



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

BOOK OF ABSTRACTS

**“Days Of Veterinary Medicine”
10th International Scientific Meeting
and
2nd European Conference on
Veterinary and Medical Education 2024**

22-25 September 2024,
Republic of North Macedonia

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and
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O20**DETERMINATION OF COMONLY USED ARTIFICIAL COLORS IN MEAT AND MEAT PRODUCTS USING HPLC-DAD METHOD**

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The color of the meat is the most crucial visual quality parameter from the consumer's perspective. Red color additives are used to improve the appearance of meat and meat products on the shelf. Although natural colors like red wine or beet juice are used, there are artificial colors which can withstand the temperature and pH conditions of processing. According the Food additive Regulation in North Macedonia, Carmoisine (Azorubine), Allura Red, Ponceau 4R and Carminic Acid are permitted for use. The allowed limits of these colors in meat and meat products range from 25 mg/kg to 200 mg/kg depending on the additive and whether the meat product is heat treated or not. The aim of this study is to develop a HPLC-DAD method to determine these additives in meat and meat products. Due to the different chemical properties of carminic acid compared to the others, two different HPLC-DAD methods with different sample preparation were developed. Both used RP C18 column and gradient elution with methanol and phosphate buffer, but with different pH levels. Method validation was performed according to ICH Harmonised Guideline Q2(R2) (2022). The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ), and found to be linear, with a correlation coefficient (R^2) ranging from 0.995 to 0.999. The LOD and LOQ values calculated from the calibration curve ranged from 0.10 to 0.23 mg/kg and 1.00 to 1.12 mg/kg respectively. The accuracy of the method was evaluated based on the recovery percentages at 3 spiked levels (1, 5 and 10 mg/kg), which were found to be within an acceptable range of 93.82 to 109.30%. The repeatability of the method was assessed at the same three spiking levels over two days, with relative standard deviation (RSD_R) values ranging from 2.01 % to 4.97 %. These performance parameters indicate that the developed and validated method is suitable for the quantitative determination of Carmoisine (Azorubine), Allura Red, Ponceau 4R and Carminic Acid in meat and meat products. Random meat products analysis using this method revealed that 15 out of 55 contained these additives but none exceeded the legislative limits.

Keywords: HPLC-DAD; carmoisine, azorubine, allura red, ponceau 4R, carminic acid, carmine