EVALUATION OF THE PRESENCE OF NITROFURANS IN MEAT AND CHICKEN EGGS

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Introduction

The nitrofurans are antimicrobial drugs that have been widely used as veterinary therapeutics or feed additives for treating bacterial diseases in cattle, swine and poultry production. Furazolidone, furaltadone, nitrofurazone and nitrofurantoin are veterinary drugs that belong to the nitrofuran group, which have been used in the treatment of infections caused by *Escherichia coli* and *Salmonella* in pigs, poultry and fishes. The nitrofurans are quickly metabolized and are not detected after few hours from their administration. Otherwise, nitrofuran metabolites remain during months as residues bound to tissue proteins [2].

It has been demonstrated that a proportion of the bound residues of furazolidone and furaltadone possess intact side-chains which have molecular characteristics in common with the parent compounds [2]. These side-chains can be released from the bound metabolites under mildly acidic conditions such as may occur in the stomach of the consumer. It has been suggested that furazolidone sidechain, 3-amino-2-oxazolidinone (AOZ), can be metabolized into-hydroxyethylhydrazine, which is a mutagenic and carcinogenic compound [7].

Nitrofuran antibiotics were banned within the European Union (EU) due to the toxicological hazard for human consumers, concerns over their carcinogenicity and mutagenicity provoked by these drugs and should not be used in food-producing animals or be present in foods produced in, or imported into, the EU. The all compounds were put into the Annex IV of the European Union Directive no. 90/2377/EC in 1993 and 1995 [1-2], currently approved in the European Union Regulation no. 2010/37/EC. However, nitrofurans can be illegally administrated to animals. During 2002-2003, nitrofuran residues were detected in poultry and aquaculture products imported to Europe from different countries and the residue of nitrofuran metabolite was detected in poultry meat from Portugal and pork meat from Italy and Greece [2].

The monitoring of residues of nitrofuran metabolites in the food chain it is a major focus in the international control of veterinary drug residues and constitutes a control and supervision tool for safe food production [1].

Sometimes, the identification of some drugs in food is possible only through the presence of their metabolites, presented most of the time in insignificant amounts. There are several analytical techniques for determining these residues, however, in the Republic of Moldova the most developed are the immunoenzymatic techniques with the use of putties from various manufacturers.

There have been many papers involving LC-MS or LC-MS/MS method for the determination of nitrofuran metabolites including AMOZ in various matrixes. These methods are sensitive and confirmatory, but the expensive instruments may not be available in every laboratory. Comparison with those instrumental methods, ELISA is a low cost and sensitive method capable of screening large amount of samples in a single test [8.]

Although the aforementioned analytical methods offer predominant accuracy and high-throughput screening capability, they are relatively resource demanding and multiple steps of these methods hinders their instant and filed applications. Accordingly, it still remains tremendous requirements to construct facile detection methods with satisfactory simplicity, speed and cost, results visible by naked eye, small sample volume requirement, shorter detection time, ease of mass production and portability [6].

Materials and methods

The examinations were carried out in 7 samples of broiler chicken meat and 8 samples of chicken eggs. Broiler chicken meat and eggs were purchased from stores from different farmers. The examinations were carried out in 7 samples of broiler chicken meat and 8 samples of chicken eggs. Broiler chicken meat and eggs were purchased from stores from different farmers.

Apparatus.

For the enzyme-linked immunoassay method, was used a "SUNRISE" model absorbance reader, for the LC/MS/MS method, was used the "Shimadzu" model chromatograph.

Reagents and chemicals.

Ridascreen kits from R-biofarm were used for the enzyme-linked immunoassay method. The standard solutions and all reagents used were of high purity. For the LC/MS/MS method only reagents of >99.9% purity.

Test principle for the enzyme-linked immunoassay method.

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-nitrofuran metabolites antibodies. The measurement is made photometrically at 450nm. The absorbtion is inversely proportional to the metabolite concentration in the sample.

LC/MS/MS analysis.

The LC/MS/MS system is composed of Liquid Chromatograph Shimadzu System (Shimadzu Coorporation, Japan) connected to a quadrupole mass spectrometer in eletrospray positive ionisation mode.

Sample preparation for the enzyme-linked immunoassay method.

The samples homogenise, prepare 10mM metabolite in dimethylsulfoxide directly before use. Mix 1g of the homogenized sample with 4 ml distilled water, 0,5ml 1M HCL and 100µl for the metabolite solution by shaking thoroughly.

The derivatization procedure was performed according to the protocol.

Sample preparation for the LC/MS/MS analysis.

A 1,0 g portion of sample was transferred to a 15 mL centrifuge tube. The samples were submitted to hydrolysis and derivatisation processes, by adding 40µL of internal standard mixture for the AOZ, AMOZ, AHD, SEM and DNSAH. The samples were centrifuged for 10 min. After centrifugation was transferred to a glass tube and let evaporate to dryness at 45 ◦C in an evaporation station. The residues were redissolved in acetonitrile–water and 0.1% acetic acid mixture and centrifuged for 5 min.

Calibration curve.

Calibration curve was prepared with blank samples which were fortified with a standard solution mixture (AOZ, AMOZ, AHD, SEM and DNSAH) (Fig.1).

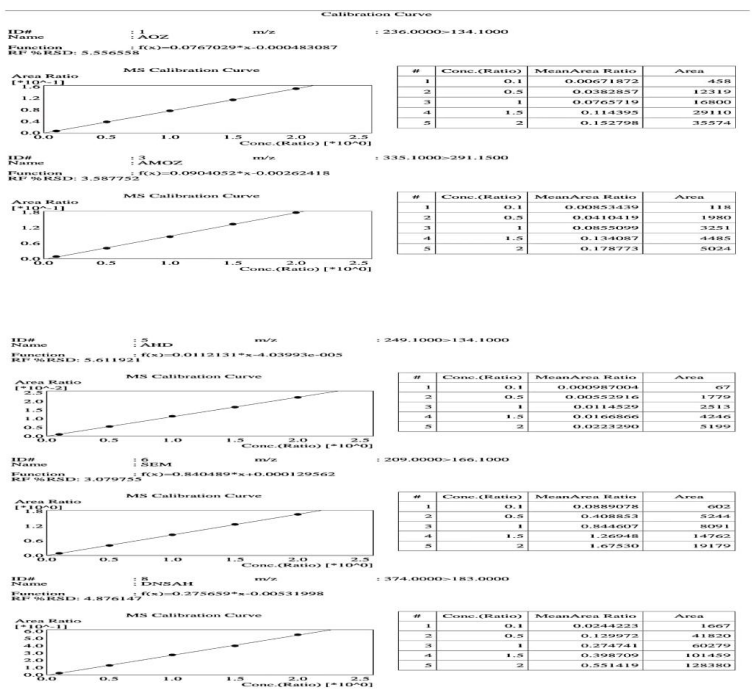

Figure 1. Calibration curve.

Results and Discussion

According to REGULATION (EU) NO. 37/2010 of the Comission of December 22.2009 regarding pharmacologically active substances and their classification according to the maximum residual limits in food products of animal origin, nitrofurans including furazolidone are included in table 2 of the Annex where the prohibited substances are mentioned, for which the maximum allowed limit cannot be established.

To determine the amount of residues of substances for which a limit is not established, the method was validated in the laboratory and the limits of detection and quantification were established through tests. The detection limit was determined as the arithmetic mean of the analyte concentration plus three times the standard deviation, and the quantification limit as the mean of the analyte concentration plus ten times the standard deviation. The Decision Limit (CCα) and the detection capacity (CCβ) were also calculated. The given parameters allow us to demonstrate what the residue content is and if it is necessary to receive a decision regarding the product with the given quantity of the substance.

Table 1. Value calculated LOD and LOQ.

Nr.	*Compound*	*Matrix*	*MRL/MRPL*	*LOD*	*LOQ*
1	*AOZ*	*Muscle*	*0.5ppb*	*0.135 ppb*	*0.2ppb*
2	*AOZ*	*Egg*	*0.5ppb*	*0.133 ppb*	*0.2ppb*
3	*AMOZ*	*Muscle*	*0.5ppb*	*0.246 ppb*	*0.4ppb*
4	*AMOZ*	*Egg*	*0.5ppb*	*0.198 ppb*	*0.3ppb*
5	*AHD*	*Muscle*	*0.5ppb*	*0.196 ppb*	*0.3ppb*
6	*AHD*	*Egg*	*0.5ppb*	*0.201 ppb*	*0.3ppb*
7	*SEM*	*Muscle*	*0.5ppb*	*0.376 ppb*	*0.6ppb*
8	*SEM*	*Egg*	*0.5ppb*	*0.350 ppb*	*0.5ppb*
9	*DNSAH*	*Muscle*	*0.5ppb*	*0.284 ppb*	*0.6ppb*
10	*DNSAH*	*Egg*	*0.5ppb*	*0.263 ppb*	*0.6ppb*

MRL/MRPL established by Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC in all metabolites it is 0.5ppb. After validating the ELISA immunoenzymatic screening method performed on the kits from R-Biopharm LOD values are between 0.13ppb and 0.37 ppb, the LOQ values are between 0.2ppb and 0.6ppb. These data allow us to draw a conclusion regarding the results obtained when examining the samples. (Tab. nr.2, nr.3).

Table 2. Concentration of nitrofurans in egg samples.

Nr.	AOZ/conc. µg/kg	AMOZ/conc. µg/kg	AHD/conc. µg/kg	SEM/conc. µg/kg	DNSAH/conc. µg/kg
1	0,32	0,20	0,07	0,01	0,02
2	0,13	0,18	0,24	0,14	0,02
3	0,25	0,08	0,01	0,30	0,03
4	0,16	0,13	1,01	0,06	0,02
5	0,17	0,15	0,17	0,28	0,02
6	0,14	0,11	0,03	0,18	0,03
7	0,16	0,14	0,06	0,24	0,01
8	0,29	0,07	1,02	0,17	0,03

Table 3. Concentration of nitrofurans in muscle samples.

Nr.	AOZ/conc. µg/kg	AMOZ/conc. µg/kg	AHD/conc. µg/kg	SEM/conc. µg/kg	DNSAH/conc. µg/kg
1	0,06	0,24	0,07	0,02	0,01
2	0,01	0,21	0,21	0,06	0,01
3	0,03	0,05	0,01	0,03	0,03
4	0,08	0,08	0,02	0,06	0,02
5	0,07	0,04	0,11	0,07	0,01
6	0,10	0,06	0,06	0,12	0,03
7	0,10	0,14	0,05	0,09	0,01

Of all the meat samples examined, no sample has a metabolite content higher than the LOD and LOQ. Egg samples no. 1, no. 3 and no. 8 have a higher content of AOZ, sample no. 4 and no. 8 a higher content of AHD, sample no. 3, no. 5 and no. 7 more SEM content.

Conclusions

Examining meat and egg samples allows us to draw conclusions on the use of substances from the nitrofuran group. So, the meat samples are free of residues of these substances. In some egg samples, contents of AOZ, AHD and SEM metabolites higher than the Limit of Quantification but lower than the MRL/MRPL.

References:

1. Merve Bacanlı, Nurşen Başaran, Importance of antibiotic residues in animal food, Food. Chem. Toxicol. *2019, Mar;125:462-466*. doi: 10.1016/j.fct.2019.01.033.
2. K. M. Cooper, R. J. Mccracken, M. Buurman, & D. G. Kennedy, Residues of nitrofuran antibiotic parent compounds and metabolites in eyes of broiler chickens, Food Additives and Contaminants, May 2008; 25(5): 548–556
3. Jane Kelly Finzi, Jose Luiz Donato, Mauro Sucupira, Gilberto De Nucci, Determination
of nitrofuran metabolites in poultry muscle and eggs by liquid chromatography-tandem mass spectrometry, Journal of Chromatography B, 824 (2005) 30–35 , doi:10.1016/j.jchromb 2005.05.01
4. R.J. McCracken and D.G. Kennedy, The detection, accumulation and distribution of The detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell, Food Additives and Contaminants, 14-Aug-2006
5. Noelia M.Valera-Tarifa, Patricia Plaza-Bolaños, RobertoRomero-González, José L.Martínez-Vidal, AntoniaGarrido-Frenich, Determination of nitrofuran metabolites in seafood by ultra high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry, Journal of Food Composition and Analysis Volume 30, Issue 2, June 2013, Pages 86-93
6. SijieLiu, LeinaDou, XiaolinYao, WentaoZhang, BingxinZhao, ZonghanWang, YanweiJi, JingSun, BaochengXu, DaohongZhang, JianlongWang, Polydopamine nanospheres as high-affinity signal tag towards lateral flow immunoassay for sensitive furazolidone detection, Food Chemistry, Volume 315, 15 June 2020, 126310
7.Eric Verdon, Pierrick Couedor, Pascal Sanders, Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, itrofurantoine,
nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry — In-house validation in line with Commission Decision 657/2002/EC,
Analytica Chimica Acta 586 (2007) 336–347 2007 doi:10.1016/j.aca.2007.01.024
8.Xiao Dong Yan , Xiu Zhi Hu , Hui Cai Zhang , Jing Liu & Jian Ping Wang Xiao Dong Yan , Xiu Zhi Hu , Hui Cai Zhang , Jing Liu & Jian Ping Wang, Direct determination of furaltadone metabolite, 3-amino-5-morpholinomethyl - 2-oxazolidinone, in meats by a simple immunoassay, Food and Agricultural Immunology, Vol. 23, No. 3, September 2012, 203-215, DOI: 10.1080/09540105.2011.615060.
9.Regulation (EU) NO. 37/2010 of the Comission of December 22.2009 regarding pharmacologically active substances and their classification according to the maximum residual limits in food products of animal origin. (access
10.Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC.