizzard) and 1 ml of 0.1 M phosphate buffer pH 7.4 were added. After adding 5 ml dichloromethane, the tube was stoppered and gently shaken at 100 cycle/min for 10 minutes and centrifuged at 4000 rpm for 10 minutes at room temperature.

After removing the aqueous layer, the organic layer was transferred into another tube and dried under nitrogen at 50°C.

The residues were dissolved in 1 ml mobile phase using a vortex mixer and sonication, before HPLC analysis. Depending on concentration, 5-20 ul was injected.

Standard preparation

Norfloxacin standard solution was prepared from drug pure 100% by dissolving a weighed amount of drug in distilled water to make stock solution.

Statistical analysis

It was carried out according to **Snedecor and Cochran (1967)**.

RESULTS AND DISCUSSION

Residues in meat of animals and poultry forms a great problem facing food hygienists and constitute a real hazard to human consumers in the recent years. These residues are responsible for inducing allergic reactions such as urticaria, eczema and other forms of dermatitis as well as increasing resistance of pathogenic microorganisms in man. In addition to their harmful effects on the microflora and consequently the produced vitamins **(Mol, 1971)**. Growth promoters are mostly given during the whole life time, while propbylatic and therapeutic regimen of antibiotics are given only for a short period **(Van Schothors et al, 1978)**. Organs such as liver and kidney, remove residual drug and greatly reduce the content in meat. These organs and tissues are tested for detecting drug residues **(Glott et al, 1979)**.

In the present study, norfloxacin administered intramuscularly to chicken at dose of 5mg/Kg weight for 5 suceessive days. Table (1) revealed that the highest concentration level of norfloxa-

cin was detected in breast muscle at the 1st day from administration of last dose (39.21±3.012 ug/g) and its level showed significant decrease (P<0.05) in the 3rd, 5th and 7th days till not detected at 9th day (22.52±2.351, 9.85±0.835 and 0.23±0.016 ug/g) respectively. The effect of boiling (100°C for 30 minutes) showed significant decrease (10.567±0.889 ug/g) at 1st day and its level decreased signifieantly (P<0.05) in 3rd and 5th till not detected at 7th day (5.863± 0.651, 1.210±0.629 ug/g) with reduction percent respectively.

The effect of frozen storage (Table 2, Fig. 6) showed significant decrease at 1st week (6.55± 0.764 ug/g) and its level decline till not detected at 5th week (4.833±0.828, 1.486 – 0.982, 0.3± 0.051 ug/g) at (2nd, 3rd and 6th week) respectively. Then followed by fat and liver at 1st week (4.607±0.561, 4.230±0.421ug/g) and its level decline till not detected at 5th week. Norfloxacin concentration level in liver showed significant decrease (Table 1, Fig. 3) till not detected at 9th day (27.23±2.321, 13 76±1.411, 1.447±0.695, 0.04±0.023 ug/g) Regarding the effect of boiling, liver concentration level was decrease at 1st day till not detected at 7th day (5.830±0.320, 3.713±0.663, 0.72± 0.053, 0.04±0.023ug/g) at (1st, 3rd, 5th, 7th) days respectively with reduction rate. Norfloxacin concentration level showed significant decrease in fat (Table 1, Fig. 4) till not detected at 7th day (25.15±1.951, 16.83 ±1.223, 0.293 ±0.012 ug/g) while boiled fat sample showed decline in norfloxacin concentration level till not detected at 7th day (4.494±0.290, 16.83±1.233, 0.293±0.012 and 0.04±0.473mg/g) at 1st, 3rd, 5th and 7th day.

Norfloxacin level in frozen liver and fat revealed significant decrease (P < 0.05) till not detected at 5th week. Table (2).

Lowest norfloxacin concentration was detected in (Table 1 & Fig. 1) at 1st day (12.5±1.033 ug/g) followed by gizzard (17.32±1.615 ug/g) showed significant decrease (P<0.05) till not detected at 7th day. Meanwhile in boiled thigh muscles the concentration level was (4.357±0.755 ug/g) and decreased till not detected at 5th day and in boiled gizzard concentration level was 3.600±0.420 ug/g till not detected at 5th day.

Norfloxacin residues were gradually disappeared with time elapsed from the onset of freezing till complete disappearance after 4th week in thigh (3.897 ± 0.484, 2.623 ± 0.162 and 0.340 ± 0.139 ug/g) and Gizzard (Table 2, Fig. 5) (4.230 ± 0.421, 0.436 ± 0.53, 0.251 ± 0.420 ug/g).

Our results are in agreement with Anadon et al (1995) they found that when norfloxacin was administered orally the concentration in breast, fat, and liver was 0.05 ug/g on the second day after the end of dosing. **Bergeron et al (1985)** reported that the concentration of norfloxacin in kidney parenchyma was 4-12 times of the serum concentration. **Scheer (1987)** reported that intravenous injection of baytril showed highest concentration in live and kidney. **Alesting (1990)** found that highest concentration were in liver, breast muscles. **Elin, (1999)** found that liver con-

tains highest concentration of ciprofloxacin followed by fat and muscles where the residues disappeared at 10th day of administration of the last dose **Elinstein et al (1994)** reported that all of the fluoroquinolones are well absorbed after oral administration fluroquinolones (FQS) are minimally protein bound and widely distributed in body tissues. **Schelbner (1972)** concluded that high concentration of penicillin G and oxytetracycline were completely inactivated by an hour heating at 90°C. Available literatures are lacking any figures concerning the effect of boiling and freezing of norfloxacin. Norfloxacin is fluoroquinolones and are similar to antibiotics in their distribution and activity. **Schelbner (1969)** stated that heating of meat at 60°C for 60 minutes had no effect on antibiotic residues, but heating to 90°C minimized to some extent the antibiotic activity. The antibiotic residues completely disappeared immediately if the meat was cooked at cooking temperature for 20 minutes. **Chunha (1972)** mentioned that normal methods of cooking destroyed aureomycin and terromycin. **Katz et al (1972)** reported that cooking of broiler tissues and organs containing chlorotetracycline residues converted the residues to isochlorotetracycline which had no known biological activity. **Vandenbrande et al (1972)** stated that cold storage of meat reduced the activity of penicillin residue. **Jukes (1973)** concluded that cooking temperature destroyed chlorotetracycline residues in meat. **Inglis and Katz (1978)** recorded that heating may cause some loss of the antibacterial activity of aminoglycosides, depending on the system in which heating was studied. **O'brein et al (1981)** studied the effects of cooking and cold storage on antibiotic residues in meat. They recorded that cooking and cold storage caused degradation of antibiotic. **Nashwa (1995)** reported that oral administration of tylosine 25 mg/Kg b.wt. twice daily for 5 successive days and boiling of chicks tissues and organs for 30 minutes completely degraded tylosine residues in all tissue samples and at 5^{th} week of freezing tylosine resides were completely disappeared from gizzard and heart and at 6^{th} week from all tissue samples. **Haagsma (1993)** concluded that the content of residues of many veterinary drugs decreased not only as a result of food preparing and processing, but also at cooled and frozen storage. **Amer et al (1994)** stated that gentamicine or netilimicine at dose of 6 mg/Kg b.wt. intramuscularly daily for 7 successive days disappeared by boiling the muscle samples for 45 minutes and freezing for one week. Moreover boiling of kidney and liver samples for 45 minutes did not destroy the residues completely. Freezing for 3 months also failed to destroy the residues in kidney completely. **Gyhan (1997)** found that apramycin sulphate residues in chicken tissues after boiling at 100°C for 45 minutes were failed to detect in liver, kidney, gizzard and fat after 48 hours from drug administration and were failed to detect in breast and thigh muscles after 72 hours from last oral dose and residues disappeared from breast and thigh muscles, liver, kidney, gizzard and fat after the third day from freezing and disappeared from skin after 2 day from freezing samples. **Poulliquen and Morvan (2002)** determined the residues of oxolinic acid (OA) and flumequine (Flu) in freeze-dried salmon muscle with attached skin, using reversed-phase

high performance liquid chromatography. They concluded that, the limits of detection and quantitative were 3.2 and 16 ng/g wet weight tissue respectively. Mean extraction recoveries of OA and Flu freeze-dried tissue were 85.5 and 85.2% respectively.

Table (1): Effect of heat treatment boiling on tissue concentrations (ug/g) of norfloxacin injected intramuscularly at dose of 5 mg/kg body weight for 5 successive days to broiler chicks (Mean ± SD) (n=4)

Time	1st D		3rd D		5th D		7th D		9th D	
	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
Breast muscles	39.21[a] ± 3.012	10.567[a] ± 0.889	22.52 ± 2.351	5.863[b] ± 0.651	9.85 ± 0.835	1.210[c] ± 0.629	0.23 ± 0.016	0	0	0
Thigh muscles	12.5 ± 1.033	4.357[b] ± 0.755	7.31 ± 0.531	1.460[d] ± 0.883	0.05 ± 0.01	0	0	-	0	0
Fat	25.15 ± 1.951	4.494[b] ± 0.290	16.83 ± 1.223	2.900[b] ± 0.269	0.293 ± 0.012	0.04[b] ± 0.473	0	0	0	0
Liver	27.23 ± 2.321	5.830[c] ± 0.320	13.76 ± 1.411	3.713[c] ± 0.663	1.447 ± 0.695	0.72[a] ± 0.053	0.04 ± 0.023	0	0	0
Gizzard	17.32 ± 1.615	3.600[d] ± 0.420	8.43 ± 0.801	1.610[d] ± 0.198	0.02 ± 0.013	0	0	-	0	0

Means with the different letters are significantly at $P < 0.05$

Table (2): Effect of frozen storage at –18°C on tissue concentrations (ug/g) of norfloxacin at injected interamuscularly dose of 5 mg/kg body weight for 5 successive days to broiler chicks (Mean ± SD) (n=4)

Time / Tissue	Before freezing	1st week	2nd week	3rd week	4th week	5th week
Breast muscles	35.21^{a} ±3.012	6.55^{a} ±0.764	4.833^{a} ±0.828	1.486^{a} ±0.982	0.3^{a} ±0.051	0
Thigh muscles	11.5^{b} ±2.033	3.897^{b} ±0.484	2.623^{b} ±0.162	0.340^{b} ± 0.139	0	0
Fat	23.15^{c} ±3.951	4.607^{c} ±0.561	2.84^{b} ±0.635	1.22^{a} ±0.121	0.01^{a} ± 0.05	0
Liver	25.31^{c} ±2.531	4.230^{c} ±0.421	2.893^{b} ± 262	1.207^{a} ± 157	0.37^{b} ±0.245	0
Gizzard	15.34^{b} ± 2.033	4.230^{c} ±0.421	0.436^{c} ± 0.053	0.251^{b} ±0.420	0	0

Means with the different letters are signifieantly at $P < 0.05$

The mean represent 4 trails analysis for pooling samples of 3 individual chick

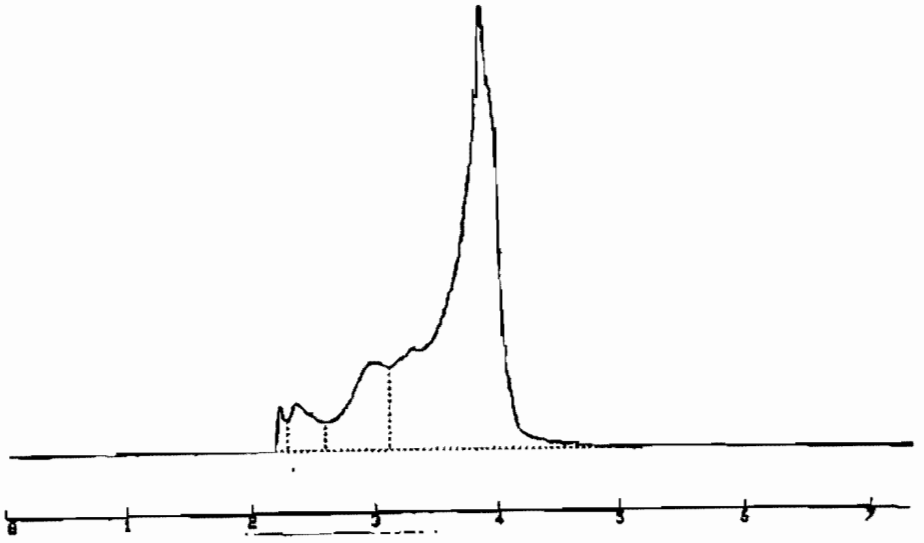


Fig. (1): Chromatograph of norfloxacin concentration (ug/g) in thigh at 1st day from last dose.

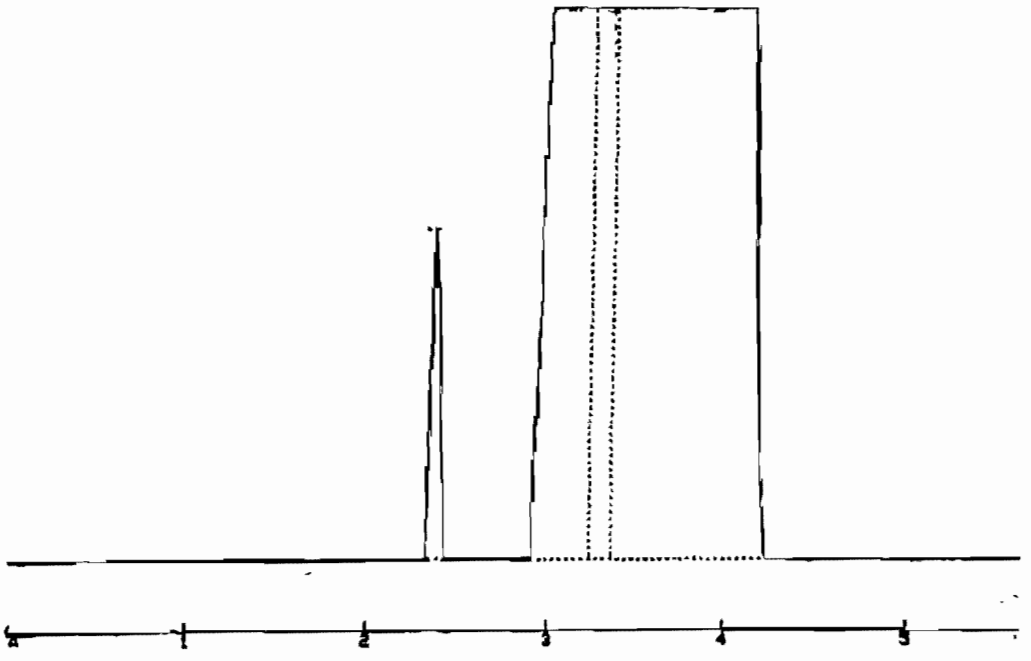


Fig. (2): Chromatograph of norfloxacin concentration (ug/g) in boiled fat at 1st day from last dose.

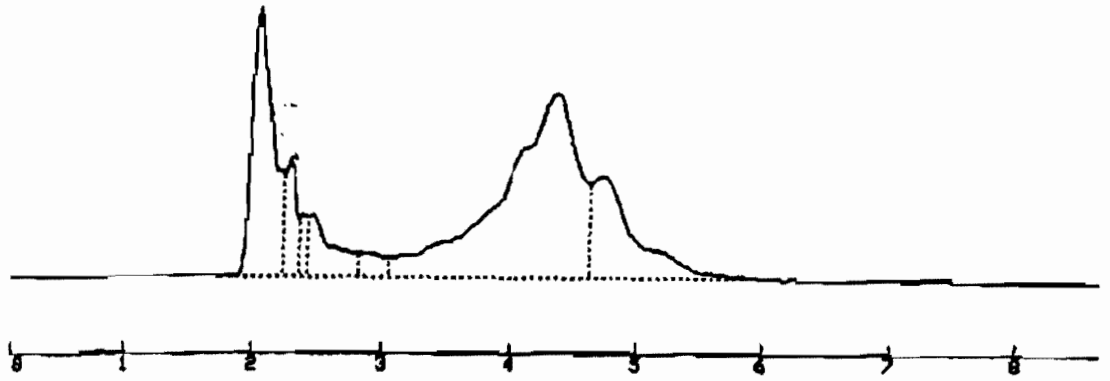


Fig. (3): Chromatograph of norfloxacin concentration (ug/g) in liver at 3rd day from last dose.

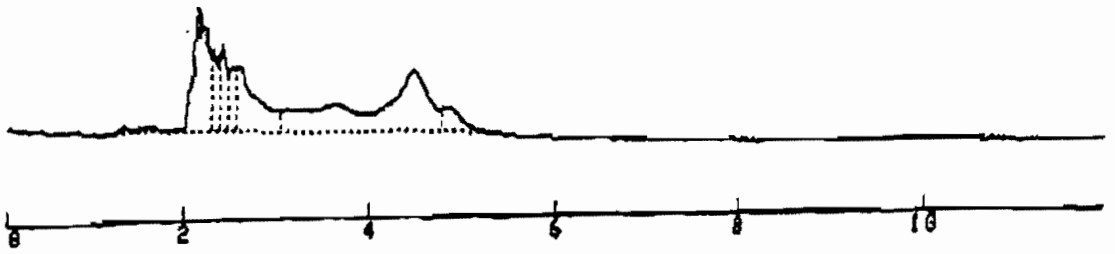


Fig. (4): Chromatograph of norfloxacin concentration (ug/g) in boiled liver at 3rd day from last dose.

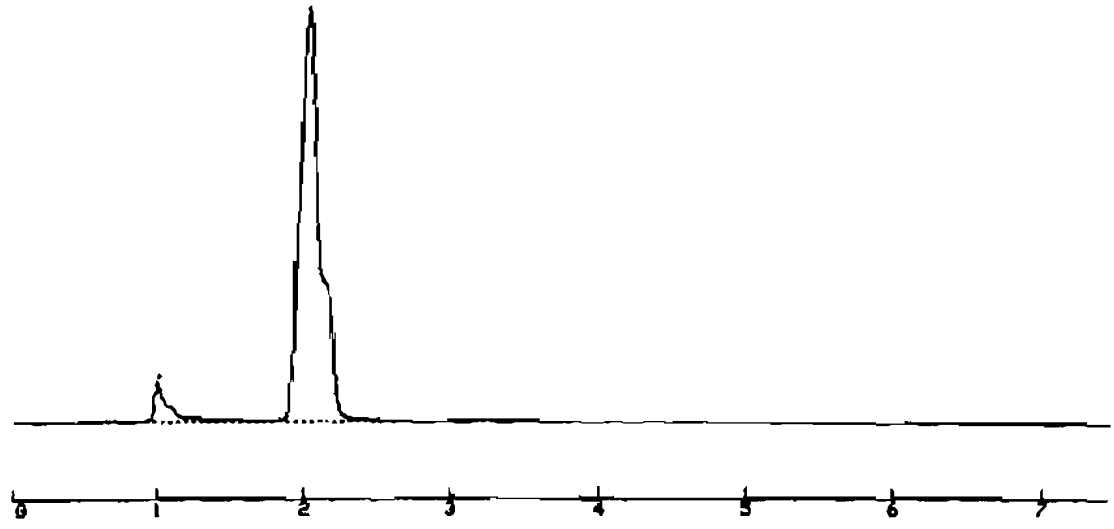


Fig. (5): Chromatograph of norfloxacin concentration (ug/g) in frozen gizzard at 1st week from last dose.

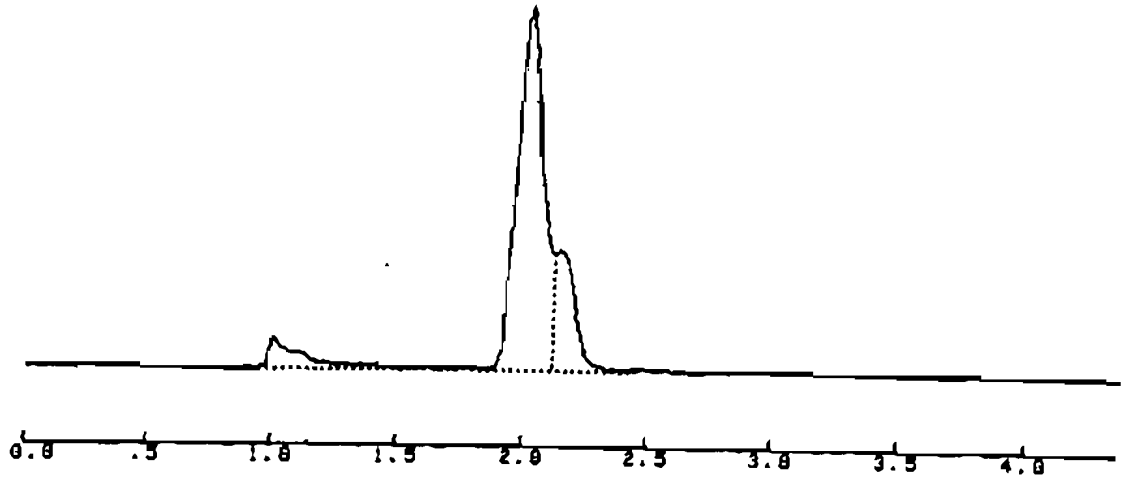


Fig. (6): Chromatograph of norfloxacin concentration (ug/g) in frozen breast muscle at 1st week from last dose.

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الملخص العربى

التقدير الكمى لمركب النورفلوكساسين وتأثير الحرارة والتجميد فى أنسجة الدجاج

المشتركون فى البحث

د/ منى عمر أبوالنيل د/ فاطمه السيد جابر*

معمل فحص الأغذية - الإسكندرية ، المعمل البيطرى - الزقازيق*

نظراً للاستخدام الواسع والمكثف للمضادات الحيوية فى مزارع الدواجن بغرض زيادة النمو ومقاومة الأمراض فإنه ينتج عنه رجود بقايا الأدوية فى لحوم وأعضاء الدواجن عند تناولها لهذا تم إجراء هذه الدراسة بغرض الكشف عن بقايا مركب النورفلوكساسين من مجموعة الفلوروكينولون وكذلك تأثير الغليان والحفظ بالتجميد على بقايا هذه الأدوية فى أنسجة وأعضاء الدواجن عقب إعطائها الجرعة العلاجية ثم قياس هذه البقايا بواسطة جهاز (الفصل الكروماتوجرافى السائل عالى الأداء).

وقد تم إستخدام إثنين وخمسين كتكوت ووضعت تحت نظام غذائى متوازن خالى من أية عقاقير، وعند الإسبوع الرابع تم إعطاء الدجاج جرعة علاجية من مركب النورفلوكساسين عن طريق الحقن العضلى لمدة ٥ أيام متتالية بجرعة ٥ ملجم/كجم من وزن الجسم.

وتم ذبح ٨ دجاجات عند اليوم (الأول - الثالث - الخامس - السابع - التاسع) بعد الجرعة الأخيرة، ثم تم تقدير بقايا المركب قبل وبعد الغليان عند درجة ١٠٠م لمدة نصف ساعة فى عضلات (الصدر - الفخذ - الدهن - الكبد - القانصة) كذلك أخذت نفس العينات من ١٢ دجاجة وتم فحصها قبل وبعد التجميد عند درجة - ١٨م إسبوعياً لمدة خمسة أسابيع متتالية ومن خلال هذه الدراسة اتضح أن أعلى تركيز لمركب الفورفلوكساسين فى عضلات الصدر عند اليوم الأول من الجرعة الأخيرة (٢١ر٣٩ + ٠ر١٢ + ٣ر٠ ميكروجرام/جم) وتناقص حتى لم يستدل عليها عند اليوم التاسع.

ثم تلاها الكبد وكان تركيز الركب ٢٣ر٢٧+٢ر٣٢١ ميكروجرام/جم ثم الدهن ١٥ر٢٥ + ١ر٩٥١ ميكروجوام) ولوحظ النقص العشوى حتى لم يستدل عليه عند اليوم التاسع، وكان أقل تركيز للمركب فى القانصة وعضلة الفخذ ٣٢ر١٧ + ١٦٥ر١، ٥ر١٢ + ٣٣ر٠ر١ ميكروجرام / جم حتى لم يستدل عليها عند اليوم التاسع..

أما بالنسبة لتأثير الغليان فكان تركيز المركب فى عضلات الصدر (٥٦٧ر٠+١٠ر٨٨٩ر٠) وقل تركيزه حتى لم يستدل عليه عند اليوم السابع، وفى الكبد والدهن المغلى كان تركيز المركب لـ٤٩٤ر٤ + ٠ر٢٩٠ ميكروجم. ٨٣٠ر٥ + ٣٢ر٠. ولم يستدل عليه عند اليوم السابع.

وقد لوحظ أيضاً أن أقل تركيز كان فى عضلات الفخذ والقابض لـ٣٥٧ر٤ + ٧٧٥ر٠ و ٦٠٠ر٣ + ٤٢٠ر٠ ميكروجرام / جم ولم يستدل عليه عند اليوم الخامس.

بينما فى عضلات الصدر التى حفظت بالتجميد عند -١٨م لوحظ أن التركيز انخفض بعد الإسبوع الأول إلى أنه لم يستدل عليه عند الإسبوع الخامس (٥٥ر٦ + ٧٦٤ر٠ و ٨٣٣ر٤ + ٨٢٨ر٠، ٤٨٦ر١ + ٩٨٢ر٠ و ٣ر٠ + ٠٥١ر١ ميكروجرام/جم) عند الإسبوع الأول والثانى والثالث والرابع وقد تلى عضلات الصدر الدهن والكبد حيث كان تركيز الفورفلوكساسين (٦٠٧ر٤ + ٦٥١ر٠ و ٢٣٠ر٤ + ٤٢١ر٠) وقل التركيز إلى أن يختفى تماماً عند الإسبوع الخامس حيث أقل تركيز كان لوحظ فى القانصة وعضلات الفخذ وانخفضت النسبة إلى أن اختفت تماماً عند الإسبوع الرابع.