QUANTITATIVE EVALUATION OF CHLORAMPHENICOL CONTENT IN CHICKEN MEAT PRODUCED BY DOMESTIC PRODUCERS WITH ELISA - TEST

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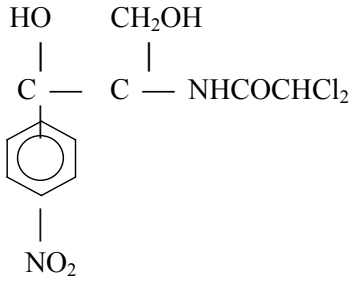
Background

Chloramphenicol is an antibiotic of wide specter. It affects Gram-positive and gram-negative bacteria, rickettsia, spirochete and Chlamydia. Because of its fantastic antibacterial effects and pharmacokinetics it has been used very often in animal treatment.
According to its chemical composition it is a derivate of nitro-phenol, molecular formula is C11H12Cl2N2O5 molecular weight M =323, 13 g/mol. Its full name is D-[-]-threo-2-dichloroacetamido-1-[p-nitro-phenyl]-1, 3-propaniol.

Structural formula:



This compound is very stable up to temperature of 100°C and it is in the wide range specter of pH (2-9). Pure substance is very liposoluble; poorly soluble in water (0, 25%) because of that it is used therapeutically in the form of palminate, stearate and sucinate. Chloramphenicol is metabolized in liver and excreted from body through urine.
Treatment of human beings by chloramphenicol is only recommended in life threatening infections. Misuse of chloramphenicol can lead to: leucopenia, trombocitopenia, irreversible plastic anemia etc. It is fully cumulative toxin because of its structure or toxic metabolites (mainly the ones that contain nitro group).
Due to chloramphenicol negative impact on human's health, treatment of animals whose products meat, milk and eggs are used for human's consumption, is strictly prohibited in EU since 1994. According to regulations, the presence of residues of this antibiotic in food must be monitored continuously in all products of animal origin.
Conventional method (radioimmunology) for quantitative evaluation of chloramphenicol has been switched by new more precise methods as: gas chromatography/MS, liquid chromatography/MS and ELISA-test. These methods are more specific and more sensible with detection level (10^{-12}g/g), or (ng/kg).

Objectives:

During March 2004 the authors of this paper were investigating presence of chloramphenicol content in samples of chicken meat from market of Canton Sarajevo. We expect higher level of self control at our producers and importers**.**

Materials and methods:

Elisa-test is analytical method which can be used for quantitative evaluation of: antibiotics, hormones, pesticides, vitamins etc; in products of animal origin. Authors of this paper were quantitatively evaluating the content of chloramphenicol residues in chicken meat during March 2004 from market of Canton Sarajevo.

Principle of the assay

Assay is based on the antigen-antibody reaction, which is being done in vitro on micro titer plate. Micro titer plate is precoated by fixed antibodies of sheep to rabbit IgG. Chloramphenicol standards or diluted chicken meat samples, chloramphenicol enzyme conjugates and antichloramphenicol antibody is added. Free chloramphenicol and chloramphenicol enzyme conjugate compete for chloramphenicole antibody binding sites (competitive enzyme immunoassay). After incubation time the non-bound (enzyme labeled) reagents are removed in a washing step. The amount of chloramphenicol enzyme conjugate is visualized by the addition of a chromogen substrate. Bound enzyme conjugate transforms the colorless chromogen into a colored product. Reaction of substrate is stopped by addition of stop solution. The color intensity is measured photometrically, where optical density is inversely proportional to the concentration of chloramphenicol in sample**.**

Preparation of sample(chicken meat)

a) Homogenize 10g of chicken meat weight 3g of the homogenized tissue sample and transfer into a glass tube.
b) Add 6ml of ethyl acetate and mix (head over head) for 10 min.
c) After centrifugation (10 min, 2000g)
d) 4ml of the ethyl acetate and evaporated at 50°C
e) The fatty residue is dissolved in 1ml iso-octane/ thrichloromethane (2:3;v/v) and 1ml of dilution buffer is added.
f) Mix with vortex 1min and centrifuged 10 min with 2000g.
g) 50µl portions of the upper layer are pipetted into the test.

Assay procedure

a) Into wells of microtitar plate, parallel have been filed with 50µl of negative probe two wells (B1 ,B2) and standard dilutient from 0,025ng/ml to 2ng/ml(well C1and C2 to well H1 and H2). In the rest of wells fill in duplicate 50µl of prepared sample.
b) In the same order every well (except A1,A2) was filed with 25µl of enzyme conjugate and 25µl of diluted antibodies
c) After a mixing plate was incubated for 1 hour on 4°C.
d) Content of the wells have been washed three times with diluents.
e) Into each well was added 100µl of substrate and incubated 30min on room temperature.

Then 100µl of stop solution was filled in every well and content roughly mixed.

Spectrophotometeric reading

Optical density of generated color was read immediately by "IDEX" spectrophotometer; on wavelength λ=450nm. Blank probe was done against air.

Calculation

a) After we measured values of absorbance for each well and calculated medium value of two parallels, the calculation of parameters has been done %A (absorbance percentage) by following formulae:

$$\%A = \frac{\text{Average value of absorbance (standard or sample)}}{\text{Average value of absorbance of zero standards}} \times 100$$

b) Calibration curve was drawn for standard dilutions of chloramphenicol with known concentration.
c) Values of chloramphenicol concentration in samples (chicken meat) were read from the curve by appropriate values of %A.

Compared results

To compare these results authors have analyzed same samples by diffuse microbiological test using test species bacteria: Bacillus cereus ATCC 11778, Bacillus subtilis ATCC 6633, Staphylococcus epidermidis ATCC12228, Sarcina lutea ATCC9341 and Sarcina lutea ATCC 15957.

Results and discussion

In 25 examined samples of chicken meat from territory of Canton Sarajevo were not detected quantities of chloramphenicol greater than the limits of detection by this method, the Elisa-test (detection limit is 0,02ng/g) Samples (chicken meat) were from domestic producers and also from different foreign producers. The assay results are summarized and presented in table No: 1. Samples are grouped into next order.

a) A1- domestic chicken meat, locality 1
b) A2- domestic chicken meat, locality 2
c) B1- imported chicken meat, locality 1
d) B2- imported chicken meat, locality 2

NOTICE: Because of are professional relations with clients we did not notify producers names.

Table1. Chloramphenicol content in the samples of chicken meat

Group	Location	Number of samples	Chloramphenicol content (ng/g)
A1	1	7	< 0,02
A2	2	6	< 0,02
B1	1	6	< 0,02
B2	2	6	< 0,02

Conclusions

1. Elisa-test through assay has shown as very sensible, reliable and simple analytical method for quantitative analysis of chloramphenicol residues in chicken meat.
2. In 25 examined samples of chicken meat were not detected quantities of chloramphenicol greater than the limits of detection by this method, the Elisa-test (detection limit is 0,02ng/g)
3. Critical moment of Elisa-test for screening chloramphenicol in chicken meat is procedure of preparing sample for assay which is relatively complicated and long lasting.

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