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UCG
Univerzitet Crne Gore
FARMACEUTSKI FAKULTET



CALIMS

U SARADNJI SA AGENCIJOM ZA LJEKOVE I MEDICINSKA SREDSTVA CRNE GORE

**II KONGRES FARMACEUTA CRNE GORE SA MEĐUNARODNIM UČEŠĆEM
II CONGRESS OF PHARMACISTS OF MONTENEGRO WITH THE INTERNATIONAL PARTICIPATION**

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Zbornik sažetaka**

**Glavni urednik:
Zorica Potpara**

**Redakcijski odbor:
Nataša Duborija-Kovačević
Svetlana Ibrić
Dragica Bojović
Tea Dakić
Snežana Pantović**

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STAVOVI I INFORMISANOST OPŠTE JAVNOSTI O KLINIČKIM ISPITIVANJIMA	139
PUBLIC ATTITUDES AND AWARENESS OF CLINICAL TRIALS	140
DETERMINATION OF CAPSAICINOIDS IN DIFFERENT GENOTYPES OF CAPSICUM SPECIES BY VALIDATED HPLC METHOD	141
KARAKTERIZACIJA I KVANTIFIKACIJA HIDROKSICIMETNIH KISELINA I FLAVANOLA U-ZRNU SORTI EVROPSKE HELJDE	141
CHARACTERIZATION AND QUANTIFICATION OF HYDROXYCINNAMIC ACIDS AND FLAVANOLS IN SEEDS OF EUROPEAN BUCKWHEAT CULTIVARS	142
ODREĐIVANJE SADRŽAJA FENOLNIH SPOJEVA I ISPITIVANJE..... ANTIOKSIDATIVNOG KAPACITETA PIVA NA TRŽIŠTU BOSNE I HERCEGOVINE	143
DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF BEER AT MARKET IN BOSNIA AND HERZEGOVINA	144
ODREĐIVANJE SADRŽAJA FENOLNIH SPOJEVA I ISPITIVANJE..... ANTIOKSIDATIVNOG KAPACITETA VINA AUTOHTONIH HERCEGOVAČKIH SORTI	145
DETERMINATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF INDIGENOUS VARIETIES OF WINES FROM HERZEGOVINA	146
KORISNOST ODREĐIVANJA FREEPSA/TOTALPSA OMJERA KOD PACIJENATA S VRIJEDNOŠĆU TOTAL PSA OD 2.1 DO 10NG/ML	146
THE USEFULNESS OF DETERMINING FREEPSA/TOTALPSA RATIO IN PATIENTS WITH TOTAL PSA VALUE FROM 2.1 TO 10 NG/ML.	147
LEKOVI U ŽIVOTNOJ SREDINI-POTENCIJALNI RIZICI ZA ČOVEKA PHARMACEUTICALS IN THE ENVIRONMENT-POTENTIAL HUMAN RISK	148
RODITELJI I NJIHOVA ZNANJA O LEKOVIMA KOJI SU NAMENJENI PEDIJATRIJSKOJ POPULACIJI	149
PARENTS AND THEIR KNOWLEDGE ABOUT MEDICINES INTENDED FOR PEDIATRIC POPULATION	150
INTERPOLIMERNI KOMPLEKSI CARBOPOL®-A 940 I RAZLIČITIH TIPOVA SURFAKTANATA..... KAO MATERIJALI ZA PRIMENU U FARMACIJI	151
INTERPOLYMER COMPLEXES OF CARBOPOL® 940 AND DIFFERENT TYPES OF SURFACTANTS AS MATERIALS FOR PHARMACEUTICAL APPLICATIONS	152
HAPLOTYPES OF SLC01B1 GENE ENCODING OATP1B1 IN ALBANIAN ETHNIC GROUP	153
ANALIZA UPOTREBE LEKOVA ZA LEČENJE DIJABETES MELITUSA..... U REPUBLICI SRBIJI U PERIODU OD 2007. DO 2012. GODINE	154
THE ANALYZE OF CONSUMPTION OF DRUGS FOR THE TRÉATMENT OF DIABETES MELLITUS IN THE REPUBLIC OF SERBIA FROM 2007 TO 2012	154
SYNTHESIS OF NOVEL BENZAMIDOMETHYL DERIVATIVES OF CIPROFLOXACIN AND THEIR STRUCTURAL CHARACTERIZATION	155
POLIMORFIZAM CYP2D6 – FAKTOR RIZIKA ZA NASTANAK NEŽELJENIH DEJSTAVA BETA BLOKATORA	156
CYP2D6 POLYMORPHISM – RISK FACTOR FOR DEVELOPING ADVERSE DRUG REACTIONS OF BETA ... BLOCKERS	157
ANALIZA POTROŠNJE LJEKOVA ZA KARDIOVASKULARNE BOLESTI U ZDRAVSTVENOJ USTANOVI..... APOTEKE CRNE GORE "MONTEFARM"	158
THE ANALYSIS OF DRUG UTILIZATION FOR CARDIOVASCULAR DISEASES IN HEALTH PHARMACY INSTITUTION OF MONTENEGRO "MONTEFARM"	158

DETERMINATION OF CAPSAICINOIDS IN DIFFERENT GENOTYPES OF CAPSICUM SPECIES BY VALIDATED HPLC METHOD

Viktorija Maksimova¹, Liljana Koleva Gudeva², Maja Shishovska³, Katerina Starkoska³, Zorica Arsova Sarafinovska^{1,3}

¹Faculty of Medical Sciences, University "Goce Delcev", Stip, Macedonia

²Agricultural faculty, University "Goce Delcev", Stip, Macedonia

³Institute for Public Health of the Republic of Macedonia, Skopje, Macedonia

Capsaicin is a commonly used phytochemical, well-known by its pharmacological properties as analgesic, antidiabetic, hypolipidemic and antitumor agent. Development of a simple method for extraction and quantification of capsaicin from hot pepper fruits (*Capsicum annuum* L.) gives a chance for effective exploitation of this highly represented agro culture in Republic of Macedonia, and brings an opportunity for further investigations on its pharmacological activity. Therefore, the aim of this study was to determine capsaicinoids (capsaicin, dihydrocapsaicin and nordihydrocapsaicin) in ethanolic extracts of 11 different genotypes of hot pepper fruits from Republic of Macedonia with a validated simple and sensitive HPLC method.

Fruits, dried and grounded, were used as a plant material for Soxhlet extraction by using a 96 % (V/V) ethanol as a solvent (70°C, for 5 hours). Quantification of a capsaicin and dihydrocapsaicin was performed on a RP-HPLC (reverse phase-high performance liquid chromatography) system by using a Zorbax SB-C18 column, (5 µm, 250 × 4.6 mm), mobile phase: H₂O/CH₃CN, 50:50 (V/V), flow rate: 1.5 mL/min and UV detection at 220 nm.

The analytical method was validated by using the protocols set out in the International Conference on Harmonization (ICH) guidelines. The required validation parameters, specificity, linearity, accuracy, precision, limit of detection, and limit of quantification, were studied for capsaicin and dihydrocapsaicin. The linearity range was found to be 1.52 – 380.00 µg/mL for capsaicin, and 1.12 – 279.00 µg/mL for dihydrocapsaicin, respectively. Limit of detection for capsaicin and dihydrocapsaicin was 0.075 and 0.109 µg/mL, and limit of quantification was 0.230 and 0.331 µg/mL for capsaicin and dihydrocapsaicin, consequently. The highest concentration of capsaicin, 2835 µg/g and for dihydrocapsaicin 2443 µg/g was found in the extract obtained from genotype Feferona.

The results showed that this method can be employed as quantification method for determination of capsaicinoids in the *Capsicum* oleoresins.

Key words: capsaicinoids, peppers, liquid chromatography, validation parameters.

KARAKTERIZACIJA I KVANTIFIKACIJA HIDROKSICIMETNIH KISELINA I FLAVANOLA U ZRNU SORTI EVROPSKE HELJDE

Biljana Kiprovska¹, Djordje Malenčić¹, Dejan Prvulović¹, Maja Mikulič-Petkovšek², Ana Slatnar², Robert Veberič² and Franci Štampar²

¹Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za ratarstvo i povrtarstvo, Trg Dositeja Obradovića 8, 21000 Novi Sad, Srbija

²Univerzitet u Ljubljani, Biotehniški fakultet, Departman za agronomiju, Katedra za uzgoj voća,