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RESEARCH ARTICLE

Effects of Boiling on Nitrofuran AOZ Residues in Commercial Eggs

Artun YIBAR [1] Bülent OKUTAN [2] Saime GÜZEL [3]

[1] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Uludag, TR-16059 Gorukle Campus, Bursa - TURKEY
[2] Department of Pharmacology, Pendik Veterinary Control and Research Institute, TR-34890 Pendik, Istanbul - TURKEY
[3] Department of Biochemistry, Faculty of Veterinary Medicine, University of Uludag, TR-16059 Gorukle Campus, Bursa - TURKEY

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Summary

The aim of this study was to determine the effects of boiling on nitrofuran 3-amino-2-oxazolidinone (AOZ) residues in eggs. The use of furazolidone in food-producing animals is banned within the EU and Turkey. The nitrofuran AOZ residues in raw and boiled eggs were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in a chromotographic run of 20 min. The method validation was done according to the criteria laid down in Commission Decision No. 2002/657 EC. Linearity was proved between 0 to 1.5 µg/kg, decision limit (CCα) was 0.70 µg/kg, detection capability (CCβ) was 0.77 µg/kg, recovery values ranged between 88-97.9% and repeatability (CV) was 3-4.3%. The detected avarage nitrofuran AOZ residue level in 13 uncooked eggs by LC-MS/MS was 0.86±0.017 µg/kg which was increased to 2.42±0.037 µg/kg after boiling. In this study, it was surprisingly found that protein-bound side-chain metabolite, nitrofuran AOZ levels in eggs were significantly increased after boiling. This finding runs counter to the claim that heat process in general should decrease various antibiotic levels in food. The observed increase ($P<0.001$) in nitrofuran AOZ levels in boiled eggs relative to uncooked eggs may be due to enhanced efficiency of extraction in boiled samples. Therefore boiled eggs should be used for analysis of nitrofuran AOZ levels in order to obtain more reliable and more predictive results.

Keywords: *Nitrofuran AOZ, Residue, Egg, LC-MS/MS, Validation, Heat process*

Ticari Yumurtalarda Nitrofuran AOZ Kalıntıları Üzerine Kaynamanın Etkisi

Özet

Bu çalışmanın amacı, yumurtalarda nitrofuran 3-amino-2-okzazolidinon (AOZ) kalıntıları üzerine kaynamanın etkisini belirlemektir. Furazolidonun gıda değeri taşıyan hayvanlarda kullanımı Avrupa Birliği'nde ve Türkiye'de yasaklanmıştır. Çiğ ve haşlanmış yumurtalarda nitrofuran AOZ kalıntıları sıvı kromatografi-tandem kütle spektrometrisi (LC-MS/MS) kullanılarak 20 dakikalık süre içerisinde analiz edilmiştir. Uygulanan yöntemin validasyonu Avrupa Birliği Komisyon Kararı No 2002/657 EC 'de belirtilen kriterlere göre yapıldı. Doğrusallık 0-1.5 µg/kg, tayin limiti (CCα) 0.70 µg/kg, tayin kapasitesi (CCβ) 0.77 µg/kg, geri kazanım değerleri %88-97.9, tekrarlanabilirlik (CV) %3-4.3 arasında gerçekleşti. LC-MS/MS ile 13 adet çiğ yumurtada 0.86±0.017 µg/kg miktarında tespit edilen ortalama değer haşlama sonucu bakılan aynı yumurta örneklerinde istatistiki olarak önemli derecede artış göstererek 2.42±0.037 µg/kg ortalama değerlere yükselmiştir. Şaşırtıcı bir şekilde bu çalışmada, kaynamadan sonra yumurta içinde protein-bağlı yan-zincir metaboliti nitrofuran AOZ düzeylerinin arttığı tespit edildi. Bu bulgular genel olarak ısıl işlemin gıdalarda çeşitli antibiyotik düzeylerini azaltması gerektiği iddiası ile ters düşmektedir. Pişmemiş yumurtalara göre haşlanmış yumurtalarda nitrofuran AOZ düzeylerinde gözlenen artış ($P<0.001$) haşlanmış örneklerde ekstraksiyon etkinliğinin artmasına bağlı olabilir. Bu nedenle nitrofuran AOZ seviyelerinin analizinde daha güvenilir ve anlamlı sonuçlar elde etmek amacıyla haşlanmış yumurtalar kullanılmalıdır.

Anahtar sözcükler: *Nitrofuran AOZ, Kalıntı, Yumurta, LC-MS/MS, Validasyon, Isıl işlem*

INTRODUCTION

Veterinary drugs are used in layer flocks by addition to feed or drinking water in order to prevent and treat diseases, promote egg productions, assist in converting stress due to environmental changes, beak trimming,

İletişim (Correspondence)
+90 224 2941200
saime@uludag.edu.tr

vaccination and other management practices. Improperly and illegal administration of these drugs can cause residue problems in eggs and these residues can cause severe harmful effects on consumers. In addition, trace amounts of antibiotic compounds in eggs favor the development of antibiotic-resistant bacteria [1-3].

Nitrofurans have been widely used in the treatment of gastrointestinal infections such as fowl cholera, coccidiosis and blackheads in laying hens. Nitrofurans, including furazolidone, in veterinary practice are banned in many countries, including Turkey, because of their mutagenic, carcinogenic and genatotoxic effects [4-7].

Parent compound furazolidone is rapidly and extensively metabolized *in vivo* (*in vivo* half life is less than a few hours) [8,9]. The side chain metabolites (3-amino-2 oxazolidinone, AOZ) can bind to proteins to form bound residues. These residues are extensively formed in liver, kidney, muscle tissues and also eggs [10-12]. They have a long residence time and their extraction from the tissues is very difficult [12-14].

Short half-life of nitrofurans has made screening for parent drugs difficult in food products, but the development of assays that can detect highly stable nitrofuran metabolites have been used to demonstrate the persistence of tissue-bound residues in egg and the other animal origin foods [9-11,15,16].

Several liquid chromatography-tandem mass spectrometry (LC–MS/MS) methods have been developed for determination of nitrofuran metabolites in different tissues [17-21] and eggs [18]. The detection of AOZ in poultry eggs, was carried out by Mccracken *et al.*[11] using LC-MS.

Several studies have generally described drug residues in uncooked tissues and the other various of food product [18,20,22]. However, the effects of cooking on nitrofuran 3-amino-2-oxazolidinone (AOZ) in eggs have not been studied. It is important to determine if residues of nitrofuran AOZ are reduced or not by boiling procedure. Since egg is usually cooked before consumption, more findings about the effects of boiling on residues of nitrofuran AOZ are needed to determine the risks to the consumer from dietary exposure to these substances. We carried out this study to examine whether boiling has an effect on residues of nitrofuran AOZ in eggs.

MATERIAL and METHODS

Reagents and Water

2-NP-AOZ-D4 internal standard was purchased from Sigma–Aldrich (St. Louis, MO, USA). The other chemicals such as dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), 2-nitrobenzaldehyde, sodium hydroxide (NaOH), dipotassium hydrogen phospate (K_2HPO_4), ethyl acetate, methanol (MeOH) and acetic acid were HPLC grade and were purchased from Merck (KGaA, Darmstadt, Germany). The water used was purified with a Milli-Q water purification system from Millipore (Millex GV, 0.45 µm).

Standard Solutions

The standard stock solution and internel standart were stored at -20°C in the dark and warmed up to room temperature before use. The working standard solution was stored at +4°C in the dark. DMSO was stored at room temperature and the other chemicals were stored at room temperature (22 to 25°C).

Samples

Samples of eggs were collected from different layer flocks as part of the "National Program of Residue Control", between 1 Jan 2010 - 31 December 2010. Nitrofuran AOZ was found positive from one poultry farm's layer flocks by LC-MS/MS. In this study we used nitrofuran AOZ positive 13 egg samples from these layer flocks.

Sample Preparation

The extraction was performed as described previously [23,24] with some minor modifications. Before boiling process, each egg (liquid) was scrambled, split and 2±0.03 g of that was put into 50 ml centrifuge tubes. The remaining amount of each egg (yolk and albumen) were immersed in 100°C water for 10 min, removed and cooled immediately in a chilled water bath. We used the same extraction method for 10 min boiled (solid) eggs. 100 µl 2-NP-AOZ-D4 internal standard and 5 ml 0.1 M HCl were added. They were vortexed for 60 sec and homogenized. Then 300 µl 50 mM 2-nitrobenzaldehyde, which was dissolved in dimethylsulfoxide (DMSO), was added and mixed for 1 min by multireax vortex. They were incubated in the shaker incubator at 37°C for 16 h at 50 rpm. After incubation, samples were cooled to the room temperature. 700 µl neutralization solution was added [1 M NaOH/0.1 M K_2HPO_4 + $3H_2O$ (2:1, v/v)] [25]. They were vortexed for 1 min. 5 ml ethyl acetate was added onto it and they were mixed in the shaker incubator at room temperature for 10 min at 300 rpm, then to prevent soap forming between lipid and ethyl acetat, 3 ml n-hexane was added. Then they were centrifuged at 15°C for 10 min at 4000 x *g*. 6 ml of upper phase were taken and transfered into 15 ml glass tube, and dried under nitrogen flow in 5.0 psi pressure at 42°C. After the drying operation, 1 mL n-hexane was added into the tube and vortexed for 1 min. 750 µl MeOH/deionized water (1:4, v/v) was added. They were mixed with the multireax vortex for 5 min. They were centrifuged at 15°C for 10 min at 2500 x *g* and the sample was taken from the lower phase by the help of an injector. Then this liquid part was filtered onto a 0.45 mm filter and 50 µl filtrate is transferred to a microvial capped and adapted to the LC autosampler [23,24].

LC–MS/MS Analysis

This study was carried out by Tandem Gold Triple Quadrupole LC-MS/MS (ZIVAK, Kocaeli, Turkey) system. LC separation was achieved by using a Synergi™ Max-RP (4 µm, 150 x 2 mm) column. The mass spectrometer operated in selective reaction monitoring mode (SRM) with positive electro-spray interface (ESI). The mobile phase A and B used were 0.2% acetic acid and methanol, respectively. The column was thermostated at 40°C. The flow rate was 0.2 ml/min, with an injection volume of 50 µl. The linear gradient was: 0-3 min 80% A, 3-7min 50% A, 7-9 min 30% A, 9-9:50 min 25% A, 9:50-13 min 0% A, 13-20 min 90% A. The electrospray capillary voltage was 5000V. Nitrogen was used as curtain and collision gas. Auto sampler, MS/MS and screening parameters are shown in *Table 1, Table 2* and *Table 3*, respectively.

Table 1. *Auto sampler parameters*
Tablo 1. *Otomatik örnekleyici parametreleri*

Parameter	Value
Injection volume	50 µl
Syringe speed	8.0 µl/sn
Washing volume	1000 µl
Washing speed	250 µl/sn
Auto sampler temperature	10°C
Column temperature	40°C

Table 2. *MS/MS parameters*
Tablo 2. *MS/MS parametreleri*

Parameter	Value	
Ionization mode	ESI +	
Screening	SRM	
Spray voltage	5023	
API nebulizing gas pressure	55	
Drying gas pressure	2.0	
Aux gas pressure	15	
Drying gas temperature	400°C	
Quad MS/MS bias	-1.0	
Screening time	0.01 sec	
SIM width	0.7 amu	
Neddle	+ 5000 V	
Shield	+ 500 V	
Dedector	+ 1700 V	
CID gas pressure	1.30 mTorr	
Spray chamber temperature	65°C	
Mass peak width in Amu	Q1=0.7	Q3=0.7

Table 3. *MS/MS screening parameters*
Tablo 3. *MS/MS tarama parametreleri*

Analyte	MS MH+ (m/z)	MS-MS (m/z)	Fragmentation Energy
2-NP-SEM	209.00	166.00	13
2-NP-SEM	209.00	192.00	11
2-NP-SEM-13$C_{15}N_2$	212.00	168.00	12
2-NP-AHD	249.00	134.00	14
2-NP-AHD	249.00	104.00	19
2-NP-AOZ	236.00	134.00	15
2-NP-AOZ	236.00	104.00	18
2-NP-AOZ-D4	240.00	134.00	15
2-NP-AMOZ-D4	340.00	296.00	11
2-NP-AMOZ	335.00	262.00	19
2-NP-AMOZ	335.00	291.00	12
I. Segment	335		
II. Segment	209.00-249.00-236.00-240.00		
Screening mode	SRM		
Segmentation energy	10		

Preparation of Matrix Standard and Validation Procedure

Five pieces of 2 g blank tissue samples were taken into 50 ml centrifuge tubes. The external standard solution was added on these samples (50, 100 and 150 µl for 0.5, 1 and 1.5 µg/kg, respectively). Then they were extracted like the other samples which are described in the sample extraction section extracted like the other samples that was described in the part of sample extraction. The method validation was done according to the criteria laid down in Commission Decision No. 2002/657 EC [26].

Calibration Curves and Results

We used blank egg samples from nitrofuran AOZ negative layer flocks for the calibration process and calculation value of CCα and CCβ. Calibration curves, used in validation process, were based upon responses (analyte/internal standard peak area ratio versus concentration of analyte) from four concentration levels ranging from 0 to 1.5 µg/kg (0, 0.5, 1 and 1.5 µg/kg), were linear. The recovery values (Rec) ranged between 88-97.9%, and repeatability (CV) ranged between 3 and 4.3%. Calibration curves, used in calculation process, were made by spiking blank matrix samples of egg with nitrofuran AOZ corresponding to concentrations of 0.5, 1 and 1.5 µg/kg.

Statistical Analysis

SPSS 17.0 programme was used for calculations. Results are expressed as mean (x) and standard error (S.E.). Differences between groups were evaluated using Wilcoxon test.

RESULTS

During the validation process, different egg samples were analysed by the same instrument and the same operator. No significant differences were found between the curves. The calibration curves were linear, with the corresponding correlation coefficients (R^2) values higher than 0.998. The decision limit (CCα) and detection capability (CCβ) were calculated for m/z 236→134, ion transition for AOZ. These values were calculated using eighteen calibration curves (at four levels 0.0, 0.5, 1.0 and 1.5 µg/kg) from six different experiments in a day, on different egg samples and 3 different days. All curves, in validation and in calculation of residues, were constructed using analyte/internal standard peak area ratio versus concentration of analyte. Linearity was proved between 0.5-1.5 µg/kg, CCα was 0.70, CCβ was 0.77 and the recovery values ranged between 88-97.9% and CV was between 3-4.3%.

The within-laboratory reproducibility was calculated in different spiked egg samples at concentration of 1 µg/kg (MRPL level). They were analysed for three different days, with the same instrument and different operators. The within-laboratory reproducibility was found satisfactory for the nitrofuran AOZ. For the calculation of the residue levels we used one calibration curve *(Fig. 1)* with four spike concentrations (0.0, 0.5, 1.0 and 1.5 µg/kg). The validation results were in accordance with the performance method of the European Commission Decision 2002/657/EC and the method was successfully applied to confirm and quantify nitrofuran AOZ in eggs [26].

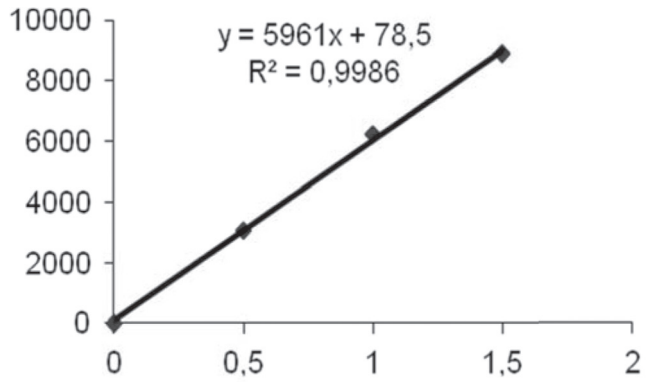

Fig 1. Calibration curve and R^2, spiked with 0.5, 1 and 1.5 µg/kg concentrations for uncooked eggs

Şekil 1. 0.5, 1 ve 1.5 µg/kg düzeyinde yüklenmiş pişmemiş yumurtaların kalibrasyon eğrisi ve R^2 değeri

The detected avarage nitrofuran AOZ residue level in 13 uncooked eggs was 0.86±0.017 µg/kg. The range of detections was between 0.76-0.97 µg/kg for uncooked eggs. After boiling, residue level was increased to 2.42±0.037 µg/kg *(Table 4)*. The range of detections in cooked eggs was between 2.04-2.55 µg/kg. Thus it was observed that the residue level of nitrofuran AOZ was higher (P<0.001) in boiled eggs than in uncooked eggs (*Fig. 2* and *3*).

DISCUSSION

Improperly and illegal administration of nitrofurans may produce residues in various foodstuffs such as eggs. The presence of these residues arises as a serious public health issue including mutagenic, carcinogenic and genatotoxic effects, induces allergic reactions in humans

Table 4. *Nitrofuran AOZ residue levels before and after boiling in eggs*
Tablo 4. *Yumurtalarda kaynamadan önce ve sonra Nitrofuran AOZ kalıntı düzeyleri*

Sample No	Before Boiling (µg/kg)	After Boiling (µg/kg)
01	0.82	2.44
02	0.81	2.04
03	0.97	2.54
04	0.76	2.31
05	0.8	2.39
06	0.87	2.49
07	0.93	2.55
08	0.88	2.49
09	0.94	2.53
10	0.85	2.42
11	0.83	2.41
12	0.82	2.46
13	0.9	2.42

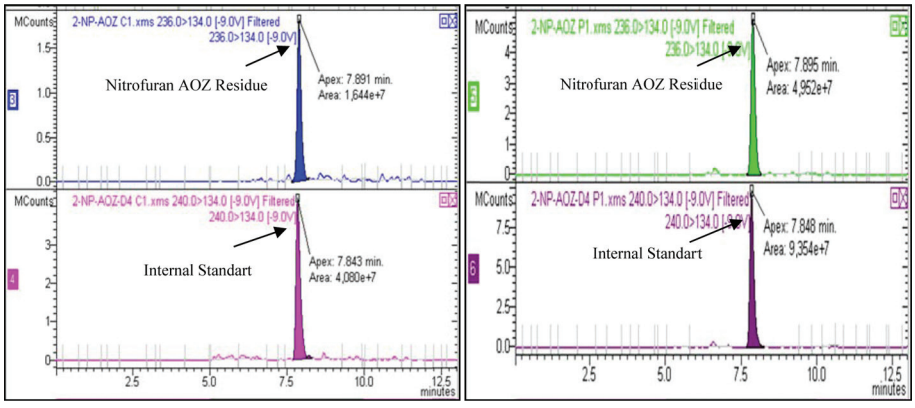

Fig 2. LC-MS/MS analysis of Nitrofuran AOZ residues and internal standart concentrations of first sample before (0.82 µg/kg) and after boiling (2.44 µg/kg) respectively

Şekil 2. Birinci örneğin haşlama öncesi (0.82 µg/kg) ve sonrası (2.44 µg/kg) LC-MS/MS analizi ile ölçülen Nitrofuran AOZ kalıntı değeri ve iç standart konsantrasyonları

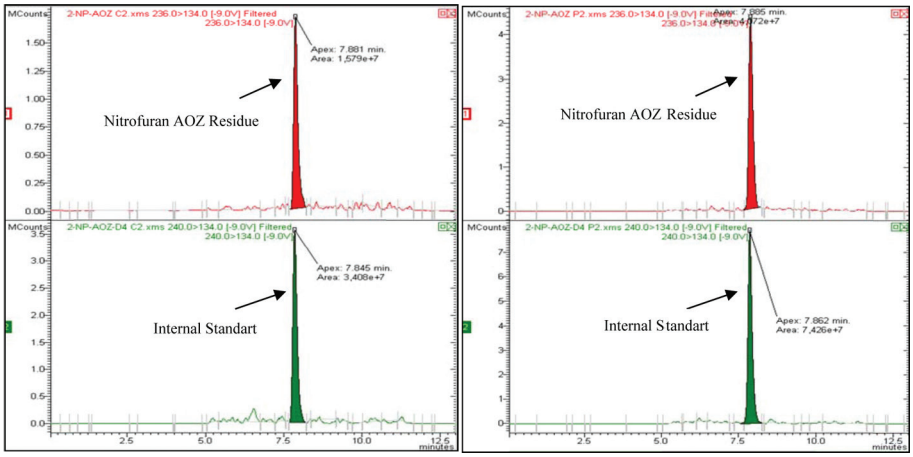

Fig 3. LC-MS/MS analysis of Nitrofuran AOZ residues and internal standart concentrations of second sample before boiling (0.81 µg/kg) and after boiling (2.04 µg/kg) respectively

Şekil 3. İkinci örneğin haşlama öncesi (0.81 µg/kg) ve sonrası (2.04 µg/kg) LC-MS/MS analizi ile ölçülen Nitrofuran AOZ kalıntı değeri ve iç standart konsantrasyonları

and give rise to an increase in the antibiotic resistance of pathogenic bacteria. Due to all these hazardous and severe problems, application of efficient detection and quantification methods and elimination of these drug residues from food matrices are important issues for consumers.

Our results run counter to the claim that heat process in general should decrease various antibiotic levels in food. In general, antibiotics in various tissues such as amphenicols residues in chicken meat [1], sulfanomides residues in chicken meat [27], ormetoprim (OMP) and sulfadimethoxine (SDM) in channel catfish muscle [28], malachite green (MG) and its major metabolite, leucomalachite green (LMG) in carp muscles [29], OTC in black tiger shrimp [30] and Japanese eel [31], chlorotetracycline and oxytetracycline in canned pork [32] are reduced by several heat process. On the contrary, some residues, such as clenbuterol in food [33] and AOZ in liver kidney and muscle [10] are stable during several heat process. Similarly, Cooper and Kennedy [34] also reported that Nitrofuran AOZ, 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ), 1-aminohydantoin (AHD) and semi-carbazide (SEM) residues remained after cooking techniques in muscle and liver of pigs and continue to pose a health risk. Furthermore, Mccracken and Kennedy [10] also reported that grilling increased the extractable AOZ content, at the expense of bound AOZ residues in both liver and kidney. The present study also showed that nitrofuran AOZ levels in eggs were significantly increased after boiling agreement with that reported by Mccracken and Kennedy [10]. These studies have also shown that the heat stability of drug residues in foodstuffs depend on type of drugs.

In conclusion, the occurrence of nitrofuran AOZ residues in eggs in Turkey was determined. Results of this study showed that nitrofuran AOZ residues were still present in the boiled eggs. In this study, the boiling procedure could not degrade residual nitrofuran AOZ in eggs. Moreover, nitrofuran AOZ residues increased with boiling. Therefore, boiling process might increase possible pharmacological and/or toxic effects of these compounds. The observed increase ($P<0.001$) in nitrofuran AOZ levels in boiled eggs relative to uncooked eggs may be due to enhanced efficiency of extraction in boiled samples. For this reason, boiled eggs should be used for analysis of nitrofuran AOZ levels in order to obtain more reliable and more predictive results.

So, it may not be assumed that heating of nitofuran residues in food will result in a safe product for human consumption. Alternative management options such as vaccinations should be applied that could reduce the frequency of antibiotics usage in laying hens, emergance of drug residues in eggs and spread of antibiotic-resistant bacteria. To ensure efficient food safety and accurate monitoring of compliance with the ban, periodic analysis to monitor the drug residues in different foodstuffs should be made more frequently and more effectively by national legal authority.

REFERENCES

1. Franje CA, Chang S, Ching-Lin S, Davis JL, Lee Y, Lee R, Chang C, Chou C: Differential heat stability of amphenicols characterized by structural degradation, mass spectrometry and antimicrobial activity. *J Pharmaceut Biomed,* 53, 869-877, 2010.

2. Goetting V, Lee KA, Tell LA: Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: A review of the literature. *J Vet Pharmacol Ther,* 34, 521-556, 2011.

3. Refsdal AO: To treat or not to treat: A proper use of hormones and antibiotics. *Anim Reprod Sci,* 61, 109-119, 2000.

4. Botsoglou NA, Fletouris DJ: Drug Residues in Food. Marcel Dekker, Inc., New York, NY, 2001.

5. CEC: Council Regulation (EEC) No. 2377/90 of 26 June 1990. Laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off J Eur Commun,* 224, 1-8, 1990.

6. Dowling PM: Miscellaneous antimicrobials: Ionophores, nitro- furans, nitroimidazoles, rifamycins, oxazolidones, and others. **In,** Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM (Eds): Antimicrobial Therapy in Veterinary Medicine. 285-300, Blackwell Publishing, Ames, IA, 2006.

7. Turkish Food Codex: Communique No. 2002/68 notification that is about the implementation of the prohibited substances on food producing animals, Official Gazette no. 24968,19.12.2002. http://www.kkgm.gov.tr/teblig/2002-68.html, 2002.

8. Nouws JFM, Laurensen J: Postmortal degradation of furazolidone and furaltadone in edible tissues of calves. *Vet Quart,* 12, 56-59, 1990.

9. Mccracken RJ, Blanchflower W, Rowan C, Mccoy M, Kennedy DG: Determination of furazolidone in porcine tissue using thermospray liquid chromatography-mass spectrometry and a study of the pharmacokinetics and stability of its residues. *Analyst,* 120, 2347-2351, 1995.

10. Mccracken RJ, Kennedy DG: Determination of the furazolidone metabolite, 3-amino-2-oxazolidinone, in porcine tissues using liquid chromatography-thermospray mass spectrometry and the occurrence of residues in pigs produced in Northern Ireland. *J Chromatogr B,* 691, 87-94, 1997.

11. Mccracken RJ, Spence DE, Floyd SD, Kennedy DG: Evaluation of the residues of furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in eggs. *Food Addit Contam,* 18, 954-959, 2001.

12. Hoogenboom LA, Berghmans MC, Polman TH, Parker R, Shaw IC: Depletion of protein-bound furazolidone metabolites containing the 3-amino-2-oxazolidinone side-chain from liver, kidney and muscle tissues from pigs. *Food Addit Contam,* 9, 623-630, 1992.

13. Vroomen lHM, Berghmans MCJ, Van Bladeren PJ, Groten JP, Wissink CJ, Kuıper HA: *In vivo* and *in vitro* metabolic studies of furazolidone: A risk evaluation. *Drug Metab Rev,* 22, 663-676, 1990.

14. Gottschall DW, Wang R: Depletion and bioavailability of [14C] furazolidone residues in swine tissues. *J Agr Food Chem,* 43, 2520-2525, 1995.

15. Cooper K, Mulder P, Rhijn VJ, Kovacsics L, Mccracken RJ, Young P, Kennedy DG: Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV. *Food Addit Contam,* 22, 406-414, 2005.

16. Stachel C, Bock C, Hamann F, Gowik P: Residues of several nitrofurans in egg. *J Vet Pharmacol Therap,* 29, 143-144, 2006.

17. Cooper K, Kennedy DG: Nitrofuran antibiotic metabolites detected at parts per million concentrations in retina of pigs - A new matrix for enhanced monitoring of nitrofuran abuse. *Analyst,* 130, 466-468, 2005.

18. Finzi JK, Donato JL, Sucupira M, De Nucci G: Determination of nitrofuran metabolites in poultry muscle and eggs by liquid chromatography-tandem mass spectrometry. *J Chromatogr B,* 824, 30-35, 2005.

19. Khong S, Gremaud E, Richoz J, Delatour T, Guy PA, Staddler RH, Mottier P: Analysis of matrix-bound nitrofuran residues in worldwide-originated honeys by ısotope dilution high-performance liquid chromatography-tandem mass spectrometry. *J Agr Food Chem,* 25, 5309-5315, 2004.

20. Leitner A, Zollner P, Lindner W: Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography-tandem mass spectrometry. *J Chromatogr,* 939, 49-58, 2001.

21. Yibar A, Cetinkaya F, Soyutemiz GE: Nitrofuran metabolite 3-amino-2-oxazolidinone residues in chicken liver. *Asian J Anim Vet Adv,* 7, 346-350, 2012.

22. Ergin Kaya S, Filazi A: Determination of antibiotic residues in milk samples. *Kafkas Univ Vet Fak Derg,* 16, 31-35, 2010.

23. Bock C, Stachel C, Gowik P: Validation of a confirmatory method for the determination of residues of four nitrofurans in egg by liquid chromatography - tandem mass spectrometry with the software Inter Val. *Anal Chim Acta,* 586, 348-358, 2007.

24. Rodziewicz L: Determination of nitrofuran metabolites in milk by liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr B,* 864, 156-160, 2008.

25. Verdon E, Couedor P, Sanders P: Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoine, 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. *Anal Chim Acta,* 586, 336-347, 2007.

26. CEC: Commission Decision 2002/657/EC of 12 August 2002 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off J Eur Commun,* 221, 8-36, 2002.

27. Furusawa N, Hanabusa R: Cooking effects on sulfonamide residues in chicken thigh muscle. *Food Res Int,* 35, 37-42, 2002.

28. Xu D, Grizzle JM, Roger WA, Santerre CR: Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel caffish. *Food Res Int,* 29, 339-344, 1996.

29. Mitrowska K, Posyniak A, Zmudzki J: The effects of cooking on residues of malachite green and leucomalachite green in carp muscles. *Anal Chim Acta,* 586, 420-425, 2007.

30. Uno K, Aoki T, Kleechaya W, Tanasomwang V, Ruangpan L: Pharmacokinetics of oxytetracycline in black tiger shrimp, *Penaeus monodon*, and the effect of cooking on the residues. *Aquaculture,* 254, 24-31, 2006.

31. Maruyama R, Uno K: Oxytetracycline residues in tissues of cultured eel and ayu and the effect of cooking procedures on the residues. *Shokuhin Eiseigaku Zasshi,* 38, 425-429, 1997 (in Japanese, with English abstract).

32. Honikel KO, Schmidt U, Woltersdorf W, Leistner L: Effect of storage and processing on tetracycline residues in meat and bones. *J Anal Chem,* 61, 1222-1227, 1978.

33. Rose MD, Shearer G, Farrington WHH: The effect of cooking on veterinary drug residues in food: 1. Clenbuterol. *Food Addit Contam,* 12, 67-76, 1995.

34. Cooper KM, Kennedy DG: Stability studies of the metabolites of nitrofuran antibiotics during storage and cooking. *Food Addit Contam,* 24, 935-942, 2007.