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


Joint meeting
$5^{\text {th }}$ International Vet-Istanbul Group Congress
\&
$8^{\text {th }}$ International Scientific Meeting Days of veterinary medicine - 2018

# BOOK OF ABSTRACTS 

23-27 September 2018 Ohrid, R. of Macedonia

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Faculty of Veterinary Medicine - Skopje, Lazar Pop Trajkov 5/7, 1000 Skopje
Tel: ++38923240700 Fax: ++ 38923114619
www. fvm.ukim.edu.mk


CIP - Каталогизација во публикација
Национална и универзитетска библиотека "Св. Климент Охридски", Скопје 636.09(062)(048.3)

INTERNATIONAL scientific meeting ( $8 ; 2018$; Ohrid)
Book of abstracts : join meeting : 5 th International Vet-Istanbul group congress \& 8 th International scientific meeting days of veterinary medicine - 2018, Ohrid, 2018 Republic of Macedonia / [editors Florina P. Percinic, Lazo Pendovski, Katerina Blagoevska]. - Skopje : Faculty of Veterinary medicine, 2018. - 200 стр. ; 23 см

Регистар
ISBN 978-9989-774-31-7
a) Ветеринарна медицина - Зборници - Апстракти COBISS.MK-ID 108195850

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It is my grea
$5^{\text {th }}$ International Days of veterinary $23^{\text {th }}$ to $27^{\text {th }}$ Septem

This year, in University, we'll b academic cooperat (VEE) partners, by the public good.

The Faculty together outstandin experts in the field researchers, veterina doctoral students, off in the veterinary edu following topics: anin and food safety.

The program for retain its usual popular student papers and pos authors, whose researc of the International Sc electing 117 papers fro followed by two poster invited speakers for the talks.

A successful confer place, where ideas abou debated, which will ope enjoyment.

On the behalf of the in Macedonia.

We look forward to w

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# DETECTION OF ARTIFICIAL SWEETENERS AND FOOD PRESERVATIVES IN ENERGY DRINKS WITH HPLC-DAD METHOD 

Stefan Jovanov ${ }^{1 *}$, Zehra Hajrulai-Musliu ${ }^{1}$, Risto Uzunov ${ }^{1}$, Velimir Stojkovski², Elizabeta Dimitrieska- Stojkovic ${ }^{1}$, Biljana Stojanovska-Dimzoska ${ }^{1}$, Dean Jankuloski ${ }^{1}$<br>${ }^{1}$ Food Institute, Faculty of Veterinary Medicine,<br>Ss. Cyril and Methodius University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, Macedonia<br>${ }^{2}$ Institute of Reproduction and Biomedicine, Faculty of Veterinary Medicine,<br>Ss. Cyril and Methodius University in Skopje,<br>Lazar Pop Trajkov 5-7, 1000 Skopje, Macedonia

Artificial sweeteners are often used in food and beverages to control calorie intake and in certain medical conditions such as diabetes, while food preservatives are used to prevent spoilage (they inhibit microbial growth). The control of sweeteners and preservatives in food and beverages is essential for the assurance of food safety and reduce the adverse health effects. The adverse effects include causing dermatological problems, headaches, mood variations, behavior changes, respiratory difficulties, seizures, allergies and cancer. The permitted levels in beverages for saccharin is $80 \mathrm{mg} / \mathrm{L}$, for acesulfame-K is $350 \mathrm{mg} / \mathrm{L}$, for aspartame is $600 \mathrm{mg} / \mathrm{L}$, for sodium benzoate is $150 \mathrm{mg} / \mathrm{L}$ and for potassium sorbate is $250 \mathrm{mg} / \mathrm{L}$. The aim of this study was determination of three sweeteners: saccharin, acesulfame-K and aspartame and two food preservatives, sodium benzoate and potassium sorbate in energy drinks using HPLC-DAD method. Total of 82 energy drinks were collected from the local stores. The preparation of the samples was performed according to the standard method EN 12856:2010. The analyses were carried out on a HPLC-DAD. The mobile phase A was acetonitrile and mobile phase B was 0.0125 M phosphate buffer, $\mathrm{pH} 3,5$. The elution was gradient and separation of the analyte was with C18 (3x150 $\mathrm{mm}, 2,7 \mu \mathrm{~m})$ column. In the validation study was included evaluation of: linearity, limit of detection (LOD), limit of quantification (LOQ), precision and reproducibility of the method. The described analytical method correctly separated the preservatives and artificial sweeteners in the samples. Calibration curve of all analytes was in the range from 10 to $100 \mathrm{mg} / \mathrm{L}$ and the correlation coefficient varies from 0.9964 to 0.9999 . The LOD for saccharin, acesulfame-K, aspartame, sodium benzoate and potassium sorbate were $0.7,0.6,12.0,12.0$ and $1.3 \mathrm{mg} / \mathrm{L}$ respectively. LOQ for all analytes was $20 \mathrm{mg} / \mathrm{L}$. The accuracy of the method was evaluated by determining the recovery of spiked energy drink samples at three concentration levels $20.0 ; 50.0 ; 100.0 \mathrm{mg} / \mathrm{L}$. The recovery was from 89.37 to $106.19 \%$. The precision and reproducibility of the method were evaluated by the relative standard deviation (RSD), obtained from the three spiked levels in two days. The RSD for the precision was from 0.221 to $2.916 \%$, and the RSD for reproducibility
was from 0.325 to $2.419 \%$. From the 82 analyzed energy drink samples acesulfam-K and saccharin were found in 65 samples in the range from $25-181.4 \mathrm{mg} / 1$ and 22-58 $\mathrm{mg} / \mathrm{l}$, respectively. Aspartam was detected in 50 samples at the concentration from $30-$ $204.7 \mathrm{mg} /$. Preservatives were found in all analyzed samples as follows: sodium benzoate at concentration levels from $50-140 \mathrm{mg} / \mathrm{l}$ and potassium sorbate from $200-220 \mathrm{mg} / 1$. In conclusion, the method was successfully validated and applied for the analysis of real samples of energy drinks. The manufacturers are use these substances in the production of energy drinks, but concentration levels were in agreement with Macedonian Regulation.

Key words: artificial sweeteners, preservatives, energy drinks, validation, HPLCDAD

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The long-ter fields, such as so banks etc. In thi techniques was to as cell transfectio properties. It wa and also storage primer embryoni freezing in liquic straws were comp 2 million cells/m straws with other after thawing thei a result of the sta in cryovial, 82.68 in vitrification m $73.75 \pm 3.45,80.6$ POF cells were slow freezing anc membrane were ( $\mathrm{p}=0.00$ ). As conc to be more effecti while slow freezi

Key words: vapor

This study was fu and TSG-2017-20

