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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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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## BOVINE

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# VALIDATION OF LC-MS/MS METHOD FOR DETECTION OF PENICILLIN ANTIBIOTICS IN MILK 

Gjylai Alija ${ }^{1 *}$, Zehra Hajrulai-Musliu², Risto Uzunov ${ }^{2}$<br>${ }^{l}$ Department of Pharmacy, Faculty of Medical Sciences, University of Tetovo, Ilinden Str. n.n., 1200 Tetovo, R. of Macedonia<br>${ }^{2}$ Food Institute, Faculty of Veterinary Medicine,<br>Ss. Cyril and Methodius University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, Macedonia

Antibiotics are widely used for therapy and prevent of diseases and as growth promoting agents in farm animals. Penicillins are group of $\beta$-lactam antibiotics and they are used for treat of bacterial infections in veterinary and human medicine. Treatments of farm animals with antibiotics results with presence of residues from these substances in milk and tissues. The consumption of food products containing penicillin drug residues may be harmful for human health because could cause allergic side effect, carcinogenic, estrogenic and neurotoxicologic effects and increase of bacterial resistance to antibiotics. Therefore, the European Union has established maximum residue levels (MRLs) for most antibiotics in milk and animal tissues. The aim of this study is validation of LC-MS/MS method for detection of 3 penicilln antibiotics (ampicillin, amoxicillin and penicillin $G$ ) in cow, sheep and goat milk. The extraction of penicillin antibiotic residues from milk was performed with acetonitrile and chromatographic separation was carried out on C18 column ( $50 \times 2.1 \mathrm{~mm}, 1.7 \mu \mathrm{~m}$ ) with mobile phase A: $0.1 \%$ formic acid in water and mobile phase B: acetonitrile. Optimization of MS/MS method was performed with standards of these 3 penicillins. Validation of the method was performed according the criteria of Commission Decision 2002/657/EC. In validation procedure were evaluated: linearity, decision limit ( $\mathrm{CC} \alpha$ ), detection capability ( $\mathrm{CC} \beta$ ), accuracy, precision and reproducibility of the method. The results for optimization of MS/MS method are with agreement with literature data. The method demonstrated good linearity on the concentration range from 2.0 to $40.0 \mu \mathrm{~g} /$ L with coefficient of correlation (r $\mathrm{r}^{2}$ ) from 0.9869 to 0.9983 . CC $\alpha$ was ranged from 2.07 to $2.75 \mu \mathrm{~g} / \mathrm{L}$, and CC $\beta$ was ranged from 3.32 to $3.86 \mu \mathrm{~g} / \mathrm{L}$. For determination of accuracy, precision and reproducibility blank milk samples were fortified with a mixture of penicillin standards at three levels ( $0.5 \times$ MRL, 1 x MRL and 1.5 x MRL).The MRL for ampicillin, amoxicillin and penicillin G is $4.0 \mu \mathrm{~g} / \mathrm{L}$. The average recoveries for all three concentration level varied from 86 to $104 \%$. The precision and reproducibility were evaluated by RSD, \% (relative standard deviation, \%). The RSD for precision ranged from $3.85 \%$ to $10.20 \%$, while the RSD for reproducibility ranged from $4.78 \%$ to $9.95 \%$. In conclusion, the LC-MS/MS method for detection of ampicillin, amoxicillin and penicillin G was validated. The validation results are in accordance with recommended criteria in 2002/657/EC and this method can be used for routine analysis for detection of these 3 antibiotics in cow, sheep and goat milk.

Key words: penicillin antibiotics, milk, validation, LC-MS/MS method

