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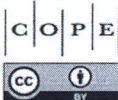
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Milan Damjanoski, M.A., Ss.Cyril and Methodius University in Skopje

Address

Macedonian Veterinary Review, Lazar Pop Trajkov 5/7, 1000 Skopje, Republic of Macedonia

Tel: ++389 2 3240 700, Fax: ++ 389 2 3114 619; e-mail: macvetrev@fvm.ukim.edu.mk; URL: www.macvetrev.mk



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aim of this study was to perform method validation of a LC-MS/MS reference method to determine the residues of amphenicol content in feed samples.

Material and methods: In the study, the samples of cattle fattening feed, not including amphenicol content, were used as research materials. The analysis was repeated with the samples of chicken fattening feed and cattle milk feed, not including amphenicol content.

Parameters of LC-MS/MS: For HPLC; flow rate: 0,2 ml/min, volume of injection: 50 µl, liquid phase: 80:20 methanol/water (v/v), for MS/MS; ionization mode: ESI -VE, API nebulizing gas pressure: 50 psi, drying gas temperature: 400°C, drying gas pressure: 35 psi, scan time: 0,4 sec, SIM width: 1,5 amu, needle: -4000V, shield: -400V, capillary: -55 V, detector: -1600V, CID gas pressure: 2 m Torr, Spray chamber temp.: 60°C, mass peak width in amu: 1,5, Quad 1: 1,5 and Quad 3: 1,5.

Parameters of Method Validation: Linearity, recovery (R) %, limit of detection (LoD) and limit of quantification (LoQ), repeatability and reproducibility were used as validation parameters.

Linearity was tested by using four different standard concentrations (0,5, 1, 1,5, and 2 µg/kg) in six repetitions. Recovery % was calculated by the spiked samples in three different standard concentrations (1, 1,5, and 2 µg/kg) in six repetitions in three days. LoD and LoQ were determined by using the calibration curve for linearity (LoD: 3 x SD/m, LoQ: 10 x SD/m, SD: smallest SD of calibration curve, m: slope of calibration curve). Repeatability were performed by using the spiked samples in three different concentrations (0,5, 1, and 2 µg/kg) in six repetitions. Reproducibility was studied by using the spiked samples of 1µg/kg by two persons in five repetitions and in four days.

Results: The results of the method validation were as follows: linearity for fluorphenicol, chloramphenicol, and tiamphenicol were 0.9975, 0.9972 and 0.9997, respectively. Recovery % for 0,5 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 103, 95.33 and 102, for 1 MRL were 97.85, 100.7 and 99.72, respectively. LoD and LoQ of fluorphenicol, chloramphenicol, and tiamphenicol were 28 and 35.01, 28.97 and 38.25, and 46.28 and 95.92, respectively. Repeatability for 0.5 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 3.7, 5.3 and 5.35, for 1 MRL were 4.3, 1.59 and 6.19, respectively. Reproducibility for 1 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 4.33, 3.74 and 5.35, respectively.

Conclusion: It was concluded that the present method validation study may be useful to formal or special laboratories, authorized by the Turkish Republic, Ministry of Agriculture for analysing the residues of amphenicol content. These laboratories, using this validated reference method, may assist exporters and importers, local producers, regulators and governments with detection of these compounds in feed and foodstuffs.

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Seasonal variations of Aflatoxin M₁ content in raw milk from Macedonia and estimation of consumers exposure

Elizabeta Dimitrieska-Stojkovic^{1*},
Zehra Hajrulai-Musliu¹, Biljana Stojanovska-Dimzoska¹, Gordana Ilievska¹, Risto Uzunov¹, Aleksandra Angeleska¹, Goran Stojkovic²

¹Ss. Cyril and Methodius University, Faculty of Veterinary Medicine, Food institute, Skopje, Macedonia

²Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute for Chemistry, Skopje, Macedonia

Introduction: Milk has the greatest demonstrated potential for aflatoxin M₁ (AFM₁) introduction in the human diet. The frequency of occurrence of AFM₁ in commercially available milk and dairy products during the Western Balkan Countries outbreak in 2013, led to an increased concern about the establishment of measures to control AFM₁ contamination. In light of these concerns, a comprehensive surveillance program for AFM₁ in raw milk was established. The determined AFM₁ levels were used to calculate between-month and between-season variations of the toxin content, as well as the fluctuations of the consumer's exposure during the surveillance period.

Material and methods: A total of 3634 raw milk samples were collected from 48 diaries in the period February 2013-January 2014. The samples were tested applying the immunochemical screening method, and the positive samples exceeding the maximum residue level (MRL) were confirmed with high performance liquid chromatography (HPLC) with fluorescence detection (FD). Testing methods were validated and confirmed to be sensitive, selective, accurate and precise according to Commission Regulation 401/2006/EC and Commission Decision 657/2002/EC requirements. To evaluate statistical differences in the means between the data series, a t-test for independent samples has been applied (95 % confidence level).

Results: Regarding the between season variations of AFM₁ concentration in milk, unlike the common AFM₁ fluctuation pattern, the highest level has been detected in Autumn (32.4 ng/kg) and accordingly the calculated EDI was 0.108 ng/kg BW/day. The highest AFM₁ average concentration was observed in October 2013 (34.8 ng/kg). Accordingly, the estimated intake revealed to be 0.116 ng/kg BW/day. This could be explained to be due to the long drought in the second half of 2013 and lack of fresh feed. Overall, the average AFM₁ concentration for the survey period was 24.2 ng/kg with the respective intake of 0.081 ng/kg BW/day. A gradual decline of the AFM₁ concentrations in milk was observed for the period studied, confirming the effectiveness of the measures taken for control of milk production facilities.

Conclusion: To ensure the safety of milk for human health, it is extremely important to avoid providing feed contaminated with AFB₁ to cows. Hence, regular

monitoring of not only the AFM₁ level in milk, but also the AFB₁ level in feed, will be required to protect the public, especially infants and young children, against AFM₁ toxicity.

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Incidence of ochratoxin A: Current situation in some food products

Biljana Stojanovska-Dimzoska*,
Katerina Davceva, Zehra Hajrulai-Musliu,
Elizabeta Dimitrieska-Stojkovic,
Risto Uzunov, Aleksandra Angeleska

Food Institute, Faculty of Veterinary Medicine, Ss Cyril and Methodius University. Lazar Pop Trajkov 5-7. Skopje, Macedonia

Introduction: Ochratoxin A, a nephrotoxic mycotoxin mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, has been shown to contaminate a wide variety of commodities (cereals and their products, grapes, wine, dried wine fruits-figs, coffee, nuts). There is growing evidence that this mycotoxin has poor effects not only on body weight, feed intake and feed conversion in animals after consumption of contaminated feed, but it is also involved in the etiology of Balkan endemic nephropathy. OTA exerts nephrotoxic, immunotoxic, teratogenic, genotoxic, mutagenic and carcinogenic effects. For this reason the International Agency for Research on Cancer evaluated OTA as a possible carcinogen in humans (group 2B). The MRL for OTA content in food has been regulated by legislation worldwide and it is in the range from 0,5 to 10 µg/kg for different commodities.

Material and methods: Total of 40 corn flour, 11 polenta, 38 wheat flour, 63 grits, 63 bread, 15 breakfast cereals, 13 green coffee, 18 frozen corn, 3 pasta (dry) and 33 strudel samples were brought to our laboratory by inspectors or from the food operators themselves during 2013-2014. The HPLC-FLD and fluorometry with immunoaffinity column clean-up were the methods used for determination of OTA. The extraction and purification of samples was done according to AOAC Official method 2000.03 (for HPLC-FLD) and according to Instruction Manual (for fluorometry).

Results: Total of 273 samples were analyzed for OTA content. Most of them (218 samples) were with OTA concentration level below LOD (79,8%). Eighteen (18) samples were positive in accordance with legislation. Among them, 10 strudel samples (30,3%) show OTA content over the MRL in the range of 3,3-9,1 µg/kg, 6 corn flour samples (15%) were with OTA concentration level in the range of 3,2-5,0 µg/kg and 2 grits samples (5,1%) were with OTA content over the MRL in the concentration range of 3,9-5,7 µg/kg. None of the following samples: polenta, wheat flour, bread, breakfast cereals, pasta, frozen corn and green coffee, surpassed the legislation limits suggested by the official agencies.

Conclusions: OTA was found in 55 samples (20,1%) tested with levels ranging from 0,14-9,1 µg/kg for different commodities. Although 79,8 % of samples were

with an OTA concentration level below LOD, the number of positive samples (6,6%) should not be neglected. The strategies for ensuring food safety should be directed to the current human exposure to OTA in relation to the safety guidelines for OTA, taking into account what can be reasonably achieved following good practices at all stages of production.

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Occurrence of ochratoxin A in Macedonian wines

Katerina Davcheva*, Biljana Stojanovska-Dimzoska, Zehra Hajrulai-Musliu, Elizabeta Dimitrieska-Stojkovic, Risto Uzunov, Aleksandra Angeleska, Basak Kucukcakan

Food Institute, Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje, Macedonia

Introduction: Ochratoxin A (OTA) is a mycotoxin produced by the fungi *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus carbonarius*. It possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. The international Agency for Cancer Research (IACR) has placed OTA into the B2 group i.e. among substances potentially carcinogenic for humans. The total intake of OTA due to wine has been provisionally estimated by the Codex Alimentarius Commission to 15%. In accordance to EU Regulations Commission (EC 123/2005) wine and other wine and/or grape must based beverages should comprise maximum concentration of 2.0 ng/ml of OTA.

Material and methods: Quantitative determination of OTA in wines and grape musts after their clean-up on immunoaffinity columns was investigated using HPLC method with fluorescence detection according to AOAC method (2001.01). In duration of 4 consecutive years (2011-2014), 189 samples of variety bottled wines and grape musts (86 red wines, 90 white wines, 11 rose wines and 2 grape musts), which originated from different parts of Macedonia, were analysed.

Results: OTA was detected in 30% of samples, in a concentration level up to: 0.349 ng/ml, 0.716 ng/ml and 0.163 ng/ml in red wines in 2012, 2013 and 2014 respectively; 0.079 ng/ml, 0.238 ng/ml in white wines in 2012 and 2013 respectively; 0.315 ng/ml in rose wines in 2013; 0.137 ng/ml in grape must in 2013. Overall OTA concentration detected in samples in 2011 and 2012 (in both red and white wines) was below LOD (0.043 ng/ml). In 2013 the mean concentration level was 0.076 ng/ml in rose wines and 0.137 ng/ml in grape must. In 2014, only in red wines the overall OTA concentration was over the LOD (0.059 ng/ml). None of the samples exceeded the maximum limit of OTA concentration.

Conclusion: In general, levels of OTA were higher in red wines than in white ones, corresponding to the comprehensive published findings. It is interpreted as a consequence of the differences in the winemaking procedures for both types. The overall OTA concentrations