



UDC 619.636/637

CODEN MVPRE

e-ISSN 1857-7415

MACEDONIAN VETERINARY REVIEW



*Proceedings of the 5th International Scientific Meeting
Days of veterinary medicine 2014
Ohrid, Macedonia
September 5-7, 2014*

Mac Vet Rev 2014; Volume 37; Supplement 1; Pages: 1-90

International Scientific Journal
MACEDONIAN VETERINARY REVIEW

An Official Publication of
the Faculty of Veterinary Medicine-Skopje
Ss.Cyril and Methodius University in Skopje

e-ISSN 1857-7415



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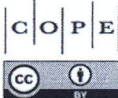
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monitoring of not only the AFM₁ level in milk, but also the AFB₁ level in feed, will be required to protect the public, especially infants and young children, against AFM₁ toxicity.

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Incidence of ochratoxin A: Current situation in some food products

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Introduction: Ochratoxin A, a nephrotoxic mycotoxin mainly produced by *Asperillus 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

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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

didn't show big fluctuation during the years and are far below the proposed limit. All samples fulfil the regulations of the European Commission regarding maximal concentration of OTA in wine and other wine and/or grape must based beverages.

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Validation of ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry method for determination of thyreostats in bovine urine

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Introduction: Thyreostats are veterinary drugs that may be used in livestock production as growth promoter. They cause retention of water in the subcutaneous and muscular tissues as well as in the gastro-intestinal tract by inhibiting thyroid hormone production; thus administration of thyreostats results in increasing of body weight. On the other hand, residues of thyreostats in meat pose a risk for human health due to the teratogenic and carcinogenic effect. For this reason, in 1981 the European Union banned their use in animal production. The aim of this study was validation of the UHPLC MS/MS method for determination of thyreostats in bovine urine.

Material and methods: Our study included four thyreostats: thyouracil (TU), methylthiouacil (MTU), propylthiouacil (PTU) and tapazole (TA). Besides these thyreostats, dimethylthiouacil was used as an internal standard. Negative bovine urine was used for validation. Before extraction, the pH of the urine was adjusted to 1. Then derivatisation of the samples was performed with 3 iodobenzylbromide, 1h at 40°C and liquid/liquid extraction was performed with ethyl acetate. The method was validated according to 2002/657/EC. The decision limit (CC α), detection capability (CC β), accuracy, precision and reproducibility were validation parameters which were obtained with validation procedures. The calibration curve for all standards was from 1.0 to 30 $\mu\text{g/kg}$.

Results: The linearity of the method showed good correlation for all standards with $r^2=0.9913$ for TA, $r^2=0.9977$ for TU, $r^2=0.9969$ MTU and for PTU $r^2=0.9946$. The accuracy was evaluated by determining the recovery of spiked urine samples on three concentration level at 5 $\mu\text{g/l}$, 10 $\mu\text{g/l}$ and 15 $\mu\text{g/l}$. The recovery for TA was from 98.22 to 123%, for TU was from 81.11 to 102.33%, for

MTU was from 92.89 to 93.67% and for PTU the recovery was from 92.33 to 106.33%. The precision of the method ranged from 4.58 to 17.45% and the reproducibility of the method was from 1.61 to 20.70%. The value from CC α were 2.26 $\mu\text{g/l}$ for TA, 10.71 $\mu\text{g/l}$ for TU, 2.79 $\mu\text{g/l}$ for MTU and 2.22 $\mu\text{g/l}$ for PTU. CC β were found to be 2.93 $\mu\text{g/l}$ for TA, 16.75 $\mu\text{g/l}$ for TU, 3.60 $\mu\text{g/l}$ for MTU and 3.04 $\mu\text{g/l}$ for PTU.

Conclusion: The UHPLC-MS/MS method for detection and identification of four thyreostats compounds in urine was developed. Good linearity, good precision, recovery and reproducibility, make this method applicable for determination of thyreostats in bovine urine.

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RP-HPLC determination of 5-hydroxymethylfurfural in honey from the Republic of Macedonia as a control parameter of its quality with some analytical aspects: a random collection study

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The decomposition of sugars produces the organic compound 5-hydroxymethylfurfural, also known as HMF. HMF affects the color and taste, thus manufacturers monitor its levels in some foods, including honey. HMF production increases with extended shelf life and/or heat exposure, and is used commonly as an indicator of heat and storage changes in honey. But, HMF is not a harmful substance in the levels present in food. According to the Codex Alimentarius Standard for Honey (1981), "HMF content of honey after processing and/or blending shall not be more than 40 mg/kg", and "in the case of honey of declared origin from countries or regions with tropical ambient temperatures, and blends of these honeys, the HMF content shall not be more than 80 mg/kg."

The aim of this work was to develop, test and implement simple, fast, accurate, rugged and robust chromatographic method for determination and quantification of 5-hydroxymethylfurfural in honey.

In this research we used HPLC system automated Varian ProStar with ternary high pressure mixing pump, Autosampler 410 with column oven and Diode Array Detector 330, controlled by software Varian-Star Version 6.31; the chemicals were Ph.Eur. grade, products of Merck Darmstadt, Germany. Several columns were tested: LiChrosphere C18 125 x 4 mm, 5mm; LiChroCart C18e 125 x 4mm, 5mm, protected with C18e pre-columns 4 x 4 mm; Eurosphere EC Knauer C18 250 x 4,6 mm, 5 mm and Perkin Elmer Brownlee C18 Pecosphere 33 x 4,6 mm with 3 mm particles.

The extraction procedure was simple, using demineralized water; the monitoring signal at 282 nm, column oven set