

UNIVERSITY "Ss. CYRIL AND METHODIUS" IN SKOPJE  
FACULTY OF VETERINARY MEDICINE - SKOPJE

# PROCEEDINGS

DAYS OF  
VETERINARY MEDICINE 2013



The 4<sup>th</sup> International Scientific Meeting

06-08 September 2013  
Struga, Republic of Macedonia

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**P16 OCURENCE OF PATULIN IN APPLE-BASED  
JUICES AND CONCENTRATES**

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**ABSTRACT**

**Introduction**

Patulin is a toxic secondary metabolite produced by a wide range of fungal species of the *Penicillium*, *Aspergillus* and *Byssochlamys* species growing on fruit, including apples, pears, grapes and other fruit. The principal risk arises when the unfit fruit is used for the production of juices and other processed fruit. Apples and apple products are excellent substrates for *Penicillium expansum*, the casual agent of "blue mould rot", to produce the patulin. According to the International Agency for Research on Cancer (IARC) it is classified in Group 3, and therefore the European Commission (EC 1881/2006) has set the maximum permitted level (MRL) of patulin for apple juice and reconstituted apple juice concentrate at 50 µg/kg.

**Materials and Methods**

Eighteen commercially available apple juice and twelve concentrated apple juices have been purchased from the market, or supplied from local producers and importers. The samples were stored in refrigerator at +4 °C prior to analysis. Sample preparation has been performed utilizing molecularly imprinted - solid-phase extraction (MIP-SPE) polymer cartridges, a product by R-Biopharm (Darmstadt, Germany). For detection and quantification a High-Performance Liquid chromatography (HPLC) with Diode Array Detector (DAD) at 276 nm has been applied. Gradient separation was carried out on reverse-phase C18 analytical column (GL-Science, Torrance, CA, USA), with 1 ml/min flow, in duration of 30 minutes.

**Results**

Applying the chromatographic conditions described above, a good separation of the patulin peak with no apparent interferences has been achieved. Linearity has been established from 10 µg/L to 500 µg/L, with a correlation coefficient ( $R^2$ ) of 0.9998, and average precision less than 1 %. The estimated limits of detection (LOD) and quantification (LOQ) were 1.8 µg/L and 5.3 µg/L, respectively. Method accuracy has been tested at 25 µg/kg and 50 µg/kg, and

the determined recoveries were > 80 %. 23.3 % of the samples being tested contained patulin less than the LOQ of the applied method. Patulin has been detected and confirmed in 23 analysis, comparing the ultraviolet spectra of standards and test samples. Concentration levels in the positive samples ranged from 15.0 to 87.8 µg/kg; 6.7 % of the tested samples have been confirmed to be non-compliant.

**Conclusion**

The performances of the HPLC-DAD method have confirmed its fitness as precise and accurate analytical technique for detection and quantification of patulin in apple juices and concentrates. The performed analysis revealed patulin's presence in over 76 % of the tested samples.

**Key words:** patulin, apple juice, molecularly imprinted polymers, HPLC-DAD