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





PROCEEDINGS

DAYS OF VETERINARY MEDICINE 2016

7th International Scientific Meeting

22-24 September 2016, Struga, Republic of Macedonia

Days of veterinary medicine 2016

Proceedings

THE 7th INTERNATIONAL SCIENTIFIC MEETING DAYS OF VETERINARY MEDICINE 2016 EXECUTIVE COMMITTEE OF

Local Organizing Committee

Prof. Lazo Pendovski, PhD, Ss.Cyril and Methodius University in Skopje, Macedonia

Stojkovik, PhD, Dr. Biljana Dimzovska Stojanovska, PhD, Dr. Radmila Crceva Nikolovska, PhD Irena Celeska, PhD, Dr. Iskra Cvetkovikj, PhD, Dr. Ksenija Ilievska, PhD, Dr. Elizabeta Dimitrievska Ass. Prof. Nikola Adamov, PhD, Ass. Prof. Kiril Krstevski, PhD, Dr. Katerina Blagoevska, PhD, Dr. Branko Atanasov, PhD, Ass. Prof. Igor Dzadzovski, PhD, Ass. Prof. Aleksandar Dodovski, PhD, Stefanovska, PhD, Ass. Prof. Florina P. Percinic, PhD, Ass. Prof. Dean Jankuloski, PhD, Ass. Prof. Igor Ulchar, PhD, Prof. Pavle Sekulovski, PhD, Prof. Blagica Sekovska, PhD, Ass. Prof. Jovana Prof. Zehra Hajrulai-Musliu, PhD, Prof. Slavcho Mrenoshki, PhD, Prof. Romel Velev, PhD, Prof.

all from the Faculty of Veterinary Medicine -Skopje (Ss. Cyril and Methodius University in Skopje, R. Macedonia)

International Scientific Committee

Prof. Vladimir Petkov, PhD, Ss.Cyril and Methodius University in Skopje, Macedonia

Prof. Geert Opsomer, PhD, University of Gent, Belgium

Prof. Jozef Laurincik, DrSc, Prof.H.C., Constantine the Philosopher University in Nitra, Slovakia

Prof. Andrej Kirbis, PhD, University of Ljubljana, Slovenia

Prof. Dr. Güven Kaşikçi, Istanbul University, Turkey

Prof. Velimir Stojkovski, PhD, Ss. Cyril and Methodius University in Skopje, Macedonia

Prof. Artur Niedzwiedz, PhD, University of Wrocław, Poland

Prof. Danijela Kirovski, PhD, University of Belgrade, Serbia

Prof. Voicilas Dan Marius, PhD, Romanian Academy of Sciences - Institute of Agricultural Prof. Halil Gunes, PhD, Istanbul University, Turkey

Economics, Romania

Dr. Kiro R. Petrovski, PhD, University of Adelaide, Australia

Prof. Ali Aydin, PhD, Istanbul University, Turkey

Prof. Bulent Alten, PhD, Hacettepe University, Turkey

Prof. Vlatko Ilieski, PhD, Ss. Cyril and Methodius University in Skopje, Macedonia

Prof. Dr. Vadims Bartkevics, PhD, Institute of Food Safety, Animal Health and Environment

"BIOR", Latvia

Dr. Hristo Daskalov, PhD, NDRVMI, Bulgarian Agency of Food Safety, Bulgaria

Prof. Tomislav Dobranic, PhD, University of Zagreb, Croatia

Prof. Giovanni M. Lacalandra, PhD, University of Bari, Italy

Dr. Benjamin Felix, French agency for food, environmental and occupational health & safety, France

Prof. Rizah Avdic, PhD, University of Sarajevo, Bosnia and Herzegovina

Prof. Serkal Gazyagci, Kirikkale University, Turkey

Dr. Els Van Pamel, PhD, Technology and Food Science Unit - Food Safety - Product Quality and

Prof. Breda Jakovac Strajn, PhD, University of Ljubljana, Slovenia

22-24 September 2016, Struga, Republic of Macedonia 7th International Scientific Meeting

2

Prof. Milka Vrecl, PhD, University of Ljubljana, Slovenia Prof. Josip Kos, PhD, University of Zagreb, Croatia Prof. Dr. Peter Vajdovich, Szent Istvan University, Hungary Prof. Piret Hussar, M.D., D.M.Sc, University of Tartu, Estonia Prof. Dine Mitrov, PhD, Ss. Cyril and Methodius University in Skopje, Macedonia Prof. IIse Schwendenwein, PhD, University of Veterinary Medicine Vienna, Austria Prof. Gregor Fazarinc, PhD, University of Ljubljana, Slovenia Prof. Gordana Ušćebrka, PhD, University of Novi Sad, Serbia Dr. Tamaš Petrović, PhD, Scientific Veterinary Institute"Novi Sad", Serbia Dr. Verica Milosevic, PhD, University of Belgrade, Serbia Prof. Plamen Trojacanec, PhD, Ss. Cyril and Methodius University in Skopje Prof. Serkan Ikiz, PhD, Istanbul University, Turkey Prof. Marlene K. Kirchner, PhD, ECAWBM, University of Copenhagen, Denmark Prof. Nenad Turk, PhD, University of Zagreb, Croatia Prof. Vladimir Ivović, PhD, University of Primorska, Slovenia Prof. Vitomir Cupic, PhD, University of Belgrade, Serbia Prof. Toni Dovenski, PhD, Ss. Cyril and Methodius University in Skopje, Macedonia Prof. Peter Dovc, PhD, University of Ljubljana, Slovenia Prof. Nihad Fejzic, PhD, University of Sarajevo, Bosnia and Herzegovina

Secretariat of the Meeting

ka Dovenska, DVM, Riste Uzunov, DVM, Marija Ratkova, DVM, Ana Cvetanovska, Dipl. Pharm. Miza, PhD, Ljupco Angelevski, MSC, Miroslav Radevski, DVM, Martin Nikolovski, DVM, Moni-Dr. Ljupco Mickov, PhD, Dr. Alesandar Cvetkovikj, PhD, Dr. Mirko Prodanov, PhD, Dr. Sandra

Technical Secretariat

Milica Tosevska Apostolova Maja Menkova, MSc

IT, DTP and Web Support

Viktor Denkovski, MSc

Topics of the Days of Veterinary Medicine 2016

Food Safety and Veterinary Public Health Basic Sciences & Clinical Sciences Animal Reproduction Animal Health

Editors

Prof. Dr. Florina P. Percinik Prof. Dr. Lazo Pendovski Monika Dovenska

Published by

Faculty of veterinary medicine - Skopje, Lazar Pop Trajkov 5/7, 1000 Skopje Tel: ++389 2 3240 700 Fax: ++ 389 2 3114 619 www. fvm.ukim.edu.mk

035 DETECTION OF BETA AGONISTS IN BIOLOGICAL MATRICES WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

Risto Uzunov*, Zehra H. Musliu, Elizabeta D. Stojkovic, Biljana S. Dimzoska, Basak Kucukcakan, Aleksandra Angeleska, Dean Jankuloski, Velimir Stojkovski

Faculty of Veterinary Medicine, University "Ss. Cyril and Methodius" Lazar Pop-Trajkov 5/7, 1000 Skopje, R. Macedonia

Introduction: β -agonists are a group of drugs which are widely used in human and veterinary medicine as a bronchodilator, cardiotonic and tocolytic agent. Moreover, β -agonists have been illegally used as a growth promoter in meat-producing livestock, because they increase the muscle mass and reduction the lipid content of the carcass. The residues of these compounds in meat and meat products present a risk for public health. Due to their side effects in human health, the use of β -agonists, as a growth promoter has been banned by the European Union at 1996.The aim of this study was detection of β -agonists in biological matrices from domestic animals with LC-MS/MS method.

Material and Methods: Total of 55 analyzed urine samples from cattle, swine, sheep and goat and 36 meat samples from bovine, swine and poultry were collected during 2015-2016 as part of national monitoring residue plan. The samples were collected and delivered from authorized veterinary inspectors. The extraction method was done according to the method of National Reference Laboratory in Berlin. The analyses were carried out on the LC-MS/MS. The chromatographic separation was achieved on a Phenomenex C18 (2.6 μ m, 2.1x50 mm) column followed by tandem mass spectrometry using an electro spray ionization source in positive mode. Validation of the method was performed according to Decision 2002/657/EC.

Results: In validation process, decision limit ($CC\alpha$), detection capability ($CC\beta$), precision, recovery, repeatability, in-house reproducibility, matrix effects and specificity were studied. The obtained results for validation are in accordance with the internationally accepted ranges. The linear regression analysis showed good correlation with R^2 from 0.9859 for cimbuterol to 0.9955 for mabuterol. Three concentration levels of 0.5, 1.0 and 1.5 times the MRPL (Minimum required performance limits) were spiked in urine and meat, and the overall recoveries were between 84 % for cimbuterol and 118 % for ractopamine. The relative standard deviation was from 9.63 to 32.64 %. The obtained values for

 $CC\alpha$ and $CC\beta$ were below MRPL. The analyses of the urine and meat sample showed that the β -agonists are not present in the samples.

Conclusion: The results of this study do not exclude the possibility of abuse of β -agonists in the future. Therefore it is still necessary to monitor these substances as a food quality control measure. The method features were found to be fit-for-purpose. Successful validation of the method according to the European Union requirements and its application to real samples demonstrated its efficiency for veterinary control of β -agonist in meat and urine.

Key words: β-agonists, urine, meat, validation, LC-MS/MS