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EFFECT OF CHLORAMPHENICOL RESIDUES ON SAFETY OF CHICKEN MEAT

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ABSTRACT

Veterinary drug residues could affect meat quality and safety. This study was undertaken to detect chloramphenicol (CAP) residues in 45-age chickens that husbandary –sacrificed at 1.5, 2.5, and 3.5 hr after CAP-individual injection dosage of 20 mg/kg . The obtained recovery was found to be 75.78 %. The detected CAP-residue was higher than the allowed MRLs for CAP-drug and Zero tolerance level. CAP-residue was acceptable at time of chicken consumption, hence most samples of chicken parts; Liver, Kidney and Internal intestines were completely CAP-depletion at 2.5 hr and 3.5 hr sacrificing. CAP-residue in muscle tissues showed higher amounts than that of MRLs at the end of the experiment which was 0.0308 ppm . So , muscle tissue was considered to be target tissue of residue analysis and long withdrawal period was required .

Keywords: Chloramphenicol, Solid phase extraction, SPE HPLC, Chickens, Residues.

INTRODUCTION

Cholramphenicol (CAP) was a bacteriostatic with a broad spectrum of activity, frequently used for therapeutic and prophylactic purposes in veterinary medicine *(Heitzman, 1994).* In addition, because it was inexpensive and until recently was not easily detected in edible products of animals, chloramphenicol became a widely used drug in the treatment of a large variety of animal diseases *(Arnold and Somogi, 1985).* CAP had a broad spectrum of activity against gram – positive and gram – negative bacteria *(Epstein et al., 1994).* Also, gram – positive pathogenic bacteria that caused mastitis were sensitive to CAP *(Allen , 1985)* . Chloramphenical was considered the most potent antibiotic for treating pneumonic and enteric conditions with respect to organisms sensitivity, and was routinely and widely used in Europe. Moreover, CAP had been recommended for the treatment of salmonella infections and for the prevention of secondary infections associated with chronic respiratory disease in poultry *(Bories et al., 1983).* Furthermore, many animal pathogens had developed resistance to other antibiotics commonly used in veterinary medicine *(Allen, 1985).* CAP was first isolated from cultures of streptomyces Venezuela in 1947, and it was produced on a large scale by chemical synthesis *(Allen, 1985).* However, in humans CAP could causes serious health problems, e.g., a plastic anemia, but there were no data indicating the minimum amount of residue, which could induce these effects *(Van Ginkel et al., 1990).* Contrary to humans, animals did not seem to be susceptible to CAP serious health problems. Therefore, on the basis of its high efficiency against a broad range of pathogenic microorganisms, CAP remained of great therapeutic value in

veterinary medicine *(Arnold and Somogi, 1985)*. Finally, adverse toxic reactions in animals treated with CAP were of low significance and had not limited the veterinary use of this drug *(Allen, 1985)*.

MATERIALS AND METHODS

1. Animal .

Day–old chicks of a commercial meat variety (Arbor Ackers) were used in this study. The broilers were mainted on the test diet up to the time that the birds were removed from the cages to be sacrificed. For water additives during the first three weeks of poultry husbandary, two types of poultry vaccines were used .

2. Tested drugs .

Drugs were obtained from Chemical Industries and Development Company " CID ", Giza, Egypt. Chloramphenicol structure was as follow:(-) threo-2-dichloro-acetamido -1-P- nitro–phenyl propane– 1, 3– diol, in its power state (CAP) .

3. Chloramphenicol study .

Total of 34 chickens were used and Six chickens where chosen from the group. These chickens were given each a single intramuscular injection of 20 µg CAP/Kg dissolved in propylene glycol–water mixture (10 %). Two birds were slaughtered after 1.5, 2.5 and 3.5 hr following administration of the drug. Also, the two birds were used to obtain sufficient tissues and organs to perform sufficient determinations .

Laboratory experiments included several steps, which could summarized in the following points: (1) Handling of tested meat samples, (2) Spiking of blank meat tissues for recoveries determination , (3) Preparation of tested drug standard solutions, (4) Procedures of drug residue identification and determination that included samples extraction and clean-up procedures; then system calibration and injection .

Concerning the extraction–purification steps of tested meat samples (Muscle tissue, kidney, liver and internal intestines) in order to determine their drug residues , the matrix solid phase dispersion extraction technique (MSPD) was used *(Le Boulaire et al., 1997)*.

Bulk C18 (40 µm; 12% Load, end capped, octadecylsilyl–derivatized silica) was used as a column packing material for easy sample–drug residue extraction. The mechanical forces applied during homogenization should be sufficient to lead to fracturing of some of the beads. However, to which degree this might occur, it did not appear to affect the flow of solvent through the column or lead to active sites wherein compounds might be lost *(Barker et al., 1989)*

4.Spiking procedures(Fortification) and recovery experiments.

For the application of spiked or fortified samples in the present study a known volumes of the identical concentration of the drug standard solution was added to blank control sample , then the calculation of the drug recovery percent (R%) could found out as shown by *Le Boulaire et al. (1997)* and *Long et al. (1990)* .

5. Determination of chloramphenicel (CAP) residues in tested tissues.

Analytical grade standard chloramphenicol (CAP) was used for preparation stock standard solutions. These solutions were used in injection on HPLC apparatus. Then, the detection and determination of CAP in tested meat samples were facilitated using the CAP standard curve plotted *(Bories et al., 1983).*

RESULTS AND DISCUSSION

1. Chloramphenicol residue determination.

For meat products in most countries residue levels of 1-10 µg / kg had been set for CAP–drug . But in the U.S.A. there was a Zero tolerance level *(Van Ginkel et al ., 1990).* Therefore , chromatographic methods or appropriate HPLC method, for determination residues of CAP–drug were important and considered the important required point to monitor the extra–label use of CAP and to ensure compliance withdrawal periods . Concerning the present investigation , it was very important to obtain calibration or standard curve using five concentrations of studied component , CAP in suitable and accurate prepared solutions (Tables 1A and 1B) . From these solutions , linear standard CAP–curve could be drawn, and this facilitated the accurate calculation of CAP–Concentrations in unknown, incurred and spiked meat samples . As shown in tables 1A and 1B , the prepared and used CAP–Concentrations were 1000, 100, 10, 1.0 and 0.1 µg/kg in methanol and sometimes in double distilled water. Injections were done immediately after preparation or after storage in refrigerator at 4 °C. Identification of CAP on HPLC instrument was the important point for CAP–residue determinations in tested meat samples.

CAP was identified on HPLC instrument at retention time (RT) of 2.781 min, and that was the mean value of approximately 15 retention times obtained from different injected CAP–standard solutions. Identification of CAP on HPLC instrument was depended on RT, and the peak areas obtained from different prepared and injected concentrations. For every injected concentration of CAP, a specific RT and an identical peak area have been obtained. Then, CAP residue in tested meat samples, whether spiked, unknown, or incurred was determined and calculated depending on peak areas obtained from the CAP–drawn standard curve. Therefore, and as described in tables 1A and 1B , the selected data were quite clear and accurate in table 1B than those reported in table 1C , and the data of table 1B were already used in CAP–standard curve calibration as shown in figure 10 .

In addition , the results obtained as described in table 1A indicated that, the recoveries (R%) obtained for CAP drug on HPLC instrument were ranged from 87.22% to 109.40% with the value 97.6% as mean level . But, for the results reported in table 1B , and for CAP–standard curve calibration, the obtained peak areas were plotted with 100% area percent in relating to 100% CAP-recovery percent for injection on HPLC-instrumentation. Moreover, and as reported in table (2), the results indicated that, the accuracy of data obtained from different injected standard solutions,

especially those used in standard curve calibration , was observed from the amount area ratio (Amt / Area) obtained. These ratios were 1.1 × 10 -1, 1.3 × 10-1, 1.2 × 10-1, 1.2 × 10-1, 1.2 × 10-1 with the CAP–standard solutions; 0.1, 1.0, 10, 100 and 1000 PPm; respectively. Also, the CAP-standard curve correlation was 1.0000 .

Table (1)A :CAP–standard curve data .

Retention time ; RT (min)	Prepared CAP Concentration (PPm)	Detected CAP amount (PPm)	Recovery percent%
2.793	1000.0000	979.1635	97.91
2.741	100.0000	99.9987	99.99
2.749	100.0000	97.7826	97.78
2.750	10.0000	9.6938	96.93
2.763	10.0000	9.6696	96.69
2.759	10.0000	9.5404	95.40
2.748	1.0000	0.9117	91.17
2.762	1.0000	0.8722	87.22
2.751	0.1000	0.1094	109.40
2.761	0.1000	0.1040	104.00

Table (1) B : Identification of used CAP by HPLC.

Retention time ; RT (min)	Prepared–CAP concentration (ppm)	Peak area mAU * S	Area percent %
2.781	1000.0000	7983.2866	99.41
2.741	100.0000	811.4476	100
2.765	100.0000	807.2812	ND
2.749	100.0000	788.0618	ND
2.750	10.0000	78.1255	100
2.759	10.0000	77.0181	ND
2.750	10.0000	80.5932	100
2.759	1.0000	8.9344	ND
2.748	1.0000	7.3484	ND
2.762	1.0000	7.0415	ND
2.761	0.1000	0.8401	ND
2.753	0.1000	1.3002	ND
2.751	0.1000	0.8818	ND

ND Not detected.

Table (1) C : HPLC–Calibration reported options

Retention time; RT (min)	CAP – Concentration; PPm.	Amount / Area *
2.781	0.1000	0.1190
	1.0000	0.1360
	10.0000	0.1279
	100.0000	0.1232
	1000.0000	0.1252

*** Correlation coefficient = 1.0000; from obtained, drawn calibration curve. Residual std. Dev. = 6.2308.**

Therefore, in order to detected CAP- residue in tested Chicken tissues, it was important to convert detected-HPLC results to accurate residue; (PPm). That was undertaken by correcting the detected residue to 100 % recovery. Regarding this , recovery results with CAP-drug using speaking (Fortification) was a vital step to obtain the conversion percent (%) for correcting the HPLC-residue, that recorded, to 100% recovery. Then, the final recovered residue were used for comparison with the MRLs or tolerances of CAP-drug. Regarding that and according to the obtained recovery as shown in table (2), the R % of 75.78 % was applicated to the tested Chicken samples. All detected samples using HPLC system were corrected to 100 % recovery using the above recorded recovery. Then, comparison with the reported legislations was undertaken to select the appropriate withdrawal time which should to be used before chicken sacrificing to minimize or preventing the occurrence of CAP residues .

Table 2: Measured CAP- residue (ppm) in chicken tissue and organs at different sacrificing time intervals (hrs).

Types of tested tissues	Sacrificing time intervals (hrs).	HPLC Detected amounts	Final recovered residue* (ppm)
Muscle tissues	1.5	17.4683	0.3309
	2.5	11.1994	0.2121
	3.5	1.6279	0.0308
Liver	1.5	0.8028	0.0152
	2.5	Zero	------
	3.5	Zero	------
Kidney	1.5	0.2568	0.0048
	2.5	Zero	------
	3.5	Zero	------
Internal intestines	1.5	10.1056	0.1914
	2.5	Zero	------
	3.5	63.0148	NR

NR Not reported; very high value.
* Recovery percent was 75.78% in tested tissues and organs

Degradation amount in of CAP-analyte ranged from 0.0242 to2.2161ppm. Therefore, sacrificing time intervals had an ideal effect on residue amount in chicken tissue. With the larger time–intervals of sacrificing, the lower residue amount for CAP–analyte was recorded . As an example, at 1.5 hr sacrificing, the residue detected was 0.4596 PPm . Then become 0.3304 PPm at 2.5 hr , and finally recorded 0.3062 PPm at 3.5 hr. As a result, the related recorded percent of deterioration and CAP-degradation was ranging from 5.26 to 28.11%. This percent related to approximately 0.0767 PPm degradation in CAP–analyte inside husbandary chicken tissues (Table 3) Total residues were found to be resulted from drug administration to an animal consisted of the parent drug plus its derived compounds, i.e. metabolites, the conjugates, and also residues bound to biological macromolecules (Livingston, 1985).

Table (3): Measured CAP – residues (ppm) in chicken muscle tissues and organs after injection with 20 mg/ kg Bodyweight.

Experiment No*.	Sacrificing time intervals (hr)	Measured concentration by HPLC**	Degradated amounts (Mean)	Degradation %
1	1.5	2.2174	-	-
	2.5	0.0013	2.2161	99.94
	3.5	ND	-	-
2	1.5	0.4596	0.1292	28.11
	2.5	0.3304	(0.0767)	16.68
	3.5	0.3062	0.0242	5.26
3	1.5	0.2363	0.0395	16.71
	2.5	0.1968	(0.0639)	27.04
	3.5	0.1085	0.0883	37.36
4	1.5	ND	ND	-
	2.5	-	-	-
	3.5	-	-	-

* Each experiment contained two chickens.ND Not determined.

** R% of tissues was ranging from 109.40 % (Not used) to 95.40% . Le Boulaire *et al* . (1997) reported 72 % recovery for CAP – analyte .

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تأثير بقايا الكلورام فينيكول على سلامة لحوم الدواجن

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المتبقى من العقاقير البيطرية فى اللحوم عامل مؤثر على جودتها وسلامتها . لذلك تم أخذ عدد ٣٤ من دواجن مزرعة الكلية المرباة تحت ظروف معينة و بعمر ٤٥ يوم و تم ذبحها على فترات زمنية ١,٥ ، ٢,٥ ، ٣,٥ ساعة بعد إعطائها جرعة واحدة من الكلورام فينيكول مقدارها ٢٠ مجم / كبم فى الفخذ . وكانت نسبة الإسترجاع المقدمة لمركب الكلورامفينيكول ٧٥,٧٨ % ، حيث إستخدمت هذه النسبة فى تعديل جميع النتائج المقدرة للمتبقى من المركب فى العينات المختبرة كـ ١٠٠ % إسترجاع . وبعد ذلك تمت المقارنة بين المتبقيات بالعينات المجهولة مع النسب القانونية الغذائية كحد أقصى مسموح به لتلك المركب (MRLs) للحفاظ على سلامة المنتج وصحة المستهلك . وتبعاً لتحلل الكلورامفينيكول ومدى تأثير فترة الـ Withdrawal time فقد كانت كمية المتبقى منه ٠,٠٠١٣ – ٢,٢٠٠ فى إحدى تجارب الحقن و ٠,١٠٨٥ – ٠,٤٥٩٦ جزء فى المليون فى تجربة تالية وكان تحلل هذا المركب واضحاً خاصة مع التركيزات المرتفعة مما يستلزم دقة زمن التحليل قبل عمل المقارنة مع الـ MRLs . وكانت نسبة التحلل للمركب ٩٩,٩٤ % مع عينات اللحوم المستخلصة والمخزنة بالتجميد حيث ظهر المركب الناتج من عمليات التحلل بجانب مركب الكلورامفينيكول الأساسى . وقد وصلت الكمية المقدرة المتحللة إلى ٠,٠٢٤٢ – ٢,٢١٦٠ جزء فى المليون بعد يومين وأقل مما يوضح أهمية إعطاء فترة مناسبة بعد الحقن وقبل الذبح مع حفظ اللحوم مجمدة لإتمام التحليل النهائى للمركب . و كانت معظم عينات الكبد والكلية والأمعاء الداخلية للدواجن بعد الذبح على ٤٥ يوماً مطابقة للـ MRLs بعد ٢,٥ ساعة ، ٣,٥ ساعة مما دل على تمام تخلص النسيج اللحمى من بقايا المركب المختبر CAP. بينما النسيج اللحمى للدواجن سجل قيمة أعلى من الـ MRLs عند تمام عمليات الذبح أى بعد ٣,٥ ساعة حيث كانت المتبقيات ٠,٠٣٠٨ جزء فى المليون . لهذا يتضح أن النسيج اللحمى أكثر تأثراً بميعاد الـ Withdrawal time .