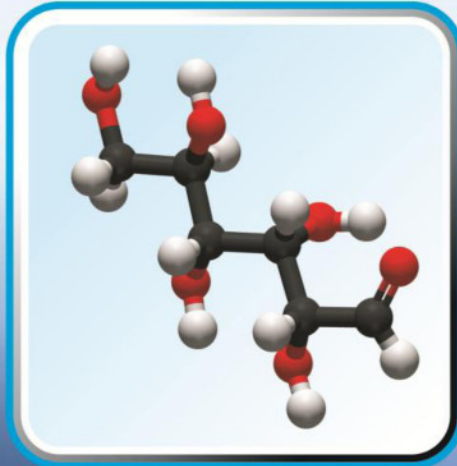



Македонски Фармацевтски Macedonian Pharmaceutical Bulletin



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5-ТИ
КОНГРЕС
НА ФАРМАЦИЈАТА
НА МАКЕДОНИЈА
СО МЕЃУНАРОДНО УЧЕСТВО

ПРЕДГОВОР

Овој број на Македонски фармацевтски билтен е специјален број на списанието посветен на Петтиот Конгрес на фармацијата на Македонија со меѓународно учество.

Во списанието се опфатени апстрактите кои од Научниот одбор се прифатени за презентација на Конгресот.

Апстрактите се печатени во оригиналната форма во која се доставени од авторите, без лекторирање и дополнително техничко обликување или пречукување па поради тоа овој број технички го нема вообичаениот стандард на Македонскиот фармацевтски билтен.

Авторите се потполно одговорни за содржините на нивните соопштенија.

PREFACE

The current issue of the Macedonian pharmaceutical bulletin is a special edition of the Fifth Congress of Pharmacy of Macedonia with International Participation.

The following issue of Macedonian pharmaceutical bulletin contains abstracts accepted by the Scientific Committee for the presentation at the Congress.

Abstracts were printed in their original form as submitted by the authors, without language corrections, technical editing or retyping.

Therefore, the layout doesn't meet the generally agreed upon standards required by the Journal.

Authors are fully responsible for the content of the submitted articles.



DEAR HONORED GUESTS, INVITED SPEAKERS, DELEGATES AND STUDENTS,

On behalf of the Scientific and Organizing Committee it is a pleasure to welcome you to Ohrid for the 5th Congress of Pharmacy in Macedonia with International Participation. The congress is organized as a joint activity of the Macedonian Pharmaceutical Association and Faculty of Pharmacy - Skopje as a quadrennial event since 1995.

This year's conference promises to be our most exciting and rewarding one yet, since more than 800 Pharmacists, among them scientists, educators, policy makers, professionals and the student community will be presenting and discussing some of the major opportunities and challenges affecting the pharmaceutical profession, today.

A great number of oral sessions and poster presentations will give both young and established scientists the opportunity to present their work and share ideas with colleagues on different topics. The opening lecture and the interdisciplinary plenary lectures presented by opinion leaders in particular fields are designed to create starting point for a fruitful congress for researchers, pharmacists and other health professionals. The scientific sessions that follow will start with opening lectures from a leading scientists in a particular field and will cover: I. Pharmaceutical Analysis / Quality Assurance / Regulatory Affairs, II. Pharmaceutical technology and biopharmacy, III. Pharmacoeconomy / Social Pharmacy, IV. Clinical biochemistry / Toxicology / Food and nutrition, V. Medicinal aromatic plants, VI. Clinical pharmacy, VII. Pharmaceutical chemistry / Biomolecular sciences. New trends in academia and education will be discussed during the half day Academic session. Two Workshops organized within the Congress, one on modern analytical techniques and one that will promote Regional ISPOR Chapter Macedonia will further enrich the activities planned for health professionals and scientists interested in those fields. The student session has a goal to introduce our students and other students from the region to experienced pharmaceutical and medical professionals, educators and scientists.

Best oral and poster presentations, selected by a Special Congress Award Committee will be awarded with prizes donated by our general sponsor.

You are invited to submit extended papers for publishing in Macedonian Pharmaceutical Bulletin immediately after the Congress.

We are especially grateful to the dedication of the entire faculty and all other colleagues and collaborators and our sponsors that have accepted to be a part of the Congress activities to ensure that this event is a success

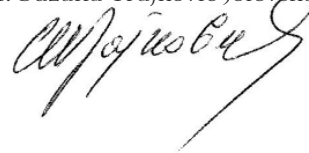
We wish you a successful Congress in Ohrid!

Regards,

Chair of the Scientific Committee
Prof. Katerina Goracinova



Chair of the Organizing Committee
Prof. Suzana Trajkovic Jolevska



ПОЧИТУВАНИ ГОСТИ, ПОКАНЕТИ ПРЕДАВАЧИ, ДЕЛЕГАТИ И СТУДЕНТИ,

Во име на Научниот и Организационен одбор задоволство е да Ви посакам добредојде во Охрид на 5-тиот Конгрес на фармацевти во Македонија со меѓународно учество. Конгресот е организиран од Македонското Фармацевтско Друштво и Фармацевтскиот факултет – Скопје, како настан кој се одржува секои четири години почнувајќи од 1995 година.

Програмата на овогодинашениот Конгрес е доказ за постојаниот напредок и промоција на овој настан. Повеќе од 800 делегати, меѓу нив и научници, професори, експерти и студенти, ќе презентираат и дискутираат за главните можности и предизвици кои ја засегаат фармацевтската професија.

Големиот број на орални и постер презентации, ќе им дадат можност како на младите така и на искусните научници да ја презентираат својата работа и меѓусебно да разменат идеи на различни теми. Воведното предавање и интередисциплинарните пленарни предавања презентирани од експерти во соодветните области ветуваат почеток на успешна работа на научниот дел од Конгресот. Научните секции кои следат, ќе започнат со воведни предавања од врвни научници во: 1. Фармацевтски анализи/ Обезбедување на квалитет/ Регулатива, 2. Фармацевтска технологија и биофармација, 3. Фармакоекономија/Социјална фармација 4. Клиничка биохемија/Токсикологија/Храна и исхрана 5. Медицински ароматични растенија 6. Клиничка фармација 7. Фармацевтска хемија/Биомолекуларни науки.

Во текот на Академската секција, ќе се дискутираат новите трендови во науката и образованието. Во рамките на Конгресот ќе се организираат две работилници. Првата ќе ги обработува современите аналитички техники, а во втората ќе се промовира регионалниот огранок на ISPOR, што дополнително ќе ги збогати активностите планирани за здравствените работници и научниците инволвирани во тие области. Студентската секција има за цел да ги промовира нашите и студентите од регионот, во професионалните, академските и научните кругови.

Најдобрите орални и постер презентации, одбрани од Комисијата за доделување награди и признанија, ќе бидат наградени од нашите генерални спонзори.

По завршување на Конгресот, ги покануваме авторите да поднесат проширена верзија на апстрактите во форма на оригинални научни трудови во Македонскиот Фармацевтски Билтен.

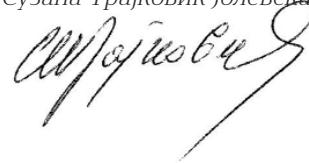
Особено сме благодарни на посветеноста на колегите од Фармацевтскиот Факултет - Скопје, сите останати соработници како и на нашите спонзори, кои прифатија да бидат дел од активностите на Конгресот.

Ви посакуваме успешен Конгрес во Охрид!
Со почит,

Претседател
на Научниот одбор
Проф. Д-р. Катерина Горачинова



Претседател
на Организационен одбор
Проф. Д-р. Сузана Трајковиќ Јолевска



PLENARY LECTURES		1
PL-1	Current trends and future prospects of pharmacoconomics and outcomes research - with special reference to health technology assessment Finn Boerlum Kristensen	2
PL-2	Perspectives of European and Global Pharmacovigilance Gunilla Sjölin-Forsberg	2
PL-3	European Legislation on Traditional Herbal Medicinal Products Werner Knöss	3
PL-5	Patients; bridging the gap between clinical theory and practical care Sue C. Jones	3
PHARMACEUTICAL ANALYSIS/QUALITY ASSURANCE/ REGULATORY AFFAIRS		5
OP-1	New LC/MS/MS techniques in pharmaceutical analysis Sebastian Kujundžić	6
OP-2	Challenges of Quality Management in Analytical Laboratories L. Ugrinova, K. Brezovska, J. Acevska, A. Poceva Panovska, J. Tonic Ribarska, S. Trajkovic Jolevska, A. Dimitrovska	7
OP-3	Review, evaluation and reporting of analytical results K. Brezovska, J. Acevska, A. Poceva Panovska, J. Tonic Ribarska, L. Ugrinova, S. Trajkovic Jolevska, A. Dimitrovska	9
OP-4	Equipment qualification for quality assurance in analytical laboratory A. Poceva Panovska, K. Brezovska, J. Tonic Ribarska, J. Acevska, L. Ugrinova, S. Trajkovic Jolevska, A. Dimitrovska	11
OP-5	Qualification and Re-qualification of analysts J. Tonic Ribarska, L. Ugrinova, K. Brezovska, J. Acevska, A. Poceva Panovska, S. Trajkovic Jolevska, A. Dimitrovska	13
OP-6	Management of Proficiency Testing Scheme (PTS) J. Acevska, L. Ugrinova, K. Brezovska, A. Poceva Panovska, J. Tonic Ribarska, S. Trajkovic Jolevska, A. Dimitrovska	15
PP-1	An RP-HPLC Study for the pKa Determination of Rizatriptan and its Impurities Biljana Otašević, Mira Zečević, Zorica Vujić and Vesna Kuntić	20
PP-2	Solid-Phase Extraction of Ibuprofen from Pharmaceutical Cream Slavica Sunaric, Milica Petkovic and Marko Denic	21
PP-3	Determination of Doxycycline in Pharmaceutical Samples by HPLC with UV detection Slavica Sunaric, Sandra Stojanovic, Snezana Mitic	21
PP-4	Determination of ketoconazole in pharmaceutical formulations Biljana Gjorgjeska	22
PP-5	Validation and quantification of Bacterial endotoxins in Solutions for haemodialysis AMINAL-100B and AMINAL-100K Elizabeta Popovska, Hristina Babunovska, Silvana Ilioska-Zlatanovic, Maja Simjanovska, Biserka Simonovska	23
PP-6	Development of an HPLC method for related and degradation products in Losartan film coated tablets Marija Spasevska, Gordana Evgenievska, Mena Ivanovska, Tatjana Bogovska, Hristina Babunovska	25
PP-7	Product Quality Review Verce Jovanovska Jankovska, Jasminka Veleska Ivanovska, Sonja Sterjevaska, Miroslava Ilievaska	26
PP-8	Five year experience in special quality control in the Croatian agency for medicinal products and medical devices Mikulčić D., Pipić-Kosanović M., Truban Žulj R., Tomić S.	28
PP-9	Analysis of purity in amlodipine tablets in Croatia G. Benković, M. Sokolić, B. Čudina, R. Truban Žulj, S. Tomić	28
PP-10	HPLC methods comparison for related and degradation products in Skopryl (Lisinopril) tablets Cveta Dolikoska, Dafinka Damcevska, Blagica Samarova-Stoev, Ana Aleksandric, Hristina Babunovska	29
PP-11	HPLC method for determination of related and degradation substances in Amlodipine alkaloid® tablets M. Dobrkovic, V. Dubrova-Koceva, S. Naumovska, N. Stojanovska, H. Babunovska	31
PP-12	HPLC method vs. UPLC method for quantitative determination of related substances in Metadon Chloride oral solution 10mg/ml Sanja Despotovska, Mena Ivanoska, Milena Strezovska, Petranka Petrusseva, Hristina Babunovska	32

PP-13	HPLC method vs. UPLC method for quantitative determination of related substances in Cefaclor capsules 500mg Milosh Todorovski, Ana Giceva, Tasika Kovacevic, Slavica Janeva, Hristina Babunovska	33
PP-14	The role of temperature dependence of chromatographic retention coefficient in separation of bile acids into congeneric groups Ana Pilipović, Mihaj Poša, Mladena Lalić	35
PP-15	Validation of Cleaning process for equipment for wet granulation Gordana Evgenievska, Ana Giceva, Miona Manasova, Vasilka Dubrova, Hristina Babunovska	36
PP-16	The quantitative structure-retention relationship (QSRR) analysis of some centrally acting antihypertensives and diuretics in cyclodextrin-mediated capillary electrophoresis Slavica Filipic, Katarina Nikolic, Irena Vovk, Mitja Krizman, Danica Agbaba	37
PP-17	The effect of pH of the medium on dissolution of Omeprazol in Omezol cps.20mg Dragana Kafedziska, Sonja Naumovska, Blagica Samarova Stoev, Hristina Babunovska	38
PP-18	Quality of glass surfaces cleaning products Olga Ortakova, Agim Ameti, Maja Shishovska, Dejan Stefanovski, Katerina Starkoska	39
PP-19	Determination of Ferulic acid with RP-HPLC method in oral solution containing extract of Ferula assafoetida L. and Viscum album L. Jasmina Slavkovska, Maja Shishovska, Dragica Doneva, Zorica Arsova –Sarafinovska, Katerina Starkoska	40
PP-20	Salting-out effects in the ether extraction of paracetamol G.M. Nikolić, J.V. Živković, A.M. Veselinović, D. Atanasković, D. Vlajin	51
PP-21	Saliva as an alternative matrix for TDM of AEDs: HPLC determination of Carbamazepine and Valproic acid in saliva of epileptic patients J.Tonic–Ribarska, Z.Sterjev, A.Haxhiu, G.Kiteva, E.Cvetkovska, I.Petrov, I.Kuzmanovski, N.Baneva, A.Arsovska, N.Tanovska, Lj.Suturkova, S.Trajkovic-Jolevska	42
PP-22	Optimization of MELC Analysis of Pramipexole and Its Impurities A. Malenović, A. Vemić, N. Kostić, T. Rakić, B. Jančić-Stojanović	45
PP-23	Central composition design in separation of ramipril and hydrochlorothiazide B. Jančić-Stojanović, B. Marković, A. Malenović, T. Rakić, A. Vemić, N. Kostić	46
PP-24	Simultaneous analysis of taxifolin (dihydroquercetin) and Vitamin C by derivative UV spectroscopy and UPLC method Nada Pavicic, Ivan Velikinac, Ljiljana Milovanovic	47
PP-25	Approaching EU - a closer look into European procedures for registration of medicinal products: MRP and DCP Zorica Pejčić, Tanja Nedjic	48
PP-26	Which one is counterfeit drug? Golocorbin-Kon S., Mikov M.	49
PP-27	Determination of all-rac-alpha-tocopherol and all-rac-alpha-tocopheryl acetate (synthetic vitamin E) in cosmetic preparations Biljana Nastova, Sandra Atanasovska, Dragica Doneva, Zorica Arsova–Sarafinovska	50
PP-28	Degradation of Mycophenolate mofetil under oxidative stress conditions Ana Protić, Ljiljana Živanović, Marina Radišić, Mila Laušević	51
PP-29	Validated HPLC method for determination of sildenafil in pharmaceutical dosage forms Z. Poposka, M. Shishovska, K. Starkoska, Z. Arsova–Sarafinovska	52
PP-30	Effect of hydrodynamics on the dissolution test of BCS class II active component formulated as solid dosage form with immediate release Irena Brašnarska, Nataša Karalija, Suzan Memed-Sejfulah, Sanja Simeonovska, jorgji Petruševski, Ema Kikovska-Stojanovska, Biljana Šapkareva, Sonja Ugarkovic	54
PP-31	HPLC method for determination of Diclofenac sodium and its impurities using “core-shell” technology M. Hadzieva, L.Ugrinova, N. Nakov, K. Brezovska, A. P. Panovska, S. T. Jolevska, A. Dimitrovska	57
PP-32	Headspace gas chromatography method for determination of propylene glycol M. Hadzieva, A. Poceva Panovska, K. Brezovska, L. Ugrinova, J. Tonic Ribarska, A. Dimitrovska	60

PP-33	Techniques for the preparation of biological samples: theoretical aspects of SPE S. Petrovski, J. Tonic-Ribarska, S. Trajkovic-Jolevska	63
PP-34	Forced degradation study on Clopidogrel 75 mg film coated tablets as useful tool during analytical method development Ksenija Brzilova, Ana Georgieva, Elena Kazandzievska, Gjorgji Petruševski, Suzan Memed-Sejfulah, Sanja Simeonovska, Biljana Šapkareva, Sonja Ugarkovic	66
PP-35	Development and optimization of a HPLC method for determination of clopidogrel-carboxylic acid, inactive metabolite of clopidogrel, in human plasma L.Bogdanovska, N.Nakov, J.Acevska, S.Trajkovic-Jolevska, A.Dimitrovska, R. Petkovska	68
PP-36	Optimization of method for determination of ACE inhibitors in human plasma based on enzyme-inhibitor binding assay followed by RP-HPLC L.Bogdanovska, N.Nakov, V. Karcev, A. Dimitrovska, R. Petkovska	71
PP-37	Comparison of the hydrodynamic effects on the dissolution rate in the paddle apparatus and its design modifications Blagica Debarlieva, Irena Brasnarska, Ema Kikovska-Stojanovska, Biljana Šapkareva, Sonja Ugarkovic	74
PP-38	Comparison of ion-pair HPLC and HILIC method for determination of cetylpyridinium chloride in pharmaceutical preparation N.Nakov, L.Bogdanovska, L.Ugrinova, M.Hadzieva, R.Petkovska, A.Dimitrovska	77
PP-39	Experimental design of stress testing study for double active component system in drug product Ivana Mickoska, Aleksandra Jovanovik, Biljana Šapkareva, Sonja Ugarkovic, Nataša Anevska-Stojanovska	79
PP-40	Comparison of extraction efficiency of different organic solvents for simplification of Dimenhydranate components quantification M.Piponski, M.Naumovska, I.S.Slavevska, T.Rusevska, I.Mitreski, G.T.-Serafimovska	82
PP-41	Adolor Gripo C as Cold Medicine Preparation – A Real and Permanent Analytical Challenge M.Piponski, T.Rusevska, I.S.Slavevska, B.Vulovska, M.Mindoseva, M.Petreska, G.T.-Serafimovska	83
PP-42	Azithromycine HPLC Quantification Method – Concepts and Testing of Experimental Variables M.Piponski, M.Globocki, I.S.Slavevska, T.Rusevska, B.Vulovska, G.Trendovska-Serafimovska	84
PP-43	Trimetazidine HPLC Method Optimization – Influence of Solubility Prescribed Medium on the Selection of Organic Modifier on Mobile Phase Composition M.Piponski, M.Globocki, I.Mitreski, E.Lazarevska, M.Petreska, K.Andonovska, G.Trendovska.-Serafimovska	86
PP-44	New bylaw on variations of marketing authorizations for medicinal products in the Republic of Macedonia, harmonization with the latest EU regulation Vesna Nasteska-Nedanovska, Ilcho Zahariev, Marija Trajchuleski, Rajna Kostoska	87
PP-45	Classification of medicines as regards their supply, prerequisite for public health protection Merjem Hadjihamza	89
PP-185	HPLC Determination of Ketoprofen residues on manufacturing equipment surfaces in cleaning validation process Katerina Kocova, Irina Batkovska Borozanova, Despina Efremova, Gordana Trendovska Serafimovska	90
PHARMACOECONOMY/SOCIAL PHARMACY/PHARMACoinFORMATICS		93
OP-7	Health Economic Aspects of Diabetes Marcin Czech	94
OP-8	Health technology assessment and pharmacoeconomics today Dinko Vitezić	94
OP-9	Pharmacoeconomy and outcome research: an industry perspective Monika Szkultecka - Debek	95
OP-10	Developing the national framework for good governance in the public pharmaceutical sector Renata Slaveska Raichki, Arta Kuli, Tatjana Kadifkova Panovska and Merjem Hadjihamza	95
OP-11	Cost analysis of the management of diabetes in Albania Besiana Bezhani, Florian Toti, Adriana Çano	97
OP-12	Adjustment and implementation of Croatian P&R model in Macedonia Tonci Buble	98

OP-13	Consumption of the prescription drugs covered by the Health insurance fund: Drugs from group C, J and N by ATC classification Kristina Hristova, Sonja Zlatanovska, Kristina Tosevska	98
OP-14	Drug consumption in Croatia 2007-2009 compared to Norway and Denmark Sasa Zezelic, Pero Draganic, Sinisa Tomic	99
OP-15	Diagnosis Related Groups (DRG) payment model in hospitals and the issue of expensive drugs Gulija M.	100
OP-16	Rational Antibiotic Use in Treatment of Respiratory Infections in Children Under Five Years of Age Marina Petric, Svetlana Nestorovic, Ljiljana Tasic, Ana Sabo	101
OP-17	Research of brand management on Macedonian pharmaceutical market Acevski Stevce	101
OP-18	Internet infovigilance – practical approach in Bulgaria Hristina Lebanova, Malina Bileva, Ilko Getov	102
OP-19	Activities of hospital pharmacists association on European level Tajda Miharija Gala	103
PP-46	Display the top ten drugs by consumption in 2009. year in RS Mirjana Đermanović, Slobodan Stanic, Saša Marić	106
PP-47	Retail and reference prices of twenty most used medicines from the Positive list after the introduction of the reference pricing system in the Republic of Macedonia Bistra Angelovska, Verica Ivanovska, Slavica Maleska Stojadinovik	106
PP-48	Analysis of the Consumption of Magistral and Galenic Medicines from 2001 to 2010 in the Public General Hospital Ohrid Slavica Maleska Stojadinovik, Bistra Angelovska, Verica Ivanovska	108
PP-49	Cost – effectiveness analysis of antimicrobial drugs used in the treatment of acute exacerbations of chronic bronchitis and chronic obstructive pulmonary disease Acevski Stevce, Sterjev Zoran, Suturkova Ljubica, Zareski Rubin	109
PP-50	Pharmaeconomic analysis of rivaroxaban for prevention of VTE in patients undergoing elective hip and knee replacement surgery Sterjev.Z, Petrovska Angelovska.A, Gavazova Kozareva.K	111
PP-51	Assessment report of adverse drug reaction received by healthcare professionals Jasmina Babic, Marko Eric, Jelena Pilic	112
PP-52	Patient Package Insert Vlacho B., Naumovska Z., Sterjev Z.	113
PP-53	Some issues in procurement of pharmaceuticals in Macedonia Wholesalers' prospective Marija Gulija	114
PP-54	Evaluation of patient from different aged groups about their satisfaction with pharmaceutical community services in R.Macedonia Sterjev Z., Vlaco B., Nestorovska K.A., Naumoska Z., Grozdanova A., Suturkova Lj.	115
PP-55	The importance of Braille alphabet application on drugs packaging Qendro Gentiana, Malaj Ledjan, Hoti Ela	118
PP-56	The role of pharmacists in decreasing antimicrobial resistance Marija Nadazdin, Lela Radakovic, Martika Ivkovic	119
PP-57	E-Marketing – new routes to approach physicians and patients Hristina Lebanova, D.K.Manolov, I. N. Getov	120
PP-58	Blood pressure control in patients with diabetes mellitus type II Roland AntoniĆ, Sandra Vezmar-Kovačević, Snežana Lukić	121
PP-59	Rational antibiotic therapy: what do our patients know? Milica Pešić, Roland AntoniĆ, Gorana Krstić, Gordana Stanisavljević, Snežana Mitić	121
PP-60	Identification of medical needs from primary health care databases in Albania N. Kellici, M. Fida, A. Dibra, M. Miraci, S. Kellici	122

CLINICAL BIOCHEMISTRY/TOXICOLOGY/ FOOD AND NUTRITION		122
OP-20	Fullerenol: Magic bullet or cell killer Rade Injac, Borut Štrukelj	124
OP-21	Derivative spectrophotometry in environmental, food and clinical samples analyses - Evaluation of EP7 monograph K.Karlijkovic-Rajic, B.Markovic	125
OP-22	Viability of L. casei in symbiotic carrot juice during fermentation and storage T. Petreska Ivanovska, L. Petrusevska Tozi, J. Hadzieva, K. Smilkov, N. Geskovski, K. Mladenovska	126
OP-23	Targeted and non-targeted LCMS methods for detection and identification of toxicologically relevant compounds Shaun Bilsborough	129
OP-24	Myeloperoxidase - risk factor for cardiovascular disease Milena Spasovska, Despina Efremova, Silvija Nesova, Tatjana Kadifkova Panovska	129
OP-25	Science as a tool for protecting the working environment: drug and alcohol abuse in the workplace Zoran Kavrakovski, Katerina Jugreva, Biljana Bauer Petrovska	131
OP-26	New trends in biomonitoring: application of RAPD-PCR and Plant Model Systems to Genetic Ecotoxicology Darinka Gjorgieva, Tatjana Kadifkova-Panovska, Saša Mitrev, Biljana Kovacevik, Emilija Kostadinovska	133
OP-27	Fingerprint based prediction of genotoxicity of thiophenes Ksenija Mickova Dimitrova, Igor Kuzmanovski	135
OP-28	Monitoring of persistent organochlorine pesticides in surface water of the Prespa region Tatjana Kadifkova Panovska, Katerina Stojkovska, Renata Slaveska Raicki	136
OP-29	Laboratory Biomarkers in Cardiovascular Medicine Hiljadnikova Bajro M., Kadifkova Panovska T.	137
PP-61	Determination of total lead content in herbal drugs grown on different locations Biljana Kaličanin, Ivana Arsić, Dragan Velimirović, Sonja Naumović	140
PP-62	Determination of Aflatoxins in granular food with Charm II Analyzer Dona Trombeva, Elizabeta Petkovska - Popovska	140
PP-63	GSTP1, GSTM1 and GSTT1 genotypes and serum GST in male COPD Žuntar Irena, Petlevski Roberta, Dodig Slavica, Popović-Grle Sanja	141
PP-64	Characteristics of the water in Lake Ohrid and Biljana springs Robertina Tomevska, Vera Novevska, Lidija Petrusevska-Tozi	142
PP-65	Determination of Aluminum content in Drinking Waters by GFAAS Suzana Angelova, Desa Jakimova, Gligor Manov	143
PP-66	Antioxidant Status and DNA Damage Induced by Heavy Metals in Matricaria recutita L. (Asteraceae) Darinka Gjorgieva, Tatjana Kadifkova-Panovska, Tatjana Ruskovska	144
PP-67	Assessment of Genotoxicity of Xenobiotics by RAPD-PCR Darinka Gjorgieva, Tatjana Kadifkova-Panovska, Saša Mitrev, Emilija Kostadinovska, Biljana Kovacevik	146
PP-68	Drugs may contribute oral-facial clefts in newborn baby Velickova, N., Gacova, M., Kamcev, N., Angelovska, B., Dimova, C.	147
PP-69	Oxidative damage of proteins in plasma of rats exposed to acute oral or intraperitoneal treatment with cadmium Aleksandra Buha, Jelena Kotur-Stevuljevic, Zorica Bulat, Danijela Djukic-Cosic, Jasmina Ivanišević, Vesna Matovic	149
PP-70	Carbon monoxide levels in blood of smokers and non-smokers determined by gas chromatography Aleksandra Buha, Zorica Bulat1, Veljko Petrović, Ivana Jakovljević, Teodora Đikić, Aleksandra Grković, Vesna Matovi	149
PP-71	Trace elements levels in patients on treatment with atypical antipsychotics Bojana Vidović, Brižita Đorđević, Zorica Bulat	150
PP-72	Serum immune response for IgA and IgG antibodies of Helicobacter pylori from patients with gastric difficulties Biserka Simonovska, Elizabeta Popovska, Nikola Simonovski, Slavko Kostoski	151
PP-73	IgA and IgG antibodies - response in diagnosis of Helicobacter pylori infection in patients with gastric symptoms Biserka Simonovska, Elizabeta Popovska, Nikola Simonovski	153

PP-74	CLA-containing food supplements on Bulgarian market Assena Stoimenova, Lili Peykova, Maria Kostova, Stefka Pankova, Kosta Todorov, Lidia Stoycheva, Danka Obreshkova	154
PP-75	Healing of duodenal ulcer after eradication of Helicobacter pylori S. Efremova, D. Efremova	155
PP-76	Examination of glutathione protective role in acute intoxicity by lead and cadmium via activity of the liver endonucleases R.S. Nikolić, J.M. Jovanović, N.S. Krstić, M.N. Stanković, G.M. Kocić	156
PP-77	Quality and safety of additives in meat products Jakimova D., Angelova S., Kadifkova Panovska T.	158
PP-78	Enhanced Health Benefits of Macedonian Red Wines Associated to Color Characteristics, Anthocyanins and Total Polyphenols Maja Dimitrovska, Mirjana Bocevska, Igor Spiroski	159
PP-79	Determination of Coenzyme Q10 Content in Dietary Supplements and Raw Materials using HPLC-DAD Maja Dimitrovska, Biljana Culeva, Anita Najdenkoska	160
PP-80	Justification of the use of fungi in nutrition Mileva Elena, Stefanoska Anita, Biljana Bauer Petrovska	161
PP-81	Biochemical composition of the beverage produced by Japanese crystals Biljana Bauer Petrovska, Svetlana Cekovska, Ljiljana Ugrinova, Biljana Chuleva, Gordana Jankoska	163
PP-82	Levels of some trace elements in some Macedonian Thymus spp. Biljana Bauer Petrovska, Olga Kirovska Cigulevska	165
PP-83	Protective role of salivatic urea in cariogenic processes D. Efremova, M. Spasovska, S. Neshova, T. Kadifkova Panovska	167
PP-84	Creatin Kinaza - MB (CK-MB) isoenzyme, AMI evaluation marker S. Neshova, M. Spasovska, D. Efremova, T. Kadifkova Panovska	169
PP-85	The values of parameters of oxidative status as a possible factor in predicting premature retinopathy Gorica Banjac, Predrag Stojakovic, Marija Slavkovic, Jasmina Ivanisevic, Milica Miljkovic, Tamara Gojkovic, Jelena Kotur-Stevuljevic, Lidija Banjac	170
PP-86	Paraoxonase-1 (PON-1) status in the premature newborn Milica Miljkovic, Tamara Gojkovic, Predrag Stojakovic, Gorica Banjac, Marija Slavkovic, Jasmina Ivanisevic, Jelena Kotur-Stevuljevic, Lidija Banjac	171
PP-87	Longitudinal oxidative stress changes in heart disease children during surgical treatment and acute heart disease exacerbation Slavkovic Marija, Stojakovic Predrag, Banjac Gorica, Ivanišević Jasmina, Miljković Milica, Kotur-Stevuljević Jelena, Vuličević Irena, Vunjak Nevenka, Peco-Antic Amira	171
PP-88	Oxidative stress status in children with pyelonephritis Predrag Stojakovic, Marija Slavkovic, Gorica Banjac, Milica Miljkovic, Jasmina Ivanišević, Jelena Kotur-Stevuljević, Amira Peco-Antić, Duško Paripović, Stanislava Petrović	172
PP-89	Detection of non-dairy fats and oils in dairy products using gas chromatography-mass spectrometry Natalija Krušaravska, Jane Bogdanov	172
PP-90	Validated HPLC-PDA method for determination of tramadol in human serum Snežana Đorđević, Vesna Kilibarda	173

MEDICINAL AND AROMATIC PLANTS

OP-30	Application of Nuclear Magnetic Resonance (NMR) methods for structure elucidation of natural products Slobodan Milosavljević	176
OP-31	The survey of species, belonging to Gentianeae family of central Balkans, pharmacognostically interesting Nebojša Menković	177
OP-32	Approaches in pharmacognosy research Gjose Stefkov, Svetlana Kulevanova	177
OP-33	Toxicological risks of herbal medicines Tatjana Kadifkova Panovska and Svetlana Kulevanova	179

OP-34	Antiaggregatory Effect Of Flavonoids Marica Medić-Šarić, Mirza Bojić	180
OP-35	Antimicrobial activity of Pinus peuce Griseb. essential oils Marija Karapandzova, Gjose Stefkov, Elena Trajkovska-Dokik, Ana Kaftandzieva, Svetlana Kulevanova	181
OP-36	Development of novel yeast strains as cell factories for the production of plant terpenoids Ivana Cvetkovikj, Antonios Makris, Codruta Ignea, Gjose Stefkov, Svetlana Kulevanova	183
OP-37	Tools for experimental design and analysis of extraction process for plant matrices Jana Klopcheska, Renata Slaveska Raichki, Vesna Rafajlovska	184
PP-91	Quantitative and multivariate analysis of polyphenolic compounds in Satureja montana L. and S. subspicata Vis. R. Jurišić Grubešić, D. Kremer, I. Kosalec, G. Šepić	188
PP-92	Evaluation of spontane and cultivated flora of "Brigade Palace" park Ajola Mesiti, Liri Dinga	189
PP-93	Fagopyrum esculentum-abiotic elicitation Tůmová L., Martin J.	189
PP-94	Screening of selected Papaveraceae species for cholinesterases inhibition and free radical scavenging activity Macáková Kateřina, Kratochvílová Eva, Chlebek Jakub, Opletal Lubomír	190
PP-95	Flavonoid production in Scutellaria baicalensis bioreactor culture Martin J., Kršková Z., Tůmová L.	191
PP-96	Composition and antimicrobial activity of Heracleum verticillatum essential oils Silvana Petrović, Milica Pavlović-Drobac, Marina Milenković, Marjan Niketić	192
PP-97	Essential oil of Seseli rigidum root Mirjana Marčetić, Branislava Lakušić, Nada Kovačević	192
PP-98	Antioxidant activities of extracts from some Carduus species Dimitrina Zheleva-Dimitrova, Iliya Zhelev, Gerassim Kitanov, Ivanka Dimitrova-Dyulgerova	193
PP-99	Ex-situ collections of endangered medicinal plants Vessela Balabanova, Antonina Vitkova, Ekaterina Kozuharova	193
PP-100	Study of plant extracts activity on different cell lines S. Kellici, B. Jucja, V. Toska, B. Basha, E. Balestrieri, R. Sorrentino, E. Troja, E. Hoti, P. Sinibaldi Vallebona	194
PP-101	Composition of the essential oils of Artemisia alba Turra Dragana Stanisavljević, Mihailo Ristić, Sofija Đorđević, Dragan Veličković, Novica Randelović	195
PP-102	Different methods of extracting capsaicin from Albanian's Capsicum fruits Enkelejda Goci, Ledjan Malaj, Rezarta Shkreli	196
PP-103	Triterpenoid saponins from the roots and in vitro root cultures of Gypsophila species Reneta Gevrenova	196
PP-104	Content of total antioxidants in infusions prepared from medicinal plants frequently used in Republic of Macedonia Tatjana Ruskovska, Darinka Gjorgieva, Marija Crcoroska, Valentina Kukovska	197
PP-105	Variations in the essential oil characteristics of Satureja montana L. (Lamiaceae) depending on environmental conditions and phenological stage Violeta Slavkovska, Mihailo Ristić, Branislava Lakušić and Dmtar Lakušić	199
PP-106	Essential oils composition variability in Salvia officinalis L. from Albania and Kosova V. Papajani-Toska, M. Hatibi, B. Basha, U. Asllani	200
PP-107	Chemical composition of rosemary oils from Albania Vilma Papajani, Klodiola Dylgjeri, Erjon Troja, Entela Haloçi	201
PP-108	Ethnobotanical and ethnomedicinal uses of plants in two regions of Albania Vilma Papajani, Dorina Shengjergji, Mirela Miraçi, Suela Kelliçi, Erina Hilaj	201
PP-109	Evaluation of several methods for the extraction of saponins from Hedera Helix leaf collected in Albania Rezarta Shkreli, Enkelejda Goci	203
PP-110	Preliminary data on analysis of methanol extraction of tubers of Gymnospermium Altaicum subsp. Scipetarum Sokol Abazi, Elmira Mehmeti, Jonida Salihila	203

PP-111	Chemical profile of olive oil with <i>Hypericum Perforatum</i> extracts Sokol Abazi, Jonida Salihila	203
PP-112	Organic compounds in tobacco (<i>Nicotiana tabacum</i> L.) from oriental varieties Klincarska-Jovanovska I, Cvetanovska L, Cvetanovska A, Srbinoska M, Bauer-Petrovska B	204
PP-113	Phytochemical and Biological Studies on Methanol Root Extract of <i>Boerhaavia diffusa</i> Milić N., Aviello G., Čanadanović-Brunet J., Mandić A., Sakač M., Borrelli F.	206
PP-114	Poisonous plants throughout history Biljana Bauer Petrovska, Zoran Kavrakovski	207
PP-115	Determination of total polyphenols in coffee (<i>Coffea</i> sp) and tea (<i>Camellia sinensis</i>) products and coffee and tea beverages Jelena Slavković, Sladjana Šobajić, Tatjana Kundaković	209
PP-116	Volatile Oil Compositions of Some Endemic <i>Hypericum</i> Species of Turkey Esra Eroğlu Özkan, Betül Demirci, Şükran Kültür, Affe Mat, Kemal Hüsnü Can Başer	210
PP-117	Distribution of bioactive flavonoids in <i>Sideritis</i> species used for medicinal "Mountain Tea" in the Balkan countries Jasmina Petreska, Gjoshe Stefkov, Svetlana Kulevanova, Kalina Alipieva, Vassya Bankova, Marina Stefova	210
PP-118	Essential oil composition of Macedonian juniper (<i>Juniperus communis</i> L., Cupressaceae) Floresha Sela, Marija Karapandzova, Gjoshe Stefkov, Svetlana Kulevanov	212
PP-119	The content of minerals in herbs and tea-infusions of Mountain tea (<i>Sideritis scardica</i> and <i>Sideritis raeseri</i>) M. Spaseska, M. Karapandzova, B. Quazimi, G. Stefkov, T. Staflov, K. Baceva, S. Kulevanova	215
PP-120	Antidiabetic and antilipidemic properties of <i>Centaurea erythraea</i> L. (Gentianaceae) Gjoshe Stefkov, Biljana Miova, Suzana Dinevska-Kjovkarovska, Jasmina Petreska, Marina Stefova, Gordana Petrusevska, Svetlana Kulevanova	217
PP-121	Essential oil composition of wild growing Sage from R. Macedonia Gjoshe Stefkov, Ivana Cvetkovikij, Marija Karapandzova, Valbona Krluku, Svetlana Kulevanova	218
PP-122	The content of macro and micronutrients in dried needles and branches of Macedonian pine (<i>Pinus peuce Griseb., Pinaceae</i>) v.s. their content in the plant residue after distillation of essential oil Marija Karapandzova, Gjose Stefkov, Trajče Staflov, Katerina Bačeva, Svetlana Kulevanova	219
PP-183	An Ethnobotanical Study in Kahramanmaraş (Turkey); Wild plants used as economic purpose in Andırın, Kahramanmaraş Serpil Demirci, Neriman Özhatay	221
PP-184	The Use of Wild Edible Plants In The South Part of İzmit (Northwest Turkey) Çağla Kızılarlan, Neriman Özhatay	222
PHARMACEUTICAL TECHNOLOGY AND BIOPHARMACY		225
OP-38	Medical Device / Drug Combination Products: A Perfect Mix for Biotherapeutics Sema Çaliş	204
OP-39	From Hollow Polymeric Nanoparticles to Entrapped DNA Ivaylo Dimitrov, Elisaveta Petrova, Rahila Kozarova, Margarita Apostolova, Christo B. Tsvetanov	204
OP-40	How well do we understand the behaviour of polymer matrix tablets? Saša Baumagrtner	206
OP-41	Current perspectives on disintegration evaluation of ODT Jachowicz R., Brniak W., Krupa A., Skórka T., Niwiński K.	207
OP-42	Identification of different solid-state modifications of atorvastatin calcium in film-coated tablets by derivative FT-IR spectroscopy Gjorgji Petruševski, Suzan Memed-Sejfulah, Sanja Simeonovska, Marina Kajdzanoska, Irena Brašnarska, Nataša Karalija, Ema Kikovska-Stojanovska, Biljana Šapkareva, Sonja Ugarkovic	209
OP-43	Molecular interactions between selected sodium salts of bile acids and morphine hydrochloride Janoš Čanadi, Mihalj Poša, Gyula Batta	210
OP-44	Swelling and drug release properties of naproxen-loaded Ca-alginate microparticles reinforced with chitosan oligosaccharide B. Calija, N. Cekic, S. Savic, B. Markovic, J. Savic, J. Milic	210

OP-45	Comparison of characteristics of granules and tablets produced in low shear and high shear granulator Sunchica Jordanoska, Krume Toshev, Natasha A. Stojanovska, Ivana Mickoska, Tina Mitrulovska, Sonja Ugarkovic	235
OP-46	Physico-chemical properties of PLGA amphipilic nanoparticles and solid tumor targeting M. Simonoska Crcarevska, N. Geskovski, M. Glavas Dodov, S. Calis, S. Dimcevska, K. Goracinova	238
OP-47	^{99m}Tc radiolabeling of nanoparticles for biodistribution and efficacy studies Geskovski N., Kuzmanovska S., Simonoska Crcarevska M., Glavas Dodov M., Kocovska Zdraveska M., Goracinova K.	240
PP-123	Depyrogenation validation Silvana Ilioska-Zlatanovic, Elizabeta Popovska, Hristina Babunovska, Maja Simjanovska, Dzoko Kungulovski	246
PP-124	The importance of monitoring polymorphic forms of pharmaceutical solids Gordana Zigic, Svetlana Mihaljica, Vesna Poprzen	247
PP-125	Risk analysis as an efficient tool for audit of starting material suppliers Borche Stamoski, Miroslav Popovski, Miroslava Ilievska, Maja Velinovska-Cadinoska, Katerina Aleksievska Beldedovska	248
PP-126	Sterile multidose lidocaine 2 % anesthetic gel – galenic preparation as a replacement of the commercial product T. Dimitrovska Manojlovikj, M. Pavlovska	250
PP-127	GMP in the production of infundibilia M. Pavlovska	251
PP-128	Document Management System as part of computerized systems in Alkaloid AD Verce Jovanovska Jankovska, Nada Popstefanova, Elizabeta Karadzinska, Darko Atanasoski, Miroslava Ilievska	253
PP-129	Risk Management for Solution for Haemodialysis Amino 100B Nada Popstefanova, Svetlana Trajkoska Dabevska, Ornela Kuzmanovska, Sonja Sterjevska	254
PP-130	Analysis of liposomes with resveratrol and vitamin C and E Atanacković M, Cvejić J, Bursać M, Lj. Gojković-Bukarica, H. Heinle	255
PP-131	Physical stability analysis of total parenteral nutrition admixture Marija Pavlovic, Mirjana Antunovic, Dusica Mirkovic, Aleksandar Petrovic	256
PP-132	The update of cosmetic legislation in the Southern Balkan countries, as a necessity in order to have safe and effective cosmetic products Mirela Miraci, Brunilda Basha Suela Kellici, Vilma Toska	257
PP-133	A comparison between Ranitidine 150mg film-coated tablets of different Balcan manufacturers with the patented drug using 'in vitro' dissolution test Ledjan Malaj, Delina Hudhra	258
PP-134	A comparison between DSC and HPLC methods in assessing drug-excipient compatibility Ela Hoti, Ledjan Malaj, Suela Kelliçi, Piera Di Martino	259
PP-135	Compounding sorbitol-mannitol irrigan for transurethral surgical procedures in hospital pharmacy Elena Najdovska, Zora Veljanova	259
PP-136	Topical preparations of Allicin and Allicin transformers: antimicrobial and anti-irritating effects Vesna Savić, Dušica Ilić, Vesna Nikolić, Ljubiša Nikolić, Mihajlo Stanković, Ljiljana Stanojević, Marija Tasić-Kostov	262
PP-137	The mineral mud in dermocosmetic preparations in Treatment of acne Potpara Zorica, Janković Slobodan	263
PP-138	Legislation and recommendations of the European Union in determining the irritation potential of cosmetic ingredients Milica Stanković, Vesna Savić, Valentina Marinković	263
PP-139	In vitro / In vivo Correlation between Two Lamotrigine Oral Formulations Lalic-Popovic M., Golocorbin-Kon S., Mikov M.	264
PP-140	Influence of particle size on the dissolution rate of atorvastatin calcium tablets E. Adamova, L. Makraduli, R. Dameska, E. Ristevska, L. Iveska, H. Litovin, D. Dimova, A. Sekovanic, M. Anevska, D. Lepčevska	265
PP-141	Formulation of gastro-resistant tablets with Eudragit® Sonja Dimchevska, Packa Antovska, Bosilka Stefanova, Sonja Ugarkovic	268
PP-142	Evidence Based Homeopathy - Scientific Framework of Homeopathy Drugs Preparations in Homeopathic Materia medica Vesna Simonova, Dora Pachova	269

PP-143	Supplier qualification – approach through risk assessment Snezana Vecerkov Vukmirovic	270
PP-144	Preformulation studies of Zolpidem tablets Packa Antovska, Gjorgji Petruševski, Sonja Dimčevska, Bosilka Stefanova, Sonja Ugarković	272
PP-145	Influence of Different Lactose Monohydrate Supplier on In-process parameters on tablets produced on a rotary tablet press Dejan Kostovski, Krume Toshev, Liljana Krsteska, Sonja Ugarkovic	274
PP-146	Static image analysis study of the morphological properties of crystalline pharmaceutically active ingredient Dejan Kostovski, Gjorgji Petruševski, Liljana Krsteska, Sonja Ugarkovic	277
PP-147	Use of descriptive statistics for evaluation of the tableting process of Zolpidem 5 mg film-coated tablets as a part of concurrent validation Bosilka Stefanova, Packa Antovska, Sonja Dimčevska, Sonja Ugarković, Blagica Debarlieva, Filip Butikoski	280
PP-148	Deviation Management in Integrated Pharmaceutical Quality System Nada Popstefanova, Marija Davcheva, Miroslava Ilievska, Svetlana Trajkoska Dabevska	283
PP-149	Effects of formulation variables on the particle size and viability of L.casei - loaded in whey protein-Ca-alginate microparticles Katarina Smilkov, Tanja Petreska Ivanovska, Lidija Petrusavska Tozi, Anita Grozdanov, Marija Glavas Dodov, Nikola Geskovski, Rumenka Petkovska, Kristina Mladenovska	285
PP-150	Qualification and process validation on blister packaging line Maja Stojevska, Biljana Pavicevic, Sonja Sterjevaska, Darko Atanasovski, Biljana Nanova, Milkica Gligorova	287
PP-151	Influence of excipients on in-process parameters on metronidazole tablets Meri Davceva	289
PP-152	Process capability indexes determination during the validation of the tablets production process Violeta Dinić Milisavljević, Nataša Anevaska Stojanovska, Sonja Ugarković, Marija Glavaš Dodov	291
PP-153	Continuous granulation, a new approach in pharmaceutical manufacturing Emilija Spaseska Aleksovska, Aleksandar Aleksovski, Emina Zahirovic	291
PP-154	Formulation optimization of ketoprofen hydrophilic matrix system by using factorial experimental design H. Litovin, R. Dameska, D. Dimova, E. Adamova, A. Sekovanic, E Ristevska, M. Anevaska, D. Lepcevska, L. Ivceska, E. Dimitrovska, L. Makraduli	292
PP-155	Formulation and evaluation of solid lipid nanoparticles loaded with budesonide for delivery to the inflamed colonic mucosa H. Litovin, K. Goracinova, N. Geskovski, M. Simonoska Crcarevska, S. Dimcevska, K. Mladenovska, M. Glavas Dodov	295
PP-156	Characterization of silica xerogel microparticles as 5-FU carriers for colon cancer therapy B. Djurdjic, N. Geskovski, G. Petruševski, M. Simonoska Crcarevska, M. Glavas Dodov, M. Cvetkovska, K.Goracinova	297
PP-157	Dissolution studies of alpha lipoic acid capsules D. Dimova, R. Dameska, L. Makraduli, E. Adamova, E. Ristevska, M.Anevaska, A. Sekovanič, D. Lepčevska, L. Ivčeska, H. Litovin	299
PP-158	Formulation and characterization of amino-functionalized silica particles for budesonide delivery V. Petrovska, K. Goracinova, M. Simonoska Crcarevska, A. Georgieva, N. Geskovski, M. Cvetkovska, M. Glavas Dodov	302
PP-159	Formulation of dental gel with micro particles loaded with tea tree oil, Melaleuca alternifolia M. Stefkova, M. Glavas Dodov, N. Geskovski, M. Simonoska Crcarevska, K. Goracinova	304
PP-160	Strategies for improvement of the EPR effect of PLGA amphiphilic nanoparticles M. S. Crcarevska, N. Geskovski, M. G. Dodov, S. Calis, S. Dimcevska, K. Goracinova	306
PP-161	Pharmacy-based survey of patient experiences and treatment effectiveness of acute diarrhea using probiotics in R. of Macedonia T. Sterjeva, K. Goracinova, M. Glavas Dodov	309
PP-162	Quality control of radiopharmaceutical agent technetium [99mTc] Mebrofenin- as a prerequisite for its application Klimentina Trajkova, Aneta Dimitorvska, Emilija Janevik- Ivanovska	311
pp-162	Quality control of radiopharmaceutical agent technetium [99mTc] Mebrofenin- as a prerequisite for its application Klimentina Trajkova, Aneta Dimitorvska, Emilija Janevik- Ivanovska	311
PP-186	Water Intrusion Test, Advantage and Practical Aspects Kukeska Silvana, Tomovska Elena, Gligorova Milkica	313

CLINICAL PHARMACY		
OP-48	Intravenous immunoglobulin in the treatment of neurological diseases Slobodan Apostolski	316
OP-49	Congenital malformations and pharmacological treatment of epilepsy in pregnancy Gordana Kiteva-Trencevska	316
OP-50	Optimization of ELISA method for determination of serum anti-GM1 antibodies Ana Poceva Panovska, Katerina Brezovska, Aleksandra Grozdanova, Slobodan Apostolski, Ljubica Suturkova	318
OP-51	Characterization of glycoproteins isolated from human peripheral nerve and two Campylobacter jejuni O:19 isolates: enteritis and GBS associated K. Brezovska, A. Poceva Panovska, A. Grozdanova, S. Apostolski, Lj. Suturkova	320
OP-52	Induction of anti-ganglioside antibodies in Guillain-Barre Syndrome animal model Grozdanova Aleksandra, Poceva Panovska Ana, Brezovska Katerina, Trajkovska-Dokić Elena, Apostolski Slobodan, Suturkova Ljubica	323
OP-53	Individualized epilepsy treatment with Carbamazepine and Valproic acid Sterjev.Z, Dimovski A.J., Suturkova Lj	325
PP-163	Potential drug-drug interactions in patients hospitalized in the Infectious Diseases Hospital 'Mother Teresa' in Tirana Klejda Hudhra, Besnik Jucja, Gjergji Minga, Vigan Saliasi	328
PP-164	The impact of antineoplastic drugs on the health of healthcare professionals Vigan Saliasi, Gjergji Minga, Dhurata Tarifa, Klejda Hudhra, Delina Hudhra	328
PP-165	Influence of immunosuppressive therapy on biochemical parameters in kidney transplant patients Nikola Stefanovic, Radmila Velickovic-Radovanovic, Aleksandra Catic-Djordjevic, Tatjana Cvetkovic	329
PP-166	Same target different pathways: Clinical pharmacist and Clinical pharmacologist Golocorbin-Kon S., Lalic-Popovic M., Mikov M.	330
PP-167	Bone mineral density in women with risk factors for osteoporosis Tamara Vojnović, Tanja Lazova, Slobodan Gigov, Ivana Indić, Jelena Živković, Jelena Gigov	330
PP-168	Monitoring the implementation and security of montelukast in children Marija Popović Milenković	331
PP-169	Angiogenesis inhibitors - New approach to treatment of malignant disease Vera Dabanović, Ana Pantović	334
PHARMACEUTICAL CHEMISTRY/BIOMOLECULAR SCIENCES		
OP-54	Electrochemical monitoring of anticancer drugs-DNA interactions by single-use sensor platforms Arzum Erdem	334
OP-55	(Benzamidomethyl)triethylammonium Chloride: A Mild and Versatile Reagent for Benzamidomethylation Emil Popovski	334
OP-56	Antiinflammatory activity and docking studies of some β-hydroxy-β-aryl propanoic acids Jelena Savić, Bojan Marković, Sanda Dilber, Marina Milenković, Milena Jovčić, Brižita Đrđević	334
OP-57	Phenylalanine derivates, new selective ligands for ampa- and kainate receptor subunits of ionotropic glutamate receptors Serafimoska Zorica, Kapedanovska Nestororvska Aleksandra, Suturkova Ljubica, Tommy N. Johansen, Karla Frydenvang	336
PP-170	Arylpiperazines active on 5HT1A receptor - QSAR analysis IvaValkova, Irini Doytchinova	340
PP-171	In vitro study of antimicrobial activity of α,β-unsaturated carboxylic acids and α-bromo-carboxylic acids Sara Filipović, Marina Milenković, Sanda Dilber, Vesna Vitnik, Tanja Jovanović, Željko Vitnik	341
PP-172	Synthesis and investigations of some 8-substituted derivatives of 1,3,7-trimethylxanthine Al. Zlatkov, Pl. Peikov	341

TABLE OF CONTENTS

PP-173	Electrochemical monitoring of anticancer drugs-DNA interactions by single-use sensor platforms Arzum Erdem	342
PP-174	(Benzamidomethyl)triethylammonium Chloride: A Mild and Versatile Reagent for Benzamidomethylation Emil Popovski	342
PP-175	Antiinflammatory activity and docking studies of some β-hydroxy-β-aryl propanoic acids Jelena Savić, Bojan Marković, Sanda Dilber, Marina Milenković, Milena Jovčić, Brižita Đrđević	343
PP-176	Phenylalanine derivates, new selective ligands for ampa- and kainate receptor subunits of ionotropic glutamate receptors Serafimoska Zorica, Kapedanovska Nestorovska Aleksandra, Suturkova Ljubica, Tommy N. Johansen, Karla Frydenvang	346
PP-177	Arylpiperazines active on 5HT1A receptor - QSAR analysis Iva Valkova, Irini Doytchinova	346
PP-178	In vitro study of antimicrobial activity of α,β-unsaturated carboxylic acids and α-bromo-carboxylic acids Sara Filipović, Marina Milenković, Sanda Dilber, Vesna Vitnik, Tanja Jovanović, Željko Vitnik Synthesis and investigations of some 8-substituted derivatives of 1,3,7-trimethylxanthine Al. Zlatkov, Pl. Peikov	348
ACADEMIC SECTION		
OP-58	PHARMINE - Pharmacy education in Europe Jeffrey Atkinson and Bart Rombaut	356
OP-59	Harmonization and reformation of the Master in Pharmacy study programme at the Faculty of Pharmacy, University of Ljubljana Irena Mlinarič-Raščan, Mirjana Gašperlin, Borut Božič	356
OP-60	The science and practice in the curricula of the Faculty of Pharmacy at the university "Ss Cyril and Methodius" in Skopje, Macedonia Aleksandar J. Dimovski, K. Mladenovska	357
PP-179	Pharmaceutical care in the curriculum of Faculty of Pharmacy and Biochemistry: past experience and future challenges Ž. Juričić, L. Bach-Rojecky, S. Vladimir-Knežević, R. Jurišić Grubešić	360
STUDENTS SECTION ORAL PRESENTATIONS		
	Titles of oral presentations (in order of appearance)	362
STUDENTS SECTION		
PP-180	Harmful effects of UV radiation on human Daliborka Jovanova, Katerina Andonovska, Biljana Bauer Petrovska	366
PP-181	Use of poppy through the ages Katerina Andonovska, Daliborka Jovanova, Biljana Bauer Petrovska	368
OTHER		
PP-182	Importance of trade marks for pharmaceutical products Katerina Ancevska Netkovska	370

In food samples analyses mainly determined compounds are colorants (Tartrazine, Sunset Yellow, Ponceau 4R in various combinations). DS was used to evaluate the efficiency of phenylalanine removal for dietary supplements. Assays for malondialdehyde contents in infant milk formulas, protein and casein in milk as well as RPHPLC-second and fourth DS for milk proteins were evaluated. Applications of DS for the control of lipid oxidation in meats along with effectiveness of carnosine and dietary supplementation with α -tocopherol as well as assay of aromatic acids in cooked meat were outlined. Content of Vitamin C, plant pigments chlorophyll a and b, soy protein isolate, energizers in energy drinks, sorbic and benzoic acids in soft drinks and antibiotics in feeds were investigated using DS.

EVALUATION OF EP7 MONOGRAPH

Within our inter-laboratory evaluation of DS monograph (Ph.Eur.7) the comparison of analogue and digital (Savitzky-Golay algorithm) of second-order derivative spectra was carried out recording spectra on GBC-Cintra 20 (digital – smoothing points 5-21) and Thermo-Scientific - Evolution - 300 (analogue – smoothing – Low, Medium, High and Very-high or digital – smoothing points 3-121) using 0.02% V/V toluene in methanol solution. Requirement for resolution power defined as ratio A/B (A-amplitude ${}^2D_{265,263}$; B-amplitude ${}^2D_{263,261}$) is not less than 0.2 corresponds to resolution of shoulder and peak within the band. The results showed that the only possible comparison of instruments is for smoothing 7, and for both analogue and digital spectra with smoothing 7 and Medium.

IN CONCLUSION, along with substantial advantages of DS the limitations are caused by: a) dependence on instrumental parameters; b) non-robust character of the selected parameters of elaborated methods-parameters can be used only for system for which they are chosen and c) lack of harmonized protocol of parameter's optimization and results presentation.

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VIABILITY OF *L. CASEI* IN SYMBIOTIC CARROT JUICE DURING FERMENTATION AND STORAGE

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INTRODUCTION

Although dairy products are generally good matrices for the delivery of probiotics to humans and traditionally the most used, fruit juices are of growing interest due to their pleasant taste profile and refreshing characteristics (1). The increased demand of fruit juices for probiotic carriers can be explained by the lack of milk allergens and cholesterol (2). In addition, fruits and vegetables inherently contain essential nutrients such as vitamins, minerals and antioxidants (3). However, the low survival rate of probiotics in fruit juices resulting from acid environment is of concern. The viability of probiotic cells in fruit juices can be improved by adding of microencapsulated cells to the juice. In this study, carrot juice was inoculated with free probiotic cells of *L. casei* and synbiotic microparticles loaded with *L. casei* to compare the survival rate of the probiotic during fermentation and storage of the synbiotic beverages at 4 °C for 6 weeks.

MATERIALS AND METHODS

Carrot juice was prepared by extraction of washed and peeled carrots with no added water or any other nutrient. Then, the juice was pasteurized at 80 °C for 20 min. The cell suspension of probiotic *Lactobacillus casei* (Chr. Hansen, Denmark) was divided into two parts: one part was used for microencapsulation and another was used as free cells for direct adding in carrot juice. Prebiotic fructooligosaccharide (FOS) (Sigma-Aldrich, USA) was added simultaneously with free probiotic cells. The initial cell concentration of $7.4 \pm 0.1 \log_{10}$ cfu/ml was applied to ferment carrot juice using *L. casei* and FOS or synbiotic microparticles. This concentration was chosen according to the recommendations for minimum counts of $7.0 \log_{10}$ cfu per g or ml of probiotic food to exert beneficial effects (4). The microparticles were prepared by modified spray-drying method (5) when aqueous dispersion of alginate (LF 10/60, Protanal, FMC Biopolymers, USA), FOS and *L. casei* was submitted to spray-drying (nozzle diameter 0.7 mm, aspirator pressure 90%, flow rate 6 ml/min, inlet and outlet temperature, 120 °C and 60 °C, Büchi Mini Spray Dryer B-290, SW) followed by subsequent cross-linking and coating in solution of CaCl₂ (Merck, Germany) and chitosan (Chitine, France) in 1% w/w acetic acid. The microparticles formed were cured at least 3 h, separated and freeze-dried (-50 °C, 0.070 mbar, 24 h, Freeze-Dryer, Labconco, USA). An optimal formulation of micro-particles was prepared, with 4% w/w alginate, 0.5% w/w chitosan and 5% w/w CaCl₂. Positively charged microparticles (21.5 ± 1.5 mV) with d_{VS} $9.1 \pm 0.8 \mu\text{m}$, Ca-content of 9.4 ± 0.1 % and high cell viability of *L. casei* ($11.3 \pm 0.15 \log_{10}$ cfu/g) were obtained. Synbiotic microparticles were added to the carrot juice on the same day of preparation. All samples of synbiotic carrot juices were packed into sterile Erlenmeyers flasks closed with cotton plugs. The samples inoculated with free cells and added particles were firstly sub-

mit to fermentation and then stored at 4 °C. The fermentation process was carried out in an incubator for 24 h at 37 °C. The viability of *L. casei* in all experiments was determined periodically during 24 h and then on a weekly basis for 6 weeks of storage. When enumeration of bacteria was performed, 1 ml of the sample was mixed with 9.0 ml of peptone water, vortexed for 15 s and serially diluted with peptone water. The viable count was determined using plate-count method on MRS agar (Merck, Germany) after 72 h of incubation at 37 °C. Regarding the samples with added microparticles, the particles were removed from juice by filtration, washed with sterile saline solution and liquefied with phosphate buffer (pH 6.9). The suspension of particles was vortexed 30 s followed by standing at room temperature and then enumerate as described above. The pH of synbiotic carrot juices were also examined (pH meter PB 11 Sartorius, Germany).

RESULTS AND DISCUSSION

During the fermentation of carrot juice *L. casei* grew rapidly with increased population of free cells compared to encapsulated ones. At the end of the fermentation period the pH value of the juice inoculated with free cells was found to be lower than that of the microparticles added juice (Fig. 1).

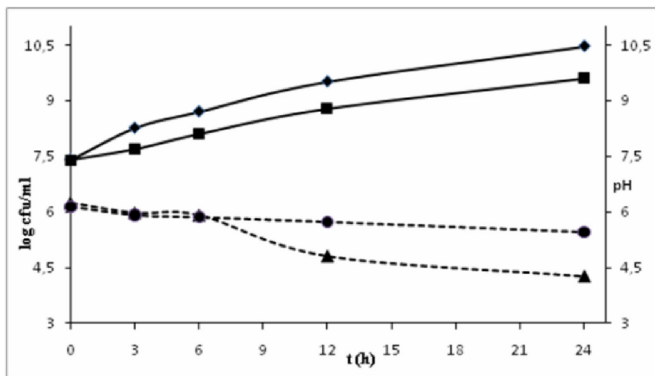


Fig. 1. Changes in viability and pH values during fermentation of carrot juice with free and microencapsulated *L. casei*

The viable cell counts of microencapsulated *L. casei* in fermented carrot juice was $8.1 \pm 0.13 \log_{10}$ cfu/ml after 6 weeks of cold storage at 4 °C, while that of free cells was only $4.89 \pm 0.1 \log_{10}$ cfu/ml. In addition, the pH values of the synbiotic carrot juices with free cells were lower than pH values of carrot juice with microencapsulated cells during all investigation period (Fig. 2).

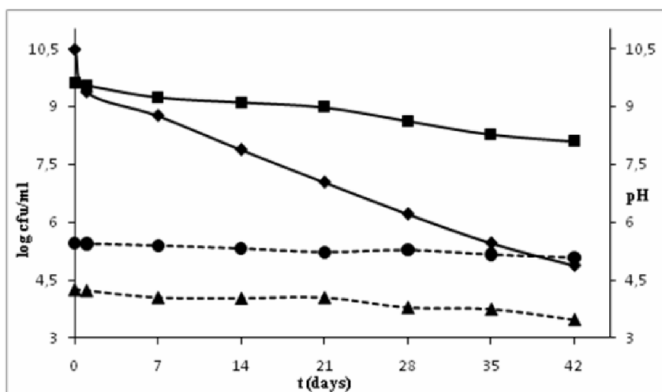


Fig. 2. Changes in viability and pH values during cold storage of fermented carrot juice with free and microencapsulated *L. casei*

The results showed that the survival rate of free probiotic cells in carrot juice was below the therapeutic level at the end of the test due to their sensitivity to the acidic conditions in the medium and need protection to maintain the viability during storage. Results also showed that adding of encapsulated *L. casei* in carrot juice as synbiotic chitosan-Ca-alginate microparticles might solve the problem. Regarding the sensory characteristics of the carrot juice with microparticles non-significant changes of the textural quality due to the low particle size was observed. Therefore, carrot juice containing synbiotic microparticles may be a new functional product and the effect of particles on the consumer acceptance should be further studied.

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ВИТАЛНОСТ НА *L. CASEI* ВО СИНБИОТСКИ СОК ОД МОРКОВ ВО УСЛОВИ НА ФЕРМЕНТАЦИЈА И ЧУВАЊЕ

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ВОВЕД

Покрај традиционалната примена на млечните производи за испорака на пробиотици во хуманиот ГИТ, пријатниот и освежувачки вкус на овошните сокови го зголемува интересот за апликација на пробиотиците во различни сокови (1). Интересот се должи на отсуството на млечни алергени и холестерол во овошните сокови (2), како и на присутните минерали, витамини и антиоксиданси во овошјето и зеленчукот (3). Киселата средина на сокот може значајно да ја намали виталноста на пробиотиците, а зголемување на виталноста може да се постигне со инкапулирање на клетките. Во овој труд, следена е виталноста на *L. casei* во синбиотски сок од морков со слободни и инкапулирани клетки за време на ферментација и чување во тек на 6 недели.

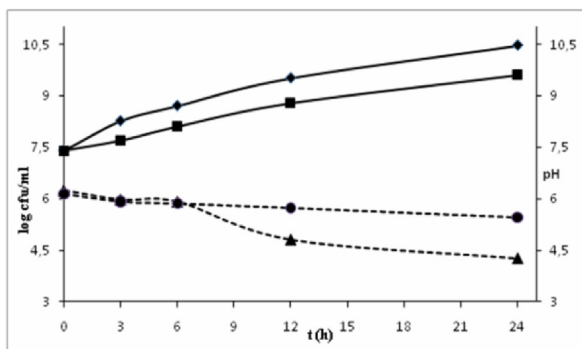
МАТЕРИЈАЛИ И МЕТОДИ

Сокот од морков беше подготвен со екстракција на измиени и излупени моркови без додадена вода и нутриенти и со примена на пастеризација (80 °C, 20 min). Клеточната суспензија на *L. casei* (Chr. Hansen, Denmark) беше поделена на два дела. Едниот беше наменет за микроинкапулирање, додека другиот беше аплициран во сокот

заедно со пребиотикот фруктоолигосахарид (ФОС) (Sigma-Aldrich, USA). Почетната концен-трација на *L. casei* ($7.4 \pm 0.1 \log_{10}$ cfu/ml) беше избрана врз основа на препораките за минимален број на витални клетки на g или ml производ за предизвикување на позитивни ефекти врз здравјето (4). Микрочестичките беа подготвени со модифициран метод на спреј сушење (5), така што водна дисперзија на алгинат (LF 10/60, Protanal, FMC Biopolymers, USA), ФОС и *L. casei* беше распрната со сушење (d диза 0.7 mm, проток 6 ml/min, аспирација 90%, влезна и излезна температура, 120 °C and 60 °C, Büchi Mini Spray Dryer B-290, SW) проследено со полиелектролитно комплексирање и вкрстено поврзување во раствор на цитозан (Chitine, France) и CaCl₂ (Merck, Germany) во 1% w/w оцетна киселина. По 3 h мешање, честичките беа одделени со центрифугирање и лиофилизирани (-50 °C, 0.070 mbar, 24 h, Freeze-Dryer, Labconco, USA). Со примена на 4% (w/w) алгинат, 0.5% (w/w) цитозан и 5% (w/w) CaCl₂, беше подготвена оптимална формулација на честички со позитивен површински полнеж (21.5 ± 1.5 mV), големина од $9.1 \pm 0.8 \mu\text{m}$, содржина на Ca од $9.4 \pm 0.1\%$ и виталност на *L. casei* од $11.3 \pm 0.15 \log_{10}$ cfu/g. Микрочестичките беа веднаш аплицирани во сокот. Потоа, примероците беа сместени во стерилни Erlenmeyer садови со памучни затворувачи и ферментирани во тек на 24 h на 37 °C во инкубатор. Виталноста на *L. casei* беше одредувана периодично во тек на 24 h и потоа еднаш неделно во услови на чување на 4 °C. Виталноста на *L. casei* беше определена со додавање на 9 ml пептонска вода на 1 ml примерок, вортексирање на содржината 15 s и сериско разредување со пептонска вода. Броенето на колониите беше вршено со примена на методот на броене на плоча на MRS agar (Merck, Germany) по инкубација на засадените плочи за време од 72 h на 37 °C. Виталноста на инкапсулираните клетки беше одредена со одделување на честичките од сокот со филтрирање. Суспензијата на честички во фосфатен пуфер (pH 6.9) беше вортексирана 30 s и оставена на собна температура. Потоа постапката за одредување на виталноста е идентична како што е претходно опишано. pH вредностите на синбиотските сокови од морков со слободни и инкапсулирани клетки од *L. casei* беа исто така испитувани (pH meter PB 11 Sartorius, Germany).

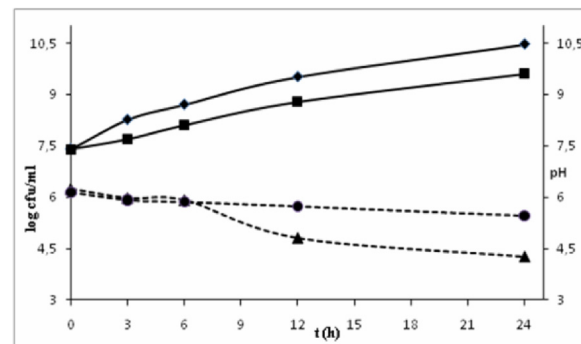
РЕЗУЛТАТИ И ДИСКУСИЈА

При ферментирање на сокот од морков беше забележан брз раст на *L. casei*, и тоа повисок раст покажаа слободните клетки. На крајот од ферментациониот период, pH вредноста на сокот со слободни пробиотски клетки беше пониска од сокот кој содржи инкапсулирани клетки (Сл. 1).



Сл. 1. Промени на виталноста на *L. casei* и pH вредноста на синбиотскиот сок од морков за време на ферментација

По 6 недели чување на 4 °C, виталноста на микроинкапсулираниот *L. casei* во фермен-тиран сок од морков беше $8.1 \pm 0.13 \log_{10}$ cfu/ml, додека виталноста на слободните клетки беше $4.89 \pm 0.1 \log_{10}$ cfu/ml. pH вредноста на сокот со слободни клетки беше пониска од pH вредноста на сокот со инкапсулирани клетки за целокупниот период на чување на 4 °C (Сл. 2).



Сл. 2. Промени на виталноста на *L. casei* и pH вредноста на ферментираниот сок од морков за време на чување на 4 °C

Резултатите покажаа дека виталноста на слободните пробиотски клетки во сок од морков не го задоволува терапевтското ниво во услови на чување што се должи на нивната осетливост кон киселата pH на сокот. Одржувањето на виталноста на *L. casei* при чување на сокот може да се подобри со инкапсулирање на клетките во облик на цитозан-Са-алгинатни честички. Со аплицирање на синбиотските микро-честички не беа забележани значајни промени на органолептичките својства и текстурата на сокот од морков како резултат на малите честички. Оттука произлегува можната употреба на сокот од морков со синбиотски микрочестички како нов функционален производ, при што следи проценка на ефектот на честичките врз прифатливоста од потрошувачите.

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