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It is our great pleasure to present this Supplement Issue on “*Macedonian Pharmaceutical Bulletin*” to the scientific and professional community. This supplement includes the short communications from the *Sixth Congress of Pharmacy in Macedonia with International participation*, as the largest gathering for the pharmacy profession held in the Republic of Macedonia. The main theme of the Congress was “Modern pharmacist - bridging science with practice”.

A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. The contributions submitted to the Congress included 6 plenary lectures, 84 section lectures, and more than 240 posters. This Congress, followed the excellent international tradition, was attended by close to 1000 domestic and foreign participants. We received 326 short paper submissions from more than 25 countries. These numbers show that our Congress is aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

We would like to thank all internationally prominent researchers for their contribution to reinforcing the overall quality of the Congress. They give the state of the art of the recent advances in the field of pharmacy research.

Sincere thanks to the hosts of the Sixth Congress of Pharmacy in Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

We acknowledge the sponsoring companies: the platinum sponsor AD ALKALOID, Skopje, the golden sponsor PLIVA, the silver sponsor EUROFARM and the bronze sponsor SEPTIMA, for the permanent support to our efforts during the organization.

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process and in the organization of the program. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank the authors, whose work was the essential part of the congress and contributed to a very successful event. Besides the many academic staff and professionals who contributed to the success of the Congress, we are grateful to the students who participated with oral presentations and posters.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

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The present issue of *Macedonian Pharmaceutical Bulletin* is a special issue of the 6<sup>th</sup> Congress of Pharmacy in Macedonia with international participation.

This issue of *Macedonian Pharmaceutical Bulletin* contains short papers accepted by the scientific committee for the presentation at the Congress.

The authors are fully responsible for the contents of their short papers.

All reviewers that were involved in the short papers revision process are sincerely acknowledged.



## Opening lecture

# The role of cocrystallization screening for the assessment of structure-activity relationship in drug development

Aleksandar Cvetkovski\*, Bistra Angelovska

Faculty of Medical Sciences, University "Goce Delcev", Krste Misirkov 10-A, P.O 201, 2000 Stip, Republic of Macedonia

## Introduction

The selection of the crystalline phases in a form of molecular co-crystals has become scientific challenge at the early stage of drug development of pharmaceutical formulations and in the late stage of synthesis and isolation of active pharmaceutical ingredients (APIs) in desirable defined crystalline forms. Optimal crystal form of API interactively interrelates and impacts its aqueous solubility and dissolution rate that are benchmark for drug delivery and absorption determining the extent of its bioavailability and pharmacokinetics profile. Determining the crystal structure and revealing the crystal packing forces and geometry of the API has impact its physicochemical properties. This approach is the criteria for assessment of the performance of the API. The range of crystal forms in which molecular co-crystals of APIs may exist is advantageous comparing to their polymorphs, salts, solvates and hydrates due to the vast number of potential co-formers which extend the limited counterions for salt formation implying the existence of more complex intermolecular interactions based on different H-bonding patterns with API that lead to conformational changes and flexibility for crystal packing in process of co-crystallization.

Co-crystallization became well known bottom-up approach starting from intermolecular interactions among either selected neutral, ionic or zwitterionic molecules to design and control the properties of the multicomponent crystals (Braga, 2004). In the scope of interest for drug design and formulation, Good et al. (2009) and Cheney et al. (2011) emphasized that the main advantage for designing pharmaceutical co-crystals (PCCs) is, through their modulating properties, to improve the performance of the native APIs such are: biopharmaceutical profile (solubili-

ty and dissolution rate), thermodynamical stability (phase transition of polymorphs, solvate/ hydrate formation, decomposition) or bulk powder processability (flowability, compressibility, particle size and shape control). Childs et al. (2007) has pointed out the necessity co-crystals (CCs) semantically to be classify based on accomplishments in research of supramolecular chemistry. This approach enlightens the complex reality of multi-component systems and the wide scope associated between salts and co-crystals, and their differences based on the location of the transferred proton within the salt - co-crystal continuum.

Biguanide drugs are well known and wide used oral antidiabetic drugs for oral therapy of diabetes type-2 that directly improve insulin action. Recent studies in the research work carried out by Vujic et al. (2015) has pointed out that biguanides in combination with targeted inhibitors in order to obtain synergy in reduction cell viability, inhibited tumor growth in the mutated neuroblastoma rat sarcoma oncogene (NRAS) protein from melanoma cells. Hence, it is expected that combination of biguanides which affect activation of the AMP-activated protein kinases (AMPK) and the regulation of energy metabolism with outcome to cell's energy sparing, in combination with other anti-cancer drug-models would influence direct blocking cell's signaling and hinder the resistance.

## Materials and methods

Co-crystallization screening reveals protocol was undertaken in order to grown single crystalline phases of PCCs composed of drug model metformin (MET), selected from biguanides class of drugs and cofomers that belong to different pharmacotherapy and functional group classes, respectively.

Co-crystallization screening was carried out on applying slow-rate solvent evaporation method for growing sin-

\* aleksandar.cvetkovski@ugd.edu.mk

gle crystalline phases at room temperature with quality absolute structure to be determined. An equimolar quantity of MET and co-crystal partner was dissolved in the minimum quantity of ethanol and left for slow evaporation at room temperature. Colorless crystals were observed after a few days.

For four MET PCC models, the methods of preparation reproducible batches were optimized. The quality of batches was controlled by Powder x-ray diffractometer comparing the obtained experimental diffractograms with the same one that was theoretically generated from the single crystal for each of four PCC models.

Single-crystal diffraction data were collected on a Nonius Kappa diffractometer equipped with a CCD detector with graphite-monochromatized MoK $\alpha$  radiation ( $\lambda = 0.71069 \text{ \AA}$ ). Intensities were corrected for Lorentz and polarization effects. The structures were solved by direct methods with the SIR97 suite of programs and refinement were performed on F2 by full-matrix least-squares methods with all non-hydrogen atoms anisotropic.

Flow-cytometry was applied for measuring viability of the two PCC models.

## Results and discussion

Vujic et al. (2015) has carried out research for both pro-cancer and anti-cancer effects of biguanides on cancer cells, indicating existence of association of the antidiabetic therapy and reduced risk of cancer in diabetic patients. Because biguanide represents the  $\pi$ -conjugated system, MET can exist in three resonance-stabilized forms, i.e. as neutral molecule (MET), monoprotonated (MET<sup>+</sup>) or diprotonated (MET<sup>2+</sup>) cation, with dissociation constants in water in range from pKa1  $\approx$  12.00 to pKa2  $\approx$  2.00.

A search of the biguanide fragments in the structural literature, both in the CCDC (Cambridge Crystallography Database Center) database and in patents, shows that in crystals MET exists as monoprotonated (MET<sup>+</sup>) or deprotonated (MET<sup>2+</sup>) but never in its neutral form MET.

We have undertaken a systematic study of the crystal chemistry of MET with the aim of understanding its properties in the solid state and finding relationships with its biopharmaceutical profile. We have determined the structures of the 29 MET PCCs. Four of this MET PCC models are “drug-drug” type of co-crystals. The ligand used for co-crystallization was from the following classes: inorganic acids (nitric, phosphoric and carbonic acid); organic NH-type acids (saccharine and acesulfame); organic OH-

type acids (squaric and picric acid); monocarboxylic acids (fumaric, acetic, trifluoroacetic, trichloroacetic, dichloroacetic, monochloroacetic, glycolic, salicylic, diclofenac) and dicarboxylic acids (oxalic, malonic, maleic, fumaric, succinic, adipic acid).

## Conclusion

In the paper are presented structure analyses for “drug-drug” type of PCCs where both API and CF exhibit pharmacological effect. This approach of designing “drug-drug” type of PCC aligned to the strategy for drug repositioning, the idea for use of a drug for treating diseases other than the drug-specified. This concept was prompted in 2012 through the Discovering New Uses for Existing Molecules program, initiated by US’s National Institute of Health (NIH).

The case study underlines the crystal growth and the method of preparation for “drug-drug” type of PCCs wherein two different APIs cocrystallized in single crystal cell, and that represent new paradigm for approaching in development of “fixed-doses” or “combo” pharmaceutical formulations. Preliminary results of the Structure-Activity Relationship study on the co-crystals composed of MET with dichloroacetic acid indicate dual and complementary anti-cancer activities of the two selected drug models for co-crystallization.

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# CONTENTS

## Opening lecture

<b>The modern pharmacist: Is the future in the past?</b> _____	<b>7</b>
Roberto Frontini	

## Closing lecture

<b>Commitment to quality means commitment to change</b> _____	<b>11</b>
Michael J. Rouse	

## Plenary lectures

<b>New psychoactive substances - analytical challenges and threats to the public health: European and Polish experience in the new drugs combating</b> _____	<b>15</b>
Zbigniew Fijalek	
<b>Green “cage” nanoparticles as efficient carriers for challenging drugs</b> _____	<b>17</b>
Ruxandra Gref	
<b>Data-driven innovation in health policy</b> _____	<b>19</b>
Ran Balicer	
<b>Clinical pharmacy - established paths and new opportunities</b> _____	<b>21</b>
Dorothea Rudolf	
<b>Brown fat induction in treatment of metabolic disorders</b> _____	<b>25</b>
Mirko Trajkovski	
<b>Bridging the computer and life sciences: the case of VI-SEEM</b> _____	<b>27</b>
Anastas Mishev	

## Pharmacoeconomy / Social pharmacy / Drug information

<b>Data-driven approaches to tackling medication adherence</b> _____	<b>33</b>
Ran Balicer	
<b>Analyzing pharmaceutical policies: Hungary as a case study</b> _____	<b>35</b>
Rok Hren	
<b>Impact of parallel trade/import of pharmaceuticals in Central East European Countries</b> _____	<b>37</b>
Zoran Sterjev, Rubin Zareski, Katerina Anchevska Netkovska, Zorica Naumovska, Aleksandra Kapedanovska Nestorovska, Aleksandra Grozdanova, Ljubica Shuturkova	
<b>The effects of the new methodology application on the method of pricing of drugs</b> _____	<b>39</b>
Rubin Zareski, Ljubica Suturkova, Zoran Sterjev	
<b>Market access of biosimilar medical products – economical, regulatory and clinical issues</b> _____	<b>41</b>
Aleksandra Grozdanova, Katerina Anchevska Netkovska, Zorica Naumovska, Aleksandra Kapedanovska Nestorovska, Zoran Sterjev, Ljubica Shuturkova	

---



<b>The importance of pharmacists in primary healthcare</b>	<b>43</b>
Lovorka Nikolic, Una Ivosevic, Natasa Lalic Cunkovic, Mirjana Savicevic	
<b>Internet and computer use amongst European pharmacy students</b>	<b>45</b>
SelenYeğeneoğlu, Bilge Sozen Sahne, Daisy Volmer, Afonso Cavaco, Maarten Postma	
<b>Activities of Macedonian Agency of medicines and medical devices in the improvement of rational use of medicines</b>	<b>47</b>
Merjem Hadjihamza Marija Darkovska Serafimovska, Mirjana Donceva	
<b>Sources of medicines information used by Lithuanian community pharmacy patients</b>	<b>49</b>
Paulius Brazauskas, Jurgita Daukšienė, Romualda Gauryliene, Raimondas Radžiūnas	
<b>Characterization of typical and atypical antipsychotics use in Albania, 2006-2012</b>	<b>51</b>
Elda Hoxha, Vilma Papajani	
<b>Developing and implementation of good pharmaceutical practices in a small private pharmacy</b>	<b>53</b>
Sead Salkoski	
<b>The research exemption in Macedonian industrial property law and its effects on the extent of patent protection for drugs</b>	<b>55</b>
Jadranka Dabovic-Anastasovska, Nenad Gavrilovic, Katerina Ancevska-Netkovska	
<b>Parallel pharmaceutical trade in Macedonia – pros and cons</b>	<b>57</b>
Goran Koevski, Jadranka Dabovik Anastasovska	
<b>Evaluation of reliability and validity of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) questionnaire (Albanian version) among breast cancer patients from Kosovo</b>	<b>59</b>
Selveta Shuleta-Qehaja, Aleksandra Grozdanova, Aleksandra Kapedanovska Nestorovska, Zorica Serafimoska, Ljubica Shuturkova, Zoran Sterjev	
<b>Additional services as a basis for the concept of pharmaceutical care</b>	<b>61</b>
Martin Gigovski, Yiannis E. Polychronakis	
<b>Protection of public interest in the area of health through compulsory licenses of patents for pharmaceuticals under the Macedonian legislation</b>	<b>63</b>
Neda Zdraveva, Valentin Pepljugoski	
<b>Attitudes of pharmacists about professional practice and work with patients in regard to reaching high level of adherence</b>	<b>65</b>
Milica Zeković, Dusanka Krajnović, Valentina Marinković, Tatjana Stojković, Ljiljana Tasić	
<b>The role of pharmacists and other health professionals in promotion of reproductive health of young people</b>	<b>67</b>
Bijana Vasić, Dragana Đuričić	
<b>The use of drugs outside of approved application</b>	<b>69</b>
Svetlana Golocorbin-Kon, Mladena Lalic-Popovic, Nebojsa Pavlovic, Maja Đanić, Natasa Milosevic, Branislava Rakic, Momir Mikov	
<b>The impact of clinical effectiveness of gemcitabine on quality of life in patients with pancreatic cancer in all stages</b>	<b>71</b>
Zana Ibraimi, Ilir Kurtishi, Ardiana Murtezani, Agim Shehi, Edita Alili	
<b>Ad-hoc comparative analysis of regulatory safety information and web-based data for recombinant medicines for assisted reproduction techniques</b>	<b>73</b>
Svetoslav Stoev, Hristina Lebanova, Emilia Naseva, Vladimir Atanasov, Ilko Getov	
<b>Results from PPS of antimicrobial prescribing in University Clinical Center of Kosovo</b>	<b>75</b>
Denis Raka, Kreshnik Hoti, Naim Morina, JetëmiraBytyçi, Albiona Rashiti, Zana Deva, Besa Bahtiri, Lul Raka	
<b>Antibiotic prescribing in regional hospital Prizren</b>	<b>77</b>
Denis Raka, Kreshnik Hoti, Naim Morina, Jetëmira Bytyçi, Albiona Rashiti, Zana Deva, Besa Bahtiri, Lul Raka	

<b>Analysis of consumption of insulin in the municipality of Stip from 2011 to 2014</b>	<b>79</b>
Dijana Atanasova, Aleksandra Petrova, Elena Drakalska, Marija Atanasova, Bistra Angelovska	
<b>Medication errors in the health care delivery-a review of the literature</b>	<b>81</b>
Tatjana Stojkovic, Valentina Marinkovic, Dusanka Krajnovic, Milica Zekovic, Ljiljana Tasic	
<b>Analysis of coordination compound of germanium with nicotinic acid as potential cardioprotector</b>	<b>83</b>
Violetta P. Narokha, Iryna V. Nizhenkovska, Olena V. Kuznetsova, Olga V. Afanasenko	
<b>Importance of clinical pharmacist in system of health care in Bosnia and Herzegovina</b>	<b>85</b>
Vedina Čordalija, Fahir Bečić, Tea Mušić-Dreković, Esmā Karahmet	
<b>Perception about health promotion and smoking cessation counselling among community pharmacists in Lithuania</b>	<b>87</b>
Jurgita Dauksiene, Greta Pavasaryte, Aurelija Batakyte, Gediminas Daukšys, Daisy Volmer	
<b>ACE inhibitors, calcium antagonists, <math>\beta</math>-blockers products authorized in Albania and their availability for pediatric groups</b>	<b>89</b>
Briseida Dosti, Ledjan Malaj	
<b>The characteristics of non-chain community pharmacies in Lithuania and their owners' attitude towards professional autonomy</b>	<b>91</b>
Jurgita Dauksiene, Edita Tiurninaite, Edita Kizeviciene, Aiste Balzekiene	
<b>The impact of socio-demographic and lifestyle factors in patients diagnosed with heart failure</b>	<b>93</b>
Pamela Gruda, Mihal Tase, Mirjeta Beqiri, Suela Këllici	
<b>The approach to the pharmaceutical waste management in the world and in Serbia</b>	<b>95</b>
Svetlana Goločorbin-Kon, Mladena Lalić-Popović, Nebojša Pavlović, Maja Đanić, Jelena Cvejić, Velibor Ilić, Momir Mikov	
<b>Type 2 diabetes risk assessment in patients of a Portuguese community pharmacy</b>	<b>97</b>
Esperança Maria Simoes da Silva, Maria Margarida Duarte Caramona	
<b>A study of the public knowledge of use of antibiotics in Kosovo</b>	<b>99</b>
Arijana Dëshishku, Merita Berisha, Linda Duraku, Arjeta Dëshishku, Bujar Fetahu	
<b>Pharmaceutical care for people with depression: experiences and challenges</b>	<b>101</b>
Zahida Binakaj, Svetlana Stojkov, Bistra Angelovska	
<b>Adjuvant chemotherapy, with or without taxanes, among women with breast cancer in Albania</b>	<b>103</b>
Erina Hilaj, Vilma Papajani, Alketa Ymeri	
<b>Bevacizumab in addition to FOLFOX chemotherapy for metastatic colorectal cancer: A Macedonian-based cost-effectiveness/utility analysis</b>	<b>105</b>
Aleksandra Kapedanovska-Nestorovska, Zorica Naumovska, Aleksandra Grozdanova, Aleksandar Dimovski, Ljubica Suturkova, Zoran Sterjev	
<b>The advertising influence on pharmacist recommendations and consumer selection of over-the-counter drugs</b>	<b>107</b>
Aleksandra Kapedanovska-Nestorovska, Zorica Naumovska, Zoran Sterjev, Ljubica Suturkova, Aleksandra Grozdanova	
<b>The relationship of law and pharmacy</b>	<b>109</b>
Katerina Anchevska Netkovska, Aleksandra Grozdanova	
<b>Pharmaceutical waste management: a necessity to position a pharmacist as a pillar of public awareness campaign</b>	<b>111</b>
Nataša Jovanović Lješević, Manda Dizdar, Branislava Rakić, Milan Ilić, Nikola Jojić, Marina Gavrančić, Slobodan Gigov	
<b>Awareness of the importance of self-management in Macedonian diabetes patients</b>	<b>113</b>
Biljana Indova, Zorica Naumovska, Aleksandra Kapedanovska Nestorovska, Aleksandra Grozdanova, Ljubica Suturkova, Zoran Sterjev	

<b>Evaluation of rational / irrational drug use at orthopedic department in Clinical Hospital Stip in the period from January to April 2013</b>	<b>115</b>
Biljana Lazarova, Aleksandra Kapedanovska Nestorovska, Zorica Naumovska, Aleksandra Grozdanova, Ljubica Suturkova, Zoran Sterjev	
<b>Intellectual property rights and patent litigation on biosimilar medicinal products</b>	<b>117</b>
Aleksandra Grozdanova, Jadranka Dabovic Anastasovska, Katerina Ancevska Netkovska	
<b>Use NSAID drugs with prescription of the doctor or without prescription in the one pharmacy in Bosnia and Hercegovina</b>	<b>119</b>
Tea Mušić-Dreković, Vedina Čordalija, Maja Malenica	
 <b>Pharmaceutical analysis / Quality assurance / Regulatory affairs</b>	
<b>QRM in the GMP environment - 10 years on since ICH Q9... Are medicines any safer now?</b>	<b>123</b>
Kevin O'Donnell	
<b>The challenges of the qualified person in a complex pharmaceutical quality system</b>	<b>125</b>
Miroslava Ilievska, Nina Ekart Oman, Jasmina Velevska Ivanovska, Verce Jovanovska Jankovska, Katerina Beldedovska Aleksievska, Maja Velinovska Chadinoska, Ksenija Brzilova, Ornela Kuzmanovska	
<b>Quality control of drug products: implementation of the new ICH Q3D guideline on elemental impurities</b>	<b>127</b>
Gregory Lecornet	
<b>Chemometrics - powerful tool in tracking the origin of cannabis samples?</b>	<b>129</b>
Slavica Ražić, Nataša Radosavljević-Stevanović	
<b>Pattern recognition techniques in preventing of API falsification</b>	<b>131</b>
Jelena Acevska, Katerina Brezovska, Natalija Nakov, Rumenka Petkovska, Aneta Dimitrovska	
<b>Orphan drugs - comparative review of FDA and EMA regulations</b>	<b>133</b>
Zoran Nakov, Jasmina Tonic-Ribarska, Suzana Trajkovic Jolevska	
<b>Development of cleaning validation master plan, including cleaning validation protocol</b>	<b>135</b>
Nade Dimovska, Nena Smiljanovska, Marina Petreska, Ketj Shapkovska, Stojne Tanevska	
<b>Method suitability test for determination of microbiological purity of Gastoguard chewable tablets</b>	<b>137</b>
Adrijana Nosacheva Trajkovska, Maja Simjanovska Daskalova, Nadezhda Stojkova, Dragi Todorovikj, Hristina Babunovska	
<b>High-performance liquid chromatography method for determination of caffeine from different matrices</b>	<b>139</b>
Nevena Grujić-Letić, Branislava Rakić, Emilia Šefer, Maja Milanović, Nataša Milić	
<b>Determination of <math>\alpha</math>-tocopheryl acetate in sunscreen lotion and cream by using the solid phase extraction and HPLC method</b>	<b>141</b>
Milica Kostić and Slavica Sunarić	
<b>Validation of analytical method for determination of microbiological purity of active pharmaceutical ingredient in Caffetin cold tablets</b>	<b>143</b>
Silvana Ilioska-Zlatanovikj, Dragi Todorovikj, Elizabeta Popovska, Hristina Babunovska	
<b>Contemporary approach in LC-MS/MS bioanalytical method development</b>	<b>145</b>
Natalija Nakov, Zoran Kavrovski, Rumenka Petkovska, Aneta Dimitrovska	
<b>Implementation of design of experiments for optimization of forced degradation of simvastatin</b>	<b>147</b>
Maja Hadzieva Gigovska, Marija Grozdanoska, Ana Petkovska, Jelena Acevska, Biljana Sapkarova, Irena Brašnarska, Sonja Ugarkovic, Aneta Dimitrovska	
<b>Residual solvent profiling in active pharmaceutical ingredients; approaches in sample preparation and method optimization</b>	<b>149</b>
Ana Poceva Panovska, Jelena Acevska, Katerina Brezovska, Rumenka Petkovska, Aneta Dimitrovska	

<b>Detecting the weakness in the hygiene - a mean for prevention of the health care-associated infections and improvement of the patients' health care in the Clinical hospital Bitola</b>	<b>151</b>
Tatjana Dimitrovska Manojlovikj, Dona Trombeva, Lenche Najdovska, Magdalena Vrchkovska, Angjela Delova, Marta Ivanovska, Ljupcho Anastasovski	
<b>Bacterial endotoxin test: a microbiological challenge</b>	<b>153</b>
Eva Troja	
<b>Quantity of disinfectants and antiseptics used in general hospital in Gevgelija in relation to appearance of intra-hospital infections</b>	<b>155</b>
Biljana Gjorgjeska, Sofija Petkovska	
<b>HPLC determination of caffeine in anti-cellulite gels after the solid phase extraction</b>	<b>157</b>
Kristina Mladenov and Slavica Sunarić	
<b>Validation and quantification of bacterial endotoxins with turbidimetric kinetic method for benzyl alcohol</b>	<b>159</b>
Elizabeta Popovska, Silvana Ilioska-Zlatanovic, Hristina Babunovska, Biserka Simonovska	
<b>A rapid and validated reverse phase liquid chromatographic method for <i>in vitro</i> dissolution test for determination of bromazepam in tablet formulations</b>	<b>161</b>
Irena Brchina, Biljana Gjorgjeska	
<b>New generation antiepileptic drugs: affordable bioanalytical method for therapeutic monitoring</b>	<b>163</b>
Arlinda Haxhiu Zajmi, Jasmina Tonic Ribarska, Emilija Cvetkovska, Rumenka Petkovska, Suzana Trajkovic Jolevska	
<b>Trend analysis in stability data for Caffetin Cold film coated tablets</b>	<b>165</b>
Sanja Despotovska, Milena Dobrkovic Shotarovska, Mena Ivanoska, Ana Aleksandric, Marina Mandzukovska, Dragana Kafedziska, Vasilka Dubrova Koceva, Hristina Babunovska	
<b>Comparative analysis of advertising and promotion of traditional herbal medicine and food supplement at different markets - case study</b>	<b>167</b>
Marjan Dzeperoski, Suzana Trajkovic-Jolevska	
<b>Validation of NIR methods for identification of ibuprofen lysine</b>	<b>169</b>
Biljana Bujaroska, Marija Spasevska, Biserka J. Trajkovska, Maja Ilijoska, Andrea Alagjozovska, Hristina Tomovska, Nada Stojanoska, Hristina Babunovska	
<b>Verification of method for determining methanol in sodium citrate with gas chromatography</b>	<b>171</b>
Marija Spasevska, Biljana Bujaroska, Gordana Mitrovska, Miona Manasova, Andrea Alagjozovska, Dafinka Damcevska, Biserka J. Trajkovska, Hristina Babunovska	
<b>Validation and quantification of bacterial endotoxins with turbidimetric kinetic method for (<i>S</i>)-Lactic acid</b>	<b>173</b>
Silvana Ilioska-Zlatanovikj, Elizabeta Popovska, Hristina Babunovska	
<b>Marketing authorization of veterinary medicinal products in Macedonia</b>	<b>175</b>
Todor Šapov, Suzana Trajković-Jolevska, Romel Velez, Jasmina Tonic-Ribarska, Nataša Krleska-Veleva, Biljana Šapova	
<b>Transfer of analytical procedures for quality control of Cilostazol 100 mg tablets</b>	<b>177</b>
Cveta Dolikjoska Trajkova, Nikola Pavleski, Ana Giceva - Pepovska, Blagica Samarova Stoev, Silvija Saveska, Maja Stojkovska, Hristina Babunovska	
<b>Counterfeit medicines - threat to worldwide public health</b>	<b>179</b>
Biljana Petrovska Jakimovska, Biljana Nanova, Milkica Gligorova	
<b>Comparative evaluation of the efficacy of local administration of doxycycline and chlorhexidine in patients with periodontal disease using multivariate chemometric data analysis</b>	<b>181</b>
Liljana Bogdanovska, Ana Poceva Panovska, Natalija Nakov, Marija Zafirova, Mirjana Popovska, Aneta Dimitrovska, Rumenka Petkovska	

<b>Development and validation of RP-HPLC-FLD method for determination of doxycycline in gingival crevicular fluid and saliva</b>	<b>183</b>
Liljana Bogdanovska, Spiro Spasovski, Mirjana Popovska, Silvana Gjoseva, Katerina Goracinova, Natalija Nakov, Marija Zafirova, Aneta Dimitrovska, Rumenka Petkovska	
<b>Phospholipids monitoring as a tool for elimination of matrix effect during LLE optimization</b>	<b>185</b>
Natalija Nakov, Jelena Acevska, Rumenka Petkovska, Zoran Kavrakovski, Aneta Dimitrovska	
<b>Evaluation of stability data on pharmaceutical dosage form in order of extending the shelf life with application of statistical methods</b>	<b>187</b>
Vasilka Dubrova - Koceva, Sonja Chortosheva, Hristina Babunovska, Sanja Despotovska, Dafinka Damcevska, Dragana Kafedziska, Marija Stojanovska	
<b>Determination of clarithromycin residues on manufacturing equipment surfaces in cleaning validation process</b>	<b>189</b>
Katerina Kochova, Elena Petrovska, Gordana Trendovska Serafimovska	
<b>Validation of RP-HPLC stability-indicating method for cilazapril and hydrochlorothiazide</b>	<b>191</b>
Jasmina Šljivić, Mira Zečević, Biljana Otašević, Ana Protić, Jelena Golubović	
<b>Development of fast, simple RP- HPLC method for determination of moxifloxacin in solid pharmaceutical dosage forms</b>	<b>193</b>
Marjan Piponski, Tanja Bakovska, Marina Naumoska, Emilija Janeva, Tatjana Rusevska Marija Globochki, Magdalena Piponska, Gordana Trendovska Serafimovska	
<b>Positive chaotropic role in development of RP- HPLC method for quantification of norfloxacin in pharmaceutical dosage forms</b>	<b>195</b>
Marjan Piponski, Tanja Bakovska, Marina Naumoska, Marija Globochki, Irena Slaveska Spirevska, Stefan Stefov, Magdalena Piponska, Elena Petrovska, Gordana Trendovska Serafimovska	
<b>Forced degradation study of moxifloxacin in tablet formulation using RP-HPLC</b>	<b>197</b>
Alma Salkić, Mira Zečević, Amra Butković, Jelena Golubović, Jasmina Šljivić	
<b>Simple RP-HPLC method for estimation of diazepam and benzyl alcohol in microclisme</b>	<b>199</b>
Maja Vragolic, Branka Ivkovic, Olivera Cudina, Sote Vladimirov, Jasmina Brboric	
<b>GC-MS method for chemical characterization of pharmaceutical packaging materials</b>	<b>201</b>
Vlado Petruševski, Suzana Trajković-Jolevska, Jasmina Tonić-Ribarska, Sonja Ugarković	
<b>Development of fast simple RP-HPLC method with UV detection for determination of Pregabalin in solid pharmaceutical dosage forms</b>	<b>203</b>
Marjan Piponski, Tanja Bakovska, Marina Namoska, Tatjana Rusevska, Irena Slaveska Spirevska, Elena Lazarevska Todevska, Stefan Stefov, Gordana Trendovska Serafimovska	
<b>Comparison of new developed UV/VIS-spectrophotometric and HPLC method with UV/VIS detection for determination of Vitamin B12 in various pharmaceutical dosage forms</b>	<b>205</b>
Tanja Bakovska, Marina Naumoska, Marjan Piponski, Emilija Janeva, Elena Petrovska, Elena Lazarevska Todevska, Hristina Andonoska, Tatjana Rusevska, Gordana Trendovska Serafimovska	
<b>Analytical approach in development of a new drug product formulation</b>	<b>207</b>
Aleksandra Petrovska, Marija Velii Veli, Veronika P. Jakimovska, Sonja Ugarkovic	
<b>Strengthening the position of OMCLs</b>	<b>209</b>
Jelena Acevska, Katerina Brezovska, Liljana Ugrinova, Suzana Trajkovic Jolevska, Aneta Dimitrovska, Richard Wanko, Kevin O'Donnell	
<b>Mathematical modeling of drug dissolution from prolonged-release drug product</b>	<b>211</b>
Blagica Manchevska, Packa Antovska, Irena Brashnarska, Sonja Ugarkovic	
<b>Validation of RP-HPLC method for determination of exemestane and its impurities in pharmaceutical dosage forms</b>	<b>213</b>
Branka Ivković, Aleksandra Jonić, Jelena Žunić, Sote Vladimirov, Milkica Crevar Sakač, Zorica Vujić	

<b>Dissolution method development for generic drug products</b>	<b>215</b>
Marija Petrovska, Ivana Mitrevska, Tina Achkoska, Irena Brašnarska, Packa Antovska, Dejan Kuneski, Sonja Ugarkovic	
<b>Validation of GC method for determination of ethanol, methanol, toluene and benzene as residual solvents in pholcodine monohydrate drug substance</b>	<b>217</b>
Olivera Blažeska, Vlado Petruševski, Ana Petkovska, Monika Stojanovska, Gjorgji Petruševski, Irena Brašnarska, Biljana Šapkareva, Sonja Ugarković	
<b>Evaluation of drug-excipient interaction in formulation of ibuprofen topical gel by High Performance Liquid Chromatography</b>	<b>219</b>
Elena Kazandzievska, Slavica Mitrevska, Irena Brasnarska, Liljana Krsteska, Dejan Kostovski, Marina Kajdzanoska, Sonja Ugarkovic	
<b>A quality by design approach for liquid chromatography method development for determination of assay of drug product</b>	<b>221</b>
Tina Achkoska, Ivana Mitrevska, Marija Petrovska, Irena Brasnarska, Sonja Ugarkovic	
<b>Comparison of method A and method B described in Ph.Eur. for determination of bacterial endotoxins in pharmaceutical preparation containing somatropine</b>	<b>223</b>
Branislava Janeva, Sandra Zinoski, Katerina Starkoska	
<b>Liability for damage caused by using medical devices</b>	<b>225</b>
Vlatko Kokolanski, Katerina Anchevska-Netkovska, Zoran Sterjev, Suzana Trajkovikj Jolevska	
<b>A quality by design based analytical method development for determination of impurities in new pharmaceutical drug product</b>	<b>227</b>
Ana Georgieva, Irena Brašnarska, Sonja Ugarković	
<b>Optimization of an UPLC method for determination of moxifloxacin hydrochloride and its related substances</b>	<b>229</b>
Marija Zafirova, Gabriela Petrovska, Liljana Ugrinova, Liljana Bogdanovska, Vasil Karcev, Katerina Brezovska, Aneta Dimitrovska, Suzana Trajkovik Jolevska	
<b>Photo stability study design of drug product containing fluoroquinolon as active compound</b>	<b>231</b>
Veronika Popovska Jakimovska, Marija Velichkovska, Aleksandra Petrovska, Irena Brašnarska, Biljana Šapkareva, Suzan Memed-Sejfulah, Sonja Ugarkovich	
<b>Investigation of chromatographic behavior of aripiprazole and its five impurities</b>	<b>233</b>
Nevena Maljurić, Ana Protić, Biljana Otašević, Jelena Golubović, Jovana Krmar, Mira Zečević	
<b>Alcohol induced dose dumping for prolonged-release drug product</b>	<b>235</b>
Elena Davitkovska, Blagica Manchevska, Dusica Angelovska, Irena Brasnarska, Packa Antovska, Biljana Šapkareva, Sonja Ugarkovic	
<b>AlkaSAP computer system validation</b>	<b>237</b>
Sonja Sterjevska, Nada Popstefanova, Darko Atanasoski, Miroslava Ilievska	
<b>Determination of cannabidiol and <math>\Delta^9</math>tetrahydrocannabinol in <i>Cannabis sativa</i> L. preparations present in the European market by HPLC/DAD</b>	<b>239</b>
Maja Shishovska, Dragica Doneva, Zorica Arsova-Sarafinovska, Katerina Starkoska	
<b>Generation and combined study on the chemical structure of nitrofurantoin radical anion</b>	<b>241</b>
Angelina Popova, Simeon Stoyanov, Denitsa Yancheva	
<b>Bioanalytical HPLC method for therapeutic drug monitoring of azathioprine metabolites during inflammatory bowel disease</b>	<b>243</b>
Bojana Danilova, Dragana Mladenovska, Matea Miceska, Jasmina Tonic Ribarska	



## Clinical biochemistry / Toxicology / Food and nutrition

<b>New <i>in vitro</i> technique for evaluation of anti-inflammatory activities of natural products and plants extracts</b>	<b>247</b>
Neda Mimica-Dukić, Ivana Beara and Dejan Orčić	
<b>Metals specificities in environmental risk assessment</b>	<b>249</b>
Dragana Vujanović	
<b>Dimethoate-induced renal toxicity in rats and the protective/ameliorative effects of <i>Laurocerasus officinalis</i> Roem. (cherry laurel) fruit extract</b>	<b>251</b>
Ayşe Eken, Burcu Ünlü-Endirlik, Elçin Özger, Ayşe Baldemir, Arzu Hanım Yay	
<b>Probiotic/synbiotic enriched ayran as functional food product – quality and therapeutic benefits</b>	<b>253</b>
Tanja Petreska Ivanovska, Zoran Zhivikj, Liljana Bogdanovska, Maja Jurhar Pavlova, Ivica Gjurovski, Trpe Ristoski, Kristina Mladenovska, Lidija Petrushevska-Tozi	
<b>Approved health claims for amino acids in/as food supplements</b>	<b>255</b>
Ermira Krasniqi, Lidija Petrusevska Tozi	
<b>Preclinical studies for evaluation of antitumor effects and normal tissue toxicity of antibody conjugates</b>	<b>257</b>
Darinka Gjorgieva Ackova, Katarina Smilkov, Emilija Janevik-Ivanovska	
<b>The pro-inflammatory effects of the organic phase obtained during Cosorb process observed in different animal strains</b>	<b>259</b>
Cristina Adriana Dehelean, Codruta Soica, Georgeta Simu, Iulia Pinzaru, Dorina Coricovac	
<b>Antioxidant versus toxic capacity of selected herbal products</b>	<b>261</b>
Blagica Jovanova, Marija Hiljadnikova-Bajro, Tatjana Kadifkova Panovska	
<b>Trend of obesity, sport and nutrition</b>	<b>263</b>
Simona Bernátová, Zuzana Hegedusová, Katarína Dostálová, Soňa Wimmerová, Zora Gerová, Eva Horváthová, Štefánia Móricaová	
<b>Turkey's highlights within inprofood (FP-7) project</b>	<b>265</b>
SelenYeegenoglu, Bilge Sozen Sahne	
<b>Assessment of cytogenetic damage and oxidative stress status in hospital staff occupationally exposed to ionizing radiation</b>	<b>267</b>
Ayşe Eken, Ahmet Aydın, Onur Erdem, Cemal Akay, Ahmet Sayal, İbrahim Somuncu	
<b>Dietary supplement use among adolescents</b>	<b>269</b>
Gordana Svonja Parezanovic	
<b>Acute and chronic renal failure related with anemia and thrombocytopenia</b>	<b>271</b>
Milena Spasovska and Tatjana Kadifkova Panovska	
<b><i>In vivo</i> study of the effects of different phases of the Cosorb process on skin's intrinsic properties</b>	<b>273</b>
Simu Georgeta-Maria, Coricovac Dorina, Cseh Liliana, Soica Codruta, Borcan Florin, Ionescu Daniela, Andoni Mihaiela, Dragos Dan, Dehelean Cristina	
<b>Thiamine and riboflavin content in infant formulas available in Serbia: Level of compliance with recommended dietary intake and adequacy of nutritional needs of infants</b>	<b>275</b>
Marko Denić, Slavica Sunarić, Jelena Lalić, Gordana Kocić	
<b>Determination of pesticide residuals by GC-ECD</b>	<b>277</b>
Ela Hoti, Lindita Qefalia, Linda Matua	
<b>The toxicity of organic solvents mixtures, containing toluene and its oxidation products</b>	<b>279</b>
Soica Codruta, Simu Georgeta-Maria, Coricovac Dorina, Mioč Marius, Borcan Florin, Ionescu Daniela, Dragos Dan, Andoni Mihaiela, Dehelean Cristina	
<b>Assessment of vitamin E content in bovine colostrum supplement by using solid phase extraction and HPLC method</b>	<b>281</b>
Slavica Sunarić, Jelena Lalić, Marko Denić, Gordana Kocić	

<b>Effects of different doses zinc gluconate on copper, iron and calcium levels in experimentally induced diabetic rabbits and type 2 diabetic patients</b>	<b>283</b>
Zorica Stanojević Ristić, Snežana Stević, Julijana Rasić, Dragana Valjarević, Momčilo Stanić	
<b>Viability and metabolic activity of <i>Lactobacillus casei</i> 01 in dairy and non-dairy products</b>	<b>285</b>
Tanja Petreska Ivanovska, Kristina Mladenovska, Lidija Petrushevska-Tozi	
<b>Effect of glucose concentration on glucose oxidase activity in a minimal model must</b>	<b>287</b>
Verica Petkova, Irina Mladenovska, Tatjana Kadifkova Panovska	
<b>Determination of lead and cadmium in foods by Graphite Furnace Atomic Absorption Spectroscopy</b>	<b>289</b>
Suzana Angelova, Biljana Mladenovski and Tatjana Kadifkova Panovska	
<b>Determination of aflatoxins in some foodstuffs by HPLC</b>	<b>291</b>
Suzana Angelova, Valide Sabani and Tatjana Kadifkova Panovska	
<b>Screening of some plant species for their antioxidant and antibacterial activity</b>	<b>293</b>
Eljona Chilku, Blagica Jovanova, Snežana Ivic Kolevska and Tatjana Kadifkova Panovska	
<b>Approach to detect possible genotoxic effects of metals in plants</b>	<b>295</b>
Darinka Gjorgieva Ackova, Tatjana Kadifkova Panovska, Katerina Bačeva Andonovska and Trajče Stafilev	
<b>Biochemical pathways in cancer progression as pharmacological targets</b>	<b>297</b>
Milena Prculovska, Ivana Angelovska, Tatjana Kadifkova Panovska, Marija Hiljadnikova-Bajro	
<b>Biomolecular mechanisms of cancer initiation as targets for therapeutic intervention</b>	<b>299</b>
Ivana Angelovska, Milena Prculovska, Tatjana Kadifkova Panovska, Marija Hiljadnikova-Bajro	
<b>Biochemical identification of <i>Helicobacter pylori</i> using the urea breath test</b>	<b>301</b>
Irena Smokvarska, Tatjana Kadifkova Panovska, Marija Hiljadnikova-Bajro	
<b>Determination of Ochratoxin A in some dried fruits by liquid chromatography</b>	<b>303</b>
Suzana Angelova, Valide Sabani and Tatjana Kadifkova Panovska	
<b>Determination of the toxic bioactivity of methanol extracts of selected commercial herbal teas</b>	<b>305</b>
Blagica Jovanova and Tatjana Kadifkova Panovska	
<b>The cancer metabolism and associated therapeutic interventions</b>	<b>307</b>
Iva Antova, Tatjana Kadifkova Panovska, Marija Hiljadnikova-Bajro	
<b>Evaluation of the toxic potential of <i>Pinus</i> species natively growing on the territory of Republic of Macedonia</b>	<b>309</b>
Blagica Jovanova, Marija Karapandzova, Tatjana Kadifkova Panovska, Svetlana Kulevanova	
<b>Exposure to organophosphates: cholinergic and non-cholinergic targets</b>	<b>311</b>
Biljana Antonijevic, Evica Antonijevic, Danijela Djukic-Cosic, Marijana Curcic, Nina Umicevic	
<b>The role of cardiac markers in the diagnosis of acute myocardial infarction and angina pectoris</b>	<b>313</b>
Emilija Kostoska, Aleksandra Crvenpanova, Tanja Angjuseva, Zan Mitrev, Tatjana Kadifkova Panovska	
<b>Challenges in interpretation of forensic toxicological findings for opiates: case report and a literature review</b>	<b>315</b>
Marija Bujaroska, Nataša Bitoljanu, Ljupčo Čakar, Renata Jankova-Ajanovska, Zlatko Jakjovski, Verica Poposka, Aleksej Duma	
<b>Drug-related deaths linked with concomitant use of methadone and benzodiazepines in the period between 2011 and 2015 in the Republic of Macedonia</b>	<b>317</b>
Marija Bujaroska, Nadica Sibinovska, Klimentina Trajkova, Verica Poposka, Goran Pavlovski, Viktorija Belokaposka Srpanova, Biljana Janeska	
<b>Unusual case of suicide with pentobarbital</b>	<b>319</b>
Natasa Bitoljanu, Verica Poposka, Elena TrajcovaKovacovska, Aleksandar Stankov, Iskra Trencavska Ivanovska, Zdravko Čakar	



<b>Evaluation of antioxidant activity of berries of <i>Juniperus excelsa</i>, <i>Juniperus communis</i> and <i>Juniperus oxycedrus</i> from Macedonian flora</b>	<b>321</b>
Leonard Kurti, Blagica Jovanova, Ariana Kelmendi, Tatjana Kadifkova Panovska and Svetlana Kulevanova	
<b>Biological variation of serum cholesterol and triglycerides</b>	<b>323</b>
Biserka Simonovska, Nikola Simonovski, Elizabeta Popovska	
<b>Biological variation of serum creatinine and urea</b>	<b>325</b>
Biserka Simonovska, Nikola Simonovski, Elizabeta Popovska	
<b>How long are opiates present in urine after consumption of product which contains poppy seeds?</b>	<b>327</b>
Danijela Đukić-Čosić, Katarina Baralić, Milka Kostadinović, Marko Antunović, Snežana Đorđević, Zorica Bulat, Marijana Čurčić, Evica Antonijević, Aleksandra Buha, Biljana Antonijević, Vesna Matović	
<b>Nutritional properties of two hybrids of dried and fresh cabbage</b>	<b>329</b>
Ljubica Karakashova, Frosina Babanovska - Milenkovska, Biljana Chuleva, Silvija Nakova	
<b>Determination of some phenolic constituents in extract of local wine species by using a validated HPLC-DAD method</b>	<b>331</b>
Ebru Türköz Acar, Mehmet Engin Celep, Mohammad Charehsaz, Gülşah Selin Akyüz, Erdem Yeşilada	
 <b>Pharmaceutical technology and biotechnology/ Cosmetology / Biopharmacy</b>	
<b>Cyclodextrin-based nanoparticles for drug encapsulation</b>	<b>335</b>
Ruxandra Gref	
<b>Geomaterials in the design of new drug delivery systems</b>	<b>337</b>
César Viseras	
<b>University Institute for positron emission tomography in Skopje - unique facility for the new challenges in the regional health care system</b>	<b>339</b>
Emilija Janevik-Ivanovska, Katerina Kolevska, Maja Velickovska, Filip Jolevski, Marija Atanasova, Marina Zdraveska-Kocovska, Meri Angeleska, Maja Chochevska, Zlatko Filipovski, Saso Nikolovski	
<b>Design and evaluation of differently produced glyceride based mini-matrices as extended release systems for highly soluble model drug</b>	<b>341</b>
Aleksandar Aleksovski, Chris Vervae, Rok Dreu	
<b>Formulation of chronotherapeutic delivery systems for delayed release of verapamil hydrochloride using polyethylene oxide polymers</b>	<b>343</b>
Sanja Bundalo, Jelena Đuriš, Svetlana Ibrić, Zorica Đurić	
<b>The role of cocrystallization screening for the assessment of structure-activity relationship in drug development</b>	<b>345</b>
Aleksandar Cvetkovski, Bistra Angelovska	
<b>Recombinant monoclonal antibody rituximab – medical uses and structural characterization</b>	<b>347</b>
Dashnor Nebija, Christian Noe, Bodo Lachmann, Kristina Mladenovska, Arlinda Daka, Pranvera Breznica	
<b>Comparison of emollient efficacy - a single centre, randomised, double-blind, bi-lateral comparison of two emollients prescribed in the UK for the management of dry skin conditions such as atopic eczema</b>	<b>349</b>
Jasmina Gallagher, Phil Rosher	
<b>Implementation of mexametry in periorbital hyperpigmentations studies</b>	<b>351</b>
Dragomirescu Anca Octavia, Simu Georgeta-Maria, Dehelean Cristina	
<b>A novel natural mixed emulsifier of alkyl polyglucoside type as liposome and skin-friendly cosmetic ingredient</b>	<b>353</b>
Mila Filipović, Milica Lukić, Sanela Đorđević, Gordana Vuleta, Snežana Savić	

<b>Development of an improved method for the <i>in vitro</i> determination of the Sun Protection Factor (SPF) for sunscreens</b>	<b>355</b>
Aleksandra Dimitrovska Cvetkovska, Valeria Dissette, Ilenia Magri, Laura Nucibella, Paola Ziosi, Silvia Vertuani, Stefano Manfredini	
<b>Emollient gels: characterisation of physical structure and behaviour in the presence of salts</b>	<b>357</b>
Samuel Owusu-Ware, Beatriz Sanchon-Lopez and Milan D. Antonijević	
<b>Emollient gels: Characterisation of textural properties and behaviour in the presence of salts</b>	<b>359</b>
Samuel Owusu-Ware, Beatriz Sanchon-Lopez and Milan D. Antonijević	
<b>Influence of diabetes and hypertension on cefuroxime permeation across placenta in pregnant women</b>	<b>361</b>
Mladena Lalić-Popović, Svetlana Goločorbin-Kon, Nebojša Pavlović, Jovana Paunković, Zorica Grujić, Momir Mikov	
<b>Placental transfer of lipophilic drug diazepam in pregnant women with diabetes and hypertension</b>	<b>363</b>
Mladena Lalić-Popović, Svetlana Goločorbin-Kon, Nebojša Pavlović, Jovana Paunković, Zorica Grujić, Momir Mikov	
<b>Self-microemulsifying drug delivery systems containing simvastatin: formulation and characterization</b>	<b>365</b>
Zora Četković, Marko Krstić, Sandra Cvijić, Dragana Vasiljević	
<b>A spectroscopic insight into the albumin structure on the nano-bio interface</b>	<b>367</b>
Nikola Geskovski, Simona Dimchevska, Rozafa Koliqi, Gjorgji Petruševski, Marina Chacorovska, Sonja Ugarkovic, Katerina Goracinova	
<b>Preliminary study concerning <i>Linum usitatissimum</i> oil as sebum-reducing agent</b>	<b>369</b>
Anca Dragomirescu, Ersilia Alexa, Georgeta Pop, Felicia Andrei, Georgeta Simu	
<b>Safety profile assessment of cosmetic anti-age creams based on natural ingredients using in vivo bioengineering techniques</b>	<b>371</b>
Ana Žugić, Nada Čujić, Jelena Živković, Gordana Zdunić, Katarina Šavikin, Nebojša Menković, Dubravka Bigović	
<b>Small-scale production and evaluation of an acetate-and a lactate -based balanced infusion solution</b>	<b>373</b>
Elena Najdovska, Zora Veljanova	
<b>Distribution coefficient of gliclazide as <i>in vitro</i> prediction model of blood brain barrier penetration</b>	<b>375</b>
Mladena Lalić-Popović, Svetlana Goločorbin-Kon, Maja Đanić, Nataša Milošević, Velibor Vasović, Boris Milijašević, Momir Mikov	
<b>Choosing the right blister packaging film</b>	<b>377</b>
Biljana Pavicevic, Maja Lazarova, Biljana Nanova, Milkica Gligorova	
<b>Qualification of cleanrooms in pharmaceutical industry</b>	<b>379</b>
Viktorija Veljanoska, Silvana Gjosheva, Elena Tomovska, Milkica Gligorova	
<b>Effect of formulation and process variables on probiotic viability after microencapsulation by spray-drying in soy protein-alginate microparticles</b>	<b>381</b>
Jasmina Hadzieva, Maja Simonoska Crcarevska, Simona Dimceska, Nikola Geskovski, Marija Glavas Dodov, Katerina Goracinova, Tanja Petreska Ivanovska, Lidija Petrushevska, Nadica Vanova, Milena Nikolovska, Kristina Mladenovska	
<b>Preparation of curcumin loaded nanoparticles: physicochemical characterization and in vitro evaluation</b>	<b>383</b>
Elena Drakalska, Denitsa Momekova, Stanislav Rangelov, Nikolai Lambov	
<b>Assessing the risk of alcohol-induced dose dumping: diclofenac sodium case</b>	<b>385</b>
Marija Lukic, Andjela Lipovac, Ivana Aleksic, Sandra Cvijic	
<b>Small scale production of gel with menthol, benzocaine and procaine HCl</b>	<b>387</b>
Slavica Maleska Stojadinovik, Bistra Angelovska	
<b>Approaches in evaluation of freeze-dried antibody conjugates</b>	<b>389</b>
Katarina Smilkov, Darinka Gjorgieva Ackova, Petre Makreski, Icko Gjorgoski, Emilija Janevik- Ivanovska	

<b>An injection method for preparation of liposomes as ketoconazole carriers</b>	<b>391</b>
Olga Popovska, Jana Simonovska, Elena Trajkoska-Bojadziska, Zoran Kavrakovski, Vesna Rafajlovska	
<b><i>In vitro</i> model for the analysis of 12-monoketocholate impact on simvastatin physico-chemical behavior in octanol/buffer system</b>	<b>393</b>
Maja Danić, Nebojša Pavlović, Mladena Lalić Popović, Bojan Stanimirov, Svetlana Goločorbin Kon, Karmen Stankov, Momir Mikov	
<b>Influence of the particle size at oleoresin extraction from red hot pepper</b>	<b>395</b>
Jana Simonovska, Olga Popovska, Elena Trajkoska-Bojadziska, Željko Knez, Zoran Kavrakovski, Vesna Rafajlovska	
<b>Development of nanoemulsion formulations of wild oregano essential oil using low energy methods</b>	<b>397</b>
Elena Trajkoska-Bojadziska, Jana Simonovska, Olga Popovska, Željko Knez, Zoran Kavrakovski, Biljana Bauer, Vesna Rafajlovska	
<b>Risk assessment in blister packaging</b>	<b>399</b>
Marija Cveevska, Irena Zdravaska, Biljana Nanova, Milkica Gligorova	
<b>Current therapeutic options and trends in drug development for Alzheimer's disease</b>	<b>401</b>
Maja Simonoska Crcarevska, Renata Slaveska Raicki, Marija Glavas Dodov	
<b>Protein corona evolution on polymer nanoparticles for targeted drug delivery</b>	<b>403</b>
Simona Dimchevska, Nikola Geskovski, Rozafa Koliqi and Katerina Goracinova	
<b>Formulation development and characterization of modified release matrix tablets with water-soluble drug</b>	<b>405</b>
Vesna Petrovska Jovanovska, Marija Velickovska, Aleksandra Petrovska, Sonja Ugarkovic, Marija Glavas Dodov	
<b>Statistical process control as a tool for process understanding and continuous process verification</b>	<b>407</b>
Violeta Dinić Milisavljević, Sonja Georgieva Jovanović, Igor Popović, Aleksandra Zeljković, Viška Miceska, Valentina Ilieva	
<b>Effects of PSD and wet granulation properties (concentration of granulation aid, temperature and humidity) on physical stability of ascorbic acid 95% granulate</b>	<b>409</b>
Oja Memed, Krume Tosev, Natasa Anevska Stojanovska, Gjorgji Petruševski, Marina Chacorovska, Sonja Ugarkovic	
<b>Preparation of doxycycline loaded chitosan microparticles for periodontal disease treatment by TPP ionic cross-linking combined with spray drying</b>	<b>411</b>
Silvana Gjoseva, Nikola Geskovski, Simona Dimchevska, Katerina Goracinova	
<b>Preformulation studies as initial phase in development of film-coated tablets with BCS class II active component</b>	<b>413</b>
Bosilka Stefanova, Packa Antovska, Sonja Ugarkovic, Gjorgji Petruševski, Marina Chachorovska	
<b>Influence of the formulation factors on the dissolution of highly dose water soluble active pharmaceutical ingredient</b>	<b>415</b>
Dejan Kuneski, Packa Antovska, Sonja Dimcevska, Bosilka Stefanova, Blagica Mancevska, Dusica Angelovska, Zoran Zivic, Sonja Ugarkovic	
<b>Trastuzumab and its radioimmunoconjugates in treatment of cancer</b>	<b>417</b>
Marija Sterjova, Paulina Apostolova, Predrag Dzodic, Katarina Smilkov, Darinka Gjorgjieva-Ackova, Emilija Janevik-Ivanovska	
<b>Trends in radiopharmacy in developing african countries</b>	<b>419</b>
Aschalew Alemu, David Mwanza Wanjeh, Joel Munene Muchira, Emilija Janevik	
<b>Solid-state compatibility screening of CaCO<sub>3</sub> and MgCO<sub>3</sub> with selection of excipients suitable for development of solid-dosage formulation</b>	<b>421</b>
Marina Chachorovska, Sonja Dimchevska, Sonja Ugarkovic, Gjorgji Petrushevski	

<b>Formulation development of immediate release tablets with water insoluble drug using fluid-bed granulation</b>	<b>423</b>
Sanja Simeonovska Gushic, Dejan Kostovski, Aleksandra Petrovska, Marija Velickovska Sonja Ugarkovic, Marija Glavas Dodov	
<b>Evaluation of physical properties on nonsteroidal anti-inflammatory gel formulation with different polymers</b>	<b>425</b>
Milka Mijalkova Dokova, Ljiljana Krsteska, Dejan Kostovski, Sonja Ugarkovic	
<b>Taste masking approach in oral suspension with nonsteroidal anti - inflammatory drug</b>	<b>427</b>
Roza Markovska Dameska, Liljana Krsteska, Milka Mijalkova Dokova, Dejan Kostovski, Sonja Ugarkovic	
<b>Influence of formulation variables on encapsulation efficiency of microsponges</b>	<b>429</b>
Maja Simonoska Crcarevska, Tanja Kjurkchieva Olumcheva, Renata Slaveska Raicki, Kristina Mladenovska, Marija Glavas Dodov	
<b>Optimization of viscosity building agent in oral paediatric suspension</b>	<b>431</b>
Eleonora Trajanovska, Suzan M. Sejfulah, Sanja Simeonovska Gushic, Vesna Petrovska Jovanovska, Ana Georgieva, Gjorgji Petrusevski, Sonja Ugarkovic	
<b>Risk assessment of excipients in medicinal drug products: a short review</b>	<b>433</b>
Borche Stamatovski, Elisaveta Adamova Abraseva, Miroslav Popovski, Suzan Memed Sejfulah, Sonja Ugarkovic, Miroslava Ilievska	
<b>Hold-time stability study - a “must-do” for pharmaceutical industry</b>	<b>435</b>
Ognjenka Rahić, Edina Vranić, Jasmina Hadžiabdić, Alisa Elezović, Marija Glavas Dodov	
<b>Comparison between some methods for solubility enhancement of lorazepam</b>	<b>437</b>
Jasmina Hadžiabdić, Edina Vranić, Ognjenka Rahić, Alisa Elezović, Marija Glavas Dodov	
<b>Comparison of biopharmaceutical properties of 5-FU loaded TEOS and TEOS/APTES microparticles for colon targeting</b>	<b>439</b>
Beti Djurdjic, Nikola Geskovski, Simona Dimchevska, Katerina Goracinova	
<b>Doxycycline hyclate-enriched gelatine nanoparticles for periodontal disease treatment: preparation and evaluation study</b>	<b>441</b>
Selestina Gorgieva, Vanja Kokol, Nikola Geskovski, Simona Dimchevska and Katerina Goracinova	
<b>Prospective of PET radiopharmaceutical development –new approach and strategy for their application</b>	<b>443</b>
Katerina Kolevska, Maja Velickovska, Marija Atanasova, Filip Jolevski, Maja Chochevska, Emilija Janevik-Ivanovska	
<b>Cosmetovigilance</b>	<b>445</b>
Irina Dukovska	
<b>Good Distribution Practice for medicinal products</b>	<b>447</b>
Fjola Hadjihamza, Eleonora Pandova, Violeta Bozinova	
<b>Medicinal and aromatic plants</b>	
<b>How to include DNA-based authentication in quality control of medicinal plants and phytomedicines?</b>	<b>451</b>
Johannes Novak	
<b>High-content screening for identification of bioactive compounds in plant extracts</b>	<b>453</b>
Laco Kacani	
<b>The application of mass spectrometry and pathway analysis in understanding the biochemistry of medicinal plants</b>	<b>455</b>
Shaun Bilsborough and Zoran Nastov	
<b>Cannabis in R. Macedonia: present situation</b>	<b>457</b>
Gjoshe Stefkov, Ivana Cvetkovikj, Marija Karapandzova, Svetlana Kulevanova	

<b>ALKMAF – Breeding opium poppy for improved alkaloid content</b>	<b>459</b>
Gjoshe Stefkov, Jelena Acevska, Mirjana Jankulovska, Marija Karapandzova, Aneta Dimitrovska, Svetlana Kulevanova, Sonja Ugarkovik, Igor Mickovski, Natasha Nasteva, Sonja Ivanovska	
<b>Possible health benefits of pine nuts as a source of omega fatty acids</b>	<b>461</b>
Marija Karapandzova, Ivana Cvetkovikj, Gjoshe Stefkov, Svetlana Kulevanova	
<b>Biogenic amines in red and white wines determined by HPTLC method</b>	<b>463</b>
Igno Tasev Jasmina Tonic Ribarska, Jürgen Fröhlich <sup>3</sup> , Donka Doneva-Sapceska	
<b>Herbal additives for extended shelf-life of processed meat products</b>	<b>465</b>
Ivana Cvetkovikj, Gjoshe Stefkov, Marija Karapandzova, Marija Glavash-Dodov, Maja Simonovska Carcarevska, Vesna Kotevska, Ana Kaftandzieva, Svetlana Kulevanova	
<b>Essential oils from Kosovar aromatic plants</b>	<b>467</b>
Avni Hajdari, Behxhet Mustafa	
<b>Homeopathic remedies - classical and complex homeopathy in Serbia</b>	<b>469</b>
Snezana Cupara, Olivera Milovanovic, Ana Barjaktarevic	
<b>Is cannabis addictive?</b>	<b>471</b>
Svetlana Golocorbin-Kon, Nebojša Pavlovic, Maja Đanić, Mladena Lalic-Popovic, Slobodan Gigov, Nikola Jojic, Momir Mikov	
<b>Macedonian bean diversity and its health benefits potential</b>	<b>473</b>
Sonja Ivanovska, Mirjana Jankulovska, Gjoshe Stefkov	
<b>Apoptotic and antioxidant activity of <i>Centaurea depressa</i> Bieb. (Asteraceae) extracts on colon colorectal adenocarcinoma (Caco-2) cell lines</b>	<b>475</b>
Özge Tarançi, Selcen Babaoğlu Aydaş, Belma Aslim	
<b>Cannabis history and timeline</b>	<b>477</b>
Biljana Bauer, Vesna Kostic, Svetlana Cekovska, Zoran Kavrakovski	
<b>Chemical composition of the essential oils of <i>Juniperus communis</i> subsp. <i>alpina</i> (Suter) Čelak (Cupressaceae)</b>	<b>479</b>
Behxhet Mustafa, Dashnor Nebija, Avni Hajdari	
<b>Chemical characterization and determination of antioxidant activity of basil (<i>Ocimum basilicum</i> L.) extracts using different types of <i>in vitro</i> tests</b>	<b>481</b>
Branislava Rakić, Nevena Grujić-Letić, Svetlana Goločorbin-Kon, Zorica Mrkonjić, Jovana Drljača, Aleksandar Rašković	
<b>Evidence-based research of plants used in cancer prevention or treatment</b>	<b>483</b>
Snezana Cupara, Ana Barjaktarevic, Olivera Milovanovic	
<b>Chemical profiling and antioxidant activity of <i>Sorbus intermedia</i> (Ehrh.) Pers fruit extracts and jam</b>	<b>485</b>
Zorica Mrkonjić, Jelena Nađpal, Branislava Rakić, Marija Lesjak, Ivana Beara	
<b>Chemical composition of volatile aroma compounds in fresh and dried spontaneous and cultivated rosette leaves of <i>Sideritis scardica</i> from R. Macedonia</b>	<b>487</b>
Bujar Qazimi, Gjoshe Stefkov, Marija Karapandzova, Ivana Cvetkovikj, Svetlana Kulevanova	
<b>Comparison of phenolic compounds between spontaneous and cultivated flowering stems of mountain tea (<i>Sideritis scardica</i> Griseb.) from R. Macedonia</b>	<b>489</b>
Bujar Qazimi, Jasmina Petreska-Stanoeva, Gjoshe Stefkov, Marina Stefova, Svetlana Kulevanova	
<b>Spectral analysis of extracts from red hot pepper (<i>Capsicum annuum</i> L.)</b>	<b>491</b>
Jana Simonovska, Denitsa Yancheva, Bozhana Mikhova, Žejko Knez, Mateja Primožić, Zoran Kavrakovski, Vesna Rafajlovska	
<b>Molecular mechanisms of capsaicin mediated cytotoxic activity</b>	<b>493</b>
Viktorija Maksimova, Zorica Arsova Sarafinovska, Liljana Koleva Gudeva	

<b>Review of critical points in quality assessment of red clover dry extract (<i>Trifolium pratense extractum siccum</i>): quantitative composition and providing of a representative sample</b>	<b>495</b>
Veljko Petrović, Nada Pavičić, Ivan Velikinac, Tamara Miladinović	
<b>Chemical composition and antimicrobial activity of <i>Chenopodium botrys</i> L. (Amaranthaceae) from Macedonian flora</b>	<b>497</b>
Ljubica Adji Andov, Marija Karapandzova, Ivana Cvetkovikj, Gjose Stefkov, Ana Kaftandzieva, Svetlana Kulevanova	
<b>The content of some biogenic elements in <i>Chenopodium album</i> L. and <i>Chenopodium botrys</i> L. (Amaranthaceae) from Macedonian flora</b>	<b>499</b>
Ljubica Adji Andov, Marija Karapandzova, Gjose Stefkov, Ivana Cvetkovikj, Katerina Baceva, Trajce Stafilov, Svetlana Kulevanova	
<b>Phytochemical study and antioxydant properties of Tunisian <i>Zizyphus lotus</i> L. extracts</b>	<b>501</b>
Simu Georgeta-Maria, Rădulescu-Grad Maria, Anca Dragomirescu, Bouani Bouthaina, Dehelean Cristina	
<b>Antimicrobial activity of Macedonian black pine</b>	<b>503</b>
Marija Karapandzova, Ivana Cvetkovikj, Gjoshe Stefkov, Elena Trajkovska-Dokik, Ana Kaftandzieva, Svetlana Kulevanova	
<b>The essential oil composition of Macedonian <i>Juniperus communis</i> L. (Cupressaceae)</b>	<b>505</b>
Ivana Cvetkovikj, Marija Karapandzova, Floresa Sela, Gjose Stefkov, Maja Simonoska Crcarevska, Marija Glavas Dodov, Svetlana Kulevanova	
<b>Polyphenolic profile of wild growing populations of <i>Salvia fruticosa</i> Mill. from Balkan Peninsula</b>	<b>507</b>
Ivana Cvetkovikj, Gjoshe Stefkov, Marija Karapandzova, Jasmina Petrevska-Stanoeva, Marina Stefova, Svetlana Kulevanova	
<b>The possibilities of application of medicinal plant materials in stomatology</b>	<b>509</b>
Elvira Kovac-Besovic, Salih Saracevic, Adnan Besovic, Kemal Duric	
<b>Investigation of chemical substances of essential oils in commercial perfumes by method of thin layer chromatography</b>	<b>511</b>
Elvira Kovac-Besovic, Azra Besovic, Haris Niksic	
<b>Chemical composition of the essential oils of some <i>Thymus</i> spp. (Lamiaceae) from Kosovo</b>	<b>513</b>
Verka Nedanova, Nebija Flurim, Marija Karapandzova, Ivana Cvetkovikj, Gjoshe Stefkov, Svetlana Kulevanova	
<b>Routes of cannabis administration: a brief review</b>	<b>515</b>
Gordana Geshtakovska, Gjoshe Stefkov	
 <b>Clinical pharmacy / Pharmaceutical chemistry / Biomolecular sciences</b>	
<b>Pharmacotherapeutic interventions and consults - Daily practice of a clinical pharmacist and academician</b>	<b>519</b>
Dorothea Rudolf	
<b>Status of clinical pharmacy in Slovenia</b>	<b>521</b>
Aleš Mrhar	
<b>Population pharmacokinetic modeling of therapeutic drug monitoring data from patients with epilepsy</b>	<b>523</b>
Daniela Milosheska, Tomaž Vovk, Iztok Grabnar	
<b>The role of drug metabolizing enzymes in personalized therapy</b>	<b>525</b>
Aleksandra Kapedanovska-Nestorovska, Zorica Naumovska, Krume Jakovski, Zoran Sterjev, Aleksandar Dimovski, Ljubica Suturkova	
<b>Influence of efflux transporter protein P-glycoprotein (<i>ABCB1/MDR1</i>) on therapeutic outcome</b>	<b>527</b>
Zorica Naumovska, Aleksandra Kapedanovska-Nestorovska, Ana Filipce, Zoran Sterjev, Aleksandar Dimovski, Ljubica Suturkova	



<b>What do we learned from new treatment of multiple myeloma?</b>	<b>529</b>
Sonja Genadieva Stavrik	
<b>Improved analgesics: BU08028 a novel, bifunctional NOP/MOP ligand</b>	<b>531</b>
Gerta Cami-Kobeci, Mei-Chuan Ko, Lawrence Toll and Stephen M. Husbands	
<b>Innovative drug discovery projects in the Latvian Institute of Organic Synthesis: from meldonium to new cardioprotective drug methyl-GBB</b>	<b>533</b>
Maija Dambrova, Edgars Liepinsh	
<b>DNA topoisomerase inhibitory activity and 3D-QSAR analysis of benzazoles</b>	<b>535</b>
Esin Aki-Yalcin, Ismail Yalcin	
<b>Development and standardization of Rituximab-conjugates for labeling with Lutetium-177 and Yttrium-90</b>	<b>537</b>
Emilija Janevik-Ivanovska, Darinka Gjorgieva Ackova, Katarina Smilkov, Icko Gjorgoski, Trajce Stafilov, Petre Makreski, Zorica Arsova-Sarafinovska, Lajos Baloch, Angela Carollo, Alberto Signore, Adriano Duatti <sup>8</sup>	
<b>Guillain Barré syndrome (GBS): new insights in the molecular mimicry between C. jejuni and human peripheral nerve (HPN) proteins</b>	<b>539</b>
Aida Loshaj - Shala, Luca Regazzoni, Armond Daci, Marica Orioli, Katerina Brezovska, Ana Poceva Panovska, Giangiacomo Beretta, Ljubica Suturkova	
<b>Impact of KRAS mutations on capecitabine adjuvant monotherapy in CRC patients</b>	<b>541</b>
Nadica Matevska-Geshkovska, Marija Staninova, Ivana Trajkovska, Aleksandar Eftimov, Milco Panovski, Natalija Petrushevska-Angelovska, Biljana Grozdanovska, Aleksandar Dimovski	
<b>Binding site description of 2-substituted benzothiazoles as potential RND efflux pump inhibitors</b>	<b>543</b>
Ismail Yalcin, Serap Yilmaz, Kayhan Bolelli, Esin Aki-Yalcin, Ufuk Over-Hasdemir	
<b>Ectoine nasal spray in treatment of allergic rhinitis</b>	<b>545</b>
Vladimir Šaranović	
<b>Monitoring of azathioprine active metabolite concentration in patients with inflammatory bowel disease in R. Macedonia</b>	<b>547</b>
Kristina Pavlovska, Maja Slaninka, Miceska Emilija Atanasovska, Marija Petrushevska, Kalina Gjorgjievska, Dragica Zendelovska, Igor Kikerkov, Jasmina Tonik Ribarska, Petranka Mishevska, Ljudmila Efremovska	
<b>The relationship between plasma protein binding and molecular properties of selected antifungal agents</b>	<b>549</b>
Jadranka Odović, Jovana Trbojević, Jasna Trbojević-Stanković, Ratimir Jelić, Biljana Stojimirović	
<b>Zileuton in treatment of patients with bronchial asthma</b>	<b>551</b>
Naim Morina, Gëzim Boçari, Ali Iljazi, Liridona Gashi, Naime Morina Shaqiri	
<b>Docking studies of neurokinin-1 receptor antagonists as an anticancer target</b>	<b>553</b>
Esin Aki-Yalcin, Özüm Öztürk, Kayhan Bolelli, Ismail Yalcin	
<b>Application of isocratic hydrophobic index obtained by RP-TLC of some succinimide derivatives in QSA(P)R studies</b>	<b>555</b>
Jelena Curcic, Natasa Milosevic, Vesna Kojic, Natasa Milic, Gordana Uscumlic, Nebojsa Banjac	
<b>Individualization of therapy in patients with renal impairment</b>	<b>557</b>
Jelena Curcic, Mladena Lalic Popovic, Svetlana Golocorbin Kon, Natasa Milic, Maja Milanovic, Natasa Milosevic	
<b>Distribution coefficients of novel coumarin derivatives</b>	<b>559</b>
Lulzime Ballazhi, Elena Dogazanska, Faik Imeri, Ahmed Jashari, Emil Popovski, Goran Stojkovikj, Bozhana Mikhova, Kristina Mladenovska	
<b>Mesenchymal stem cells as a new approach in treatment of systematic lupus erythematosus</b>	<b>561</b>
Magdalena Vrchkovska, Tatjana Dimitrovska Manojlovik, Marija Vrchkovska	

<b>May bile acids be utilized to enrich oncological armamentarium?</b>	<b>563</b>
Bojan Stanimirov, Nebojša Pavlović, Karmen Stankov, Maja Đanić, Vesna Kojić, Svetlana Goločorbin-Kon, Momir Mikov	
<b>Prediction of binding affinities of different bile acids towards multidrug transporters in <i>Lactobacillus acidophilus</i> NCFM - a pharmacoinformatic approach</b>	<b>565</b>
Maja Đanić, Nebojša Pavlović, Bojan Stanimirov, Tijana Stojančević, Svetlana Goločorbin Kon, Momir Mikov	
<b>Therapeutic drug monitoring as a tool for good clinical outcomes</b>	<b>567</b>
Suela Kellici, Anyla Bulo, Joana Mihani, Jera Kruja	
<b>Impact of SLCO1B1 521T&gt;C and 388A&gt;G polymorphisms on response to atorvastatin in the albanian population</b>	<b>569</b>
Arlinda Daka, Aleksandar Dimovski, Aleksandra Kapedanovska, Marija Vavlukis, Aleksandar Eftimov, Nadica Matevska Geshkovska, Sashko Kedev, Dashnor Nebija, Pranvera Breznica Selmani, Blerina Koshi, Kristina Mladenovska	
<b>New human Glutathione-S-transferase P1-1 inhibitors and their ligand binding site and GSH complex formation descriptions</b>	<b>571</b>
Ismail Yalcin, Tugba Ertan-Bolelli, Esin Akı-Yalcin	
<b>Physicochemical properties of novel derivatives of norfloxacin: solubility and pKa</b>	<b>573</b>
Pranvera Breznica-Selmani, Kristina Mladenovska, Bozhana Mikhova, Arlinda Daka, Dashnor Nebija, Blerina Koshi, Z. Kavrovski and Emil Popovski	
<b>Physicochemical properties of novel derivatives of norfloxacin: distribution coefficient</b>	<b>575</b>
Pranvera Breznica-Selmani, Emil Popovski, Bozhana Mikhova, Arlinda Daka, Dashnor Nebija, Blerina Koshi, Maja Stevanoska and Kristina Mladenovska	
<b>Methotrexate - an old drug with new pharmaceutical formulations and new indications</b>	<b>577</b>
Svetlana Goločorbin-Kon, Nebojša Pavlović, Bojan Stanimirov, Saša Vukmirović, Boris Milijašević, Hani Al-Salami, Momir Mikov	
<b>Targeting endoplasmic reticulum stress in diabetes</b>	<b>579</b>
Bojan Stanimirov, Karmen Stankov, Nebojša Pavlović, Maja Đanić, Svetlana Goločorbin-Kon, Momir Mikov	
<b>Detection of chromosomal abnormalities with multiplex ligation dependent probe amplification in patients with myelodysplastic syndromes</b>	<b>581</b>
Tatjana Sotirova, Borce Georgievski, Oliver Karanfilski, Aleksandar Stojanovic, Sonja Genadieva-Stavric, Aleksandar Eftimov, Aleksandar J. Dimovski	
<b>Regulatory aspects of data protection and privacy requirements in interventional biomedical studies</b>	<b>583</b>
Milica Zugic, Kristina Mladenovska	
<b>Protecting personal data in (pharmaco)epidemiological research: international regulation and macedonian law</b>	<b>585</b>
Milica Zugic and Kristina Mladenovska	
<b>Oxidative stress index in rat stomach as a measure of gastric tolerability of newly synthesized anti-inflammatory compounds</b>	<b>587</b>
Jelena Savić, Marina Milenković, Jelena Kotur-Stevuljević, Zorica Vujić, Sote Vladimirov and Jasmina Brborić	
<b>Antiproliferative effects of a betulin nanoformulation on a lung carcinoma cell line – A549</b>	<b>589</b>
Ioana Zinuca Pavel, Iulia Pinzaru, Roxana Ghiulai, Stefana Avram, Marius Mioc, Codruta Soica, Dorina Coricovac, Cristina Adriana Dehelean	
<b>Treatment of uremic pericarditis treated with intermittent hemodialysis</b>	<b>591</b>
Mirlind Behxheti, Lutfi Zylbeari, Nasir Behxheti, Gazmend Zylbeari, Zamira Bexheti	
<b>Treatment of arterial hypertension with ACE (Angiotensin-Converting-Enzyme) inhibitors for patients with chronic renal insufficiency</b>	<b>593</b>
Dorontina Bexheti, Sadi Bexheti, Nexhbedin Beadini, Lutfi Zylbeari	



**Treatment of apolipoprotein profile in patient with rheumatoid arthritis** \_\_\_\_\_ **595**  
Nasir Behxheti, Lutfi Zylbeari, Mirlind Behxheti, Gazmend Zylbeari, Zamira Bexheti.

**Treatment with L-carnitine in uremic patients treated with chronic hemodialysis - resistant erythropoietin** \_\_\_\_\_ **597**  
Lutfi Zylbeari, Driton Selmani Nexhibe Nuhii, Zamira Bexheti, Gazmend Zylbeari

**Prediction of blood–brain barrier permeation of  $\alpha$ -adrenergic and imidazoline receptor ligands using different HPLC systems and quantitative structure-permeability relationship analysis** \_\_\_\_\_ **599**  
Jelica Vucicevic, Marija Popovic, Katarina Nikolic, Slavica Filipic, Danica Agbaba

## Continual professional development

**Excellence in pharmacy practice – Quality indicators based on tradition, experience and innovations** \_\_\_\_\_ **603**  
Arijana Meštrović

**Quality of community pharmacy service in Republic of Macedonia – professional supervision** \_\_\_\_\_ **605**  
Bistra Angelovska and Jasminka Patceva

**Model framework for off label use of medicines** \_\_\_\_\_ **607**  
Blerina Koshi, Elizabeta Zisovska, Vasilka Nica, Maja Simonoska Crcarevska, Marija Glavas Dodov, Renata Slaveska-Raicki

**Developing community pharmacy practice** \_\_\_\_\_ **609**  
Vesna Stavrova, Maja Simonoska Crcarevska, Marija Glavash Dodov and Renata Slaveska-Raicki

**Lifelong learning - reality and perspective** \_\_\_\_\_ **611**  
Elizabeta Tomevska Ilievska, Jasmina Tonic Ribarska, Suzana Trajkovic Jolevska, Katerina Ancevska Netkovska, Goran Ajdinski

**Ethics, professionalism and autonomy of pharmacist – vision for the future** \_\_\_\_\_ **613**  
Arijana Meštrović

**Ethical dimensions of pharmacy** \_\_\_\_\_ **615**  
Kiril Temkov

**Continuing professional development - challenge for professional organization** \_\_\_\_\_ **617**  
Svetlana Stojkov, Dragana Rajković, Bistra Angelovska, Zahida Binakaj

**Implementation of standards for good compounding practices in hospital pharmacy** \_\_\_\_\_ **619**  
Vasilka Nica, Maja Simonoska Crcarevska, Marija Glavas Dodov, Renata Slaveska Raicki

**Role of community pharmacists in chronic disease management in the Republic of Macedonia** \_\_\_\_\_ **621**  
Donka Pankov, Maja Simonoska Crcarevska, Kristina Mladenovska, Renata Slaveska Raicki, Marija Glavas Dodov

**The role of the community pharmacist in self-medication with over-the-counter drugs: R. Macedonia survey** \_\_\_\_\_ **623**  
Marija Glavas Dodov, Ana Poceva Panovska, Maja Simonoska Crcarevska, Andrea Puzderliski, Vladimir Indov, Aneta Dimitrovska

**Relationship between management style and pharmacist job satisfaction in marketing strategy departments (MSDs) in headquarters of ten pharmaceutical companies in Bangladesh: a cross-sectional study** \_\_\_\_\_ **625**  
Dilshad Noor Lira, Abu Shara Shamsur Rouf

## Student session

**Porous microparticulated system for topical delivery of natural bioactive compounds** \_\_\_\_\_ **629**  
Lea Taneska, Elena Risteska, Blagorodna Koprivica, Marija Glavas Dodov, Maja Simonoska Crcarevska

**HPLC determination of hypericin content in Hyperici oleum** \_\_\_\_\_ **631**  
Veronika Stoilkovska, Jelena Acevska, Gjose Stefkov

<b>Optimization of HPLC method for determination of related substances in metamizole sodium using core-shell columns</b>	<b>633</b>
Belma Asanova, Filip Cvetanovski, Gabriela Petrovska, Marija Zafirova, Katerina Brezovska	
<b>Survey of community pharmacy practice in Republic of Macedonia</b>	<b>635</b>
Angela Arsovska, Maja Simonoska Crcarevska, Marija Glavas Dodov, Tatjana Sterjeva, Renata Slaveska Raicki	
<b>Antimicrobial resistance to antibacterial agents in common respiratory tract pathogens in pediatric population</b>	<b>637</b>
Stefan Matik, Ana Vavlukis, Aleksandra Kapedanovska Nestorovska, Zorica Naumovska, Aleksandra Grozdanova, Zoran Sterjev	
<b>Risperidone loaded nanostructured lipid carriers: formulation optimisation and characterisation</b>	<b>639</b>
Nikola Lazarevski, Hristina Litovin, Maja Simonoska Crcarevska, Marija Glavas Dodov	
<b>Formulation and characterization of rosmarinic extract loaded PEGylated liposomes for brain delivery</b>	<b>641</b>
Ljubica Cambuleva, Dushko Shalabalija, Ivana Cvetkovikj, Maja Simonoska Crcarevska, Marija Glavas Dodov	
<b>Determination of the arbutin content in wild growing populations of <i>Arctostaphylos uva-ursi</i> (L.) Spreng from Korab mountain</b>	<b>643</b>
Viktorija Labroska, Ivana Cvetkovikj, Gjoshe Stefkov	
<b>Influence of ABCB1 C3435T genotype on clinical cardiovascular outcomes in coronary artery disease patients on Clopidogrel treatment</b>	<b>645</b>
Ana Vavlukis, Lile Zdraveska, Viktorija Nikolovska, Aleksandra Kapedanovska Nestorovska, Zoran Sterjev, Aleksandra Grozdanova, Zorica Naumovska	
<b>Formulation development of self-microemulsifying system containing Atorvastatin</b>	<b>647</b>
Andrej Slavkovski, Maja Simonoska Crcarevska, Marija Glavas Dodov	
<b>A topical w/o/w multiple emulsions containing resveratrol: formulation and characterization</b>	<b>649</b>
Radmila Stanojkovska, Maja Simonoska Crcarevska, Marija Glavas Dodov	
<b>Probiotics and immunological disorders</b>	<b>651</b>
Spase Stojanov, Katarina Smilkov	
<b>The functions of sialic acid and its polymers and associated diseases</b>	<b>653</b>
Sofija Gicheva, Marija Hiljadnikova Bajro, Tatjana Kadifkova Panovska	
<b>Biosimilars in clinical use</b>	<b>655</b>
Martina Keshkjec, Martina Miloshevska, Aleksandra Grozdanova	
<b>The toxicology of aflatoxins and public awareness</b>	<b>657</b>
Elisaveta Durolojkova, Marija Hiljadnikova Bajro and Tatjana Kadifkova Panovska	
<b>World Health Organization standards for ethical and efficient promotion of over-the-counter pharmaceuticals</b>	<b>659</b>
Aleksandar Derlis, Rosana Bozhinovska, Elena Todorovska, Monika Mitreska, Renata Slaveska Raicki	
<b>The impact factors during proper chamomile drying</b>	<b>661</b>
Petar Dacev, Ile Canev	
<b>Development of microsponges as drug delivery carriers: Optimization of formulation variables using sequential experimental strategy</b>	<b>663</b>
Elena Markova, Monika Kostovska, Tanja Kjurkchieva Olumcheva, Marija Glavas Dodov, Maja Simonoska Crcarevska	
<b>Pharmacovigilance practice in community pharmacies</b>	<b>665</b>
Aleksandar Spirov, Aleksandra Kapedanovska Nestorovska, Aleksandra Grozdanova, Zoran Sterjev, Zorica Naumovska	
<b>Quality assurance of volumetric glassware in analytical laboratory</b>	<b>667</b>
Blagoj Achevski, Vasil Karcev, Katerina Brezovska	

<b>Application of AAS vs ICP-OES in determination of macro and microelements in dietary supplements</b> _____	<b>669</b>
Rosana Trpeska, Matilda Petrova, Liljana Bogdanovska, Aneta Dimitrovska, Rumenka Petkovska	
<b>Mineral composition of soil substrate of <i>Arctostaphylos uva-ursi</i> (L.) Spreng. fam. Ericaceae</b> _____	<b>671</b>
Elena Petrova, Mile Markoski, Gjoshe Stefkov	
<b>HPLC determination of amygdalin in different plant material</b> _____	<b>673</b>
Cvetanka Petrevska, Ivana Cvetkovikj, Gjose Stefkov	
<b>GC determination of potential phytoestrogenic compounds in alcoholic beverages</b> _____	<b>675</b>
Marjan Gjurcheski, Gjoshe Stefkov, Ivana Cvetkovikj	
<b>Essential oil composition of St. John's wort (<i>Hypericum perforatum</i> L.)</b> _____	<b>677</b>
Veronika Angelovska, Marija Karapandzova	
<b>Comparison of pharmacopoeial methods for analysis of residual solvents</b> _____	<b>679</b>
Marija Brezovska, Ana Ivcevska, Ana Poceva Panovska	
<b>Comparative analysis of EU and USA falsified medicine legislation</b> _____	<b>681</b>
Filip Cvetanovski, Belma Asanova, Katerina Brezovska	