Farmaceutska hemija i analitika lekova

Pharmaceutical Chemistry and Drug Analysis

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USAGE OF ANTHOCYANES FOR AUTHENTICATION OF ALIMENTARY PRODUCTS AND ITS DRAWBACKS

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Authentication of alimentary products based on botanical raw materials (e.g., fruits, grains, vegetables etc.) is often based on identification and quantification of anthocyanes contained therein. For authentication purpose, we need fast procedures and one of them is screening of these products with use of thin-layer chromatography (TLC).

It was the aim of this study to show vulnerability of anthocyanes analyzed by means of TLC against stationary phases of different activity (cellulose powder, RP-C18) and mixed mobile phases employing organic acids (formic acid, acetic acid) and hence, a limited performance thereof as authentication markers. To this effect, we used cyanin and keracyanin (two anthocyanines), andpelargonidin and delphinidin (two anthocyanidines) as four phytochemical standards. Partial hydrolytic decomposition of anthocyanines was demonstrated by the results originating from TLC, mass spectrometry with use of the TLC-MS interface and from using the sugars-specific visualization reagent PABA. Moreover, calibration curves were elaborated for the four test anthocyanes and then these compounds were identified and quantified in the selected alimentary samples (fruit juices and herbal infusions). It was demonstrated that authentication of alimentary products with use of anthocyanes as authenticity markers can result in a semi-quantitative response only and in practical impossibility to confirm with full confidence the presence of a particular anthocyane in a scrutinized sample of a fruit juice or herbal infusion. In conclusion, there is a warning that authentication of alimentary products containing anthocyanes can occasionally be misleading.

RAČUNARSKI PROGRAMI ZA REZOLUCIONI PARAMETAR U MONOGRAFIJI EVROPSKE FARMAKOPEJE ZA METODU DERIVATIVNE SPEKTROFOTOMETRIJE

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Evropska farmakopeja (EP 9) propisuje monografiju za metodu derivativne spektrofotometrije koju karakteriše diferencijacija osnovnih apsorpcionih spektara u derivativne spektre odgovarajućeg izvoda i od posebnog značaja je za primene u farmaceutskim analizama. U monografiji je propisan rezolucioni parametar koji predstavlja odnos amplituda (A/B) iz derivativnog spektra drugog izvoda za model sistem toluen/metanol. Rezolucioni parametar izračunat u softvreskom programu Cintral je upoređen sa vrednostima dobijenim u programu OriginPro8 i u prethodnim istraživanjima u programu Spectral Ver. 1.70.

Propisan model rastvor prema EP monografiji je toluen u metanolu 0,02% V/V. Snimljeni osnovni spektri za pet rastvora prevedeni su u derivativne drugog izvoda u programima Cintral i OriginPro8. Nakon toga birani su odgovarajući parametri za korekciju šuma u oba programa (opisni u Cintral-u: najmanji, srednji i najveći; numerički u OriginPro8: 5, 7 i 9).

Direktno izračunat drugi izvod dobijen u Cintral-u davao je srednju vrednost A/B 0,6548 uz najveću vrednost RSD. Najmanjom i srednjom korekcijom šuma povećavala se srednja vrednost A/B uz smanjenje RSD, dok je najvećom korekcijom dobijena srednja vrednost A/B bila značajno smanjena. Direktno izračunat drugi izvod u OriginPro8 davao je nešto nižu srednju vrednost A/B (0,6408) od Cintral-a i značajno manju RSD. Korekcija šuma sa 5 nije imala uticaja na srednju vrednost A/B, samo je smanjivala RSD, dok je 7 smanjivala srednju vrednost A/B i davala sličnu RSD, a vrednosti 9 i veće, za korekciju šuma, nisu bile primenljive.

Programi Cintral i OriginPro8 davali su slične vrednosti za rezolucioni parametar A/B pri čemu je zadovoljen propis EP (A/B nije manji od 0,2). Srednja korekcija šuma (Cintral) bila je uporediva sa 7 korekcijom (OriginPro8) i obe su optimalne za dobijanje digitalnih derivativnih spektara. Dobijeni rezultati značajni su za procenu mogućnosti poređenja kod međulaboratorijskih analiza i transfera metoda za određivanja primenom derivativne spektrofotometrije.

COMPUTER PROGRAMS FOR THE RESOLUTION PARAMETER IN THE EUROPEAN PHARMACOPEIA MONOGRAPH OF DERIVATIVE SPECTROPHOTOMETRY

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European Pharmacopeia (EP) prescribes the monograph for derivative spectrophotometry which is characterized by the differentiation of zero-order absorption spectra into derivative spectra of corresponding order and is therefore of great significance for application in pharmaceutical analysis. In the monograph resolution parameter, which represents the ratio of amplitudes (A/B) of the second order for the model solution toluene/methanol, is prescribed. The resolution parameter calculated in software program Cintral was compared to the values calculated in OriginPro8 and to the ones obtained from the previous research in Spectral Ver. 1.70.

The prescribed model solution according to EP monograph is toluene in methanol 0.02% V/V. Zero-order spectra for the five solutions were recorded and transformed into second order derivative spectra in both Cintral and OriginPro8. Then the smoothing parameters were chosen (descriptive in Cintral: light, medium and heavy; numeric in OriginPro8: 5, 7 and 9).

Second order spectra directly calculated by Cintral gave mean value A/B 0.6548 with the highest RSD. Light and medium smoothing increased the mean value A/B with a decrease of RSD, whereas heavy smoothing significantly decreased mean value A/B. Directly calculated second order in OriginPro8 gave slightly lower mean value A/B (0.6408) than Cintral and significantly lower RSD. Smoothing 5 didn't impact the mean value A/B, but decreased RSD, whereas 7 decreased mean value A/B and gave similar RSD, but smoothings 9 and higher were not applicable.

Programs Cintral and OriginPro8 gave similar values for resolution parameter A/B, which is as prescribed in EP (A/B not less than 0.2). Medium smoothing (Cintral) was comparable to smoothing 7 (OriginPro8) and both are optimal to obtain digital derivative spectra. Obtained results were significant for the assessment of the comparison possibility in inter-laboratory analysis and method transfer for assay analysis using derivative spectrophotometry.

MEDICINSKA HEMIJA INHIBITORA HISTON DEACTILAZE 6 – IN SILICO PRISTUP DIZAJNU LEKOVA

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Hemija postranslacionih modifikacija histona, kao i njihov uticaj na ekspresiju gena predstavlja jedan od najizazovnijih procesa koji se izučava u kancerskoj epigenetici. Kovalentne modifikacije, kao što su acetilovanje i deacetilovanje histona menjaju arhitekturu hromatina i mogu dovesti do različitih ćelijskih odgovora. Od 2006. godine, registrovano je 5 inhibitora histon deacetilaza, efikasnih u terapiji hematoloških maligniteta. Medicinski hemičari posvećuju posebnu pažnju selektivnosti novodizajniranih jedinjenja ka HDAC6 izoformi, koja je jedinstveno lokalizovana u citoplazmi i utiče na dinamske procese citoskeleta.

Metode dizajna lekova bazirane na hemijskim strukturama poznatih HDAC1 iHDAC6 inhibitora (pristup zasnovan na strukturi liganada) unapredile su naše razumevanje strukturnih karakteristika neophodnih za potentnu HDAC6 inhibiciju. Da bi se kvantifikovao odnos između strukture i potentnosti različitih HDAC inhibitora, primenili smo 3D-QSAR studiju (trodimenzionalnu studiju odnosa strukture i aktivnosti) sa već publikovanim strukturama HDAC1 i HDAC6 inhibitora. Hemijska struktura skriptaida i njegovi izračunati tridimenzionalni deskriptori su korišćeni za pretragu novih fragmenata sa sličnim hemijskim osobinama kao inaftalimidno jezgo (dizajn zasnovan na strukturi fragmenta). Predviđene HDAC1 i HDAC6 aktivnosti novodizajniranih jedinjenja su dobijene korišćenjem validiranih 3D-QSAR modela. Za dalje studije, odabrana su ona jedinjenja sa poboljšanom *in silico* selektivnošću usmerenoj ka HDAC6 izoformi. Način vezivanja novih jedinjenja je ispitan studijama molekulskog dokinga i upoređen sa načinom vezivanja poznatih HDAC inhibitora.

Kombinovani pristupi zasnovani na strukturi liganda, fragmenata i strukturi vezivnog mesta su uspešno primenjeni u našoj grupi za pronalaženje novih i hemijskih atraktivnih HDAC6 inhibitora. Uočeno je da su predviđene aktivnosti pomoću 3D-QSAR studije za najbolje rangirana dizajnirana jedinjenja u korelaciji sa rezultatima studije virtuelnog dokinga. Ovakav kombinovani protokol povećava šansu pronalaženja HIT jedinjenja kao selektivnog HDAC6inhibitora, što će u narednim istraživanjima biti potvrđeno *in vitro* studijama novosintetisanih jedinjenja u našoj laboratoriji.

MEDICINAL CHEMISTRY OF HISTONE DEACETYLASE 6 INHIBITORS – IN SILICO DRUG DESIGN APPROACHES

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The chemistry of histone posttranslational modifications and their influence on gene expression present one of the most challenging processes studied in cancer epigenetics. The covalent modifications, such as histonea cetylation and deacetylation alter the chromatin architecture and lead to different cellular responses. Since 2006, there have been five histone deacetylase (HDAC) inhibitors clinically approved for the haematological cancers. Medicinal chemists pay particular attention to the selectivity of newly designed compounds against HDAC6 isoform, as it is uniquely located in the cytoplasm and controls the dynamics of the cytoskeleton.

Drug design methodologies based on the known HDAC1 and HDAC6 inhibitors structures (ligand-based approach) improved our understanding of the structural requirements needed for potent HDAC6 inhibitors. To quantify the relation between the structure and potency in a group of diverse HDAC inhibitors, we performed 3D-QSAR (Quantitative Structure-Activity Relationship) studies with published HDAC1 and HDAC6 inhibitors. The structure of scriptaid and its derived three-dimensional descriptors were used for searching of novel fragments with the similar chemical properties as its naphthalimide core (fragment-based design).

The predicted HDAC1 and HDAC6 activities of newly designed compounds were obtained by validated 3D-QSAR models. We selected only those compounds with improved in silico selectivity toward HDAC6 isoform for further studies. The binding modes of novel compounds were introspected by molecular docking studies and compared with the known HDAC inhibitors binding modes.

The combined ligand-based, fragment-based and structure-based methodologies were successively applied in our group to discover novel and chemically attractive HDAC6 inhibitors. We observed that the predicted potency of the top-ranked designed HDAC6 inhibitors by 3D-QSAR studies are correlated with the virtual docking results. The combined protocol increases the hit rate in the discovery of selective HDAC6 inhibitors, which will be further examined by in vitro studies of synthesized compounds in our laboratory.

UTICAJ OPERATIVNIH USLOVA I METODA EKSTRAKCIJE NA ANTIOKSIDANTIVNU AKTIVNOST SMILJA (HELICHRYSUM ITALICUM (ROTH) G. DON FIL.)

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Smilje (Helichrysum italicum (Roth) G. Don fil.) je rasprostranjeno u mediteranskom području. Smilje, sadrži aktivne principe i sekundarne metabolite kao što su fenolne kiseline, flavonoidi, pironi, triterpenoidi, seskviterpeni, acetofenoni, floroglucinoli i male količine etarskog ulja. Cilj ovog rada bio je da se testira antioksidativna aktivnost ekstrakata smilja dobijenih od različitih polarnih i nepolarnih rastvarača.

Ekstrakcije su izvedene vodom, metanolom, etil acetatom, hloroformom, acetonom, n-heksanom i metil hloridom tokom različitih vremenskih interval (10, 30 i 60 minuta), a stepen usitnjenosti bio je 0,3 i 2 mm. Antioksidativna aktivnost je testirana spektrofotometrijskom metodom koja meri ukupan sadržaj fenola i flavonoida, kao i inhibitorne aktivnosti DPPH (2,2 – difenil – 1 – pikrilhidrazil) radikala.

Rezultati su pokazali da su analizirani ekstrakti imali značajnu antioksidativnu aktivnost. Ukupan sadržaj fenola kreće se od 21,26 do 53,61 mg GAE/g SE. Svi ekstrakti su pokazali dobru antioksidativnu aktivnost sa IC50 vrednošću u rasponu od 21,38 do 71,18 μg/mL. Sa druge strane, svi ekstrakti su pokazali generalno nizak sadržaj ukupnih flavonoida.

Utvrđeno je da povećano vreme ekstrakcije, polariteta rastvarača i stepen usitnjenosti povećavaju kvalitet ekstrakta u smislu sadržaja fenolnih komponenti i antioksidantivnih efekata.

INFLUENCE OF OPERATIONAL CONDITIONS AND EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY OF IMMORTELLE EXTRACTS (HELICHRYSUM ITALICUM (ROTH) G. DON FIL.)

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Immortelle (Helichrysum italicum, (Roth) G. Don fil.) is widely distributed in the Mediterranean region. Immortelle contains important metabolites such as phenolic acids, flavonoids, pyrones, triterpenoids, sesquiterpenes, acetophenones, phloroglucinols, and small amount of essential oil. The aim of this work was to test the antioxidant activity of immortelle extracts obtained from different polar and non-polar solvents.

The extraction was performed with water, methanol, ethyl-acetate, chloroform, acetone, n-hexane and methylene chloride during different periods of time (10, 30 and 60 minutes) and the degree of fragmentation was 0.3 and 2 mm. Antioxidant activity was tested by spectrophotometric method measuring total phenolic and flavonoid content and inhibitory activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The results showed that analyzed extracts had significant antioxidant activity. Total phenol content ranged from 21.26 – 53.61 mg GAE/gDE. All extracts showed good antioxidant activity with an IC50 value in the range from 21.38 to 71.18 μg/ml. On the other hand, all extracts showed generally low content of total flavonoids.

It was found that increased time of extraction, solvent polarity and degree of comminution of the drug increase the quality of the extracts in terms of the content of phenolic components and antioxidant effects.

CHEMOMETRIC EVALUATION OF SOLVENT ELUTION STRENGTH IN REVERSED-PHASE TLC ON RP2 AND RP8 PLATES USING SELECTED DRUGS AND MODEL COMPOUNDS

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To investigate in experimental conditions the eluotropic strength on RP2 and RP8 plates for 7 chromatographic grade solvents (acetone, acetonitrile, dioxane, ethanol, isopropanol, methanol, tetrahydrofuran), as well as for water itself. 35 model compounds (including drugs) were used as a test set in the investigation. The use of a modern chemometric mixture design concept allowed to estimate each solvent effect separately from the other results with a small error (about 0.05 of RM value). Principal component analysis revealed that only one parameter (mean eluotropic strength) explains overall variability in the retention datasets. The bootstrapping was also used to check the distribution and uncertainty of the obtained values. It can be concluded that all solvents have quite similar elution strength for both adsorbents. The obtained values can be used as a reference during optimization of TLC systems.

PHOTOSTABILITY STUDY OF AGOMELATINE BY UHPLC-DAD/ESI-Q-TOF

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According to the European Pharmacopoeia, over 250 active pharmaceutical ingredients are classified as photolabile. This fact involves two major risks. Firstly, exposure of the photolabile pharmaceuticals to the solar or artificial radiation could lead to loss of their pharmacological activity. Secondly, such interaction could lead to formation of transformation products (TPs), possibly more toxic than the parent molecule. The aim of this study was an investigation of the direct photolysis of agomelatine under the ICH-recommended conditions. Agomelatine, a novel atypical antidepressant, is a potent agonist of melatonin receptors, and 5-HT2C receptor antagonist.

Aqueous solution of agomelatine was irradiated (0 – 150 min) under the xenon lamp, equipped with D65 filter. The applied conditions imitated full UV-Vis solar spectrum. Obtained samples were then analyzed with the use of UHPLC-DAD/ESI-Q-TOF coupled system. RP-18 column and gradient elution of mobile phase consisting of water with addition of 0.1% formic acid and acetonitrile were used. ACD/Labs Percepta software was used to calculate acute toxicity to rodents of the identified compounds.

During the experiment over half of the initial agomelatine concentration was decomposed. Reaction kinetics fitted the first-order model, and the degradation half-life was 131.69 min. Six main TPs were found, and their chemical structures were elucidated. All of the identified TPs were found as a agomelatine photooxidation or demethylation products. Additionally, the in silico evaluation of their acute toxicity to rodents was performed. The results, obtained with the use of six models were subsequently submitted to PCA, in order to visualize relationships between the TPs properties.

Results of this study demonstrated that agomelatine is not a photostable molecule, and pharmaceutical formulations containing this compound should be protected from light. Majority of the identified TPs were formed as a consequence of agomelatine hydroxylation or oxidation. PCA facilitated comparison of toxicity of TPs and the parent compound.

COLD LABELED TRASTUZUMAB-P-SCN-BN-DTPA AND TRASTUZUMAB-P-SCN-BN-1B4M-DTPA CONJUGATES– PREPARATION AND SPECTROSCOPIC ANALYSIS

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The importance of immunoconjugates in treatment of various cancers was motivation for us to formulate a stable cold labeled trastuzumab conjugates with two bifunctional chelators (BFCAs) (*p*-SCN-Bn-1B4M-DTPA(2-(4-isothiocyanatobenzyl)-6-methyl-diethylene-triaminepentaacetic acid and *p*-SCN-Bn-DTPA(2-(4-izothiocyanatobenzyl)-diethylenetriaminepentaacetic acid)). The labeling with non-radioactive $LuCl_3$ and YCl_3 is important to determine the possible physicochemical changes in the structure of immunoconjugates after metal binding. ATR-IR (Attenuated total reflectance-infrared) and Raman spectroscopy as powerful and non-destructive techniques are appropriate for verification of possible secondary structure chances of trastuzumab after conjugation and labeling.

Anti-HER2/neu monoclonal antibody trastuzumab was conjugated with p-SCN-Bn-DTPA,*p*-SCN-Bn-1B4M-DTPA in ratio of 1:10and 1:50 and lyophilized to solid state. The freeze dried conjugates were labeled with cold $LuCl_3$ and YCl_3. The retained secondary structure of the antibody was proven by spectroscopic analysis with ATR-IR and Raman spectroscopy and compared with purified trastuzumab from commercial product Herceptin®. The ATR-IR and Raman spectra of four samples have shown the presence of characteristic amide bands and retained native IgG1 structure of the antibody principally composed of β-sheets. Characteristic amide I band at ~1670 cm^{-1} and amide III band (1230-1300 cm^{-1}) were detected in Raman spectra. IR spectra also contain the amide I (1700-1600 cm^{-1}), amide II (1480-1575 cm^{-1}) and amide III bands (1255-1244 cm^{-1}) specific for secondary structure of the proteins.

No significant changes in antibody structure after cold labeling gives us a hope for further radiolabeling of immunoconjugates with $^{177}LuCl_3$ and $^{90}YCl_3$and development of radioimmunotherapeutics and diagnostic products active against HER2 positive breast tumors.

PRIMENA PAMPA TESTA U PROCENI PERMEABILNOSTI I RETENCIJE U KOŽI DERIVATA KORTIENSKE KISELINE METILPREDNIZOLONA KAO POTENCIJALNIH SOFT GLUKOKORTIKOIDA

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Amidi ili estri kortienske kiseline metilprednizolona predstavljaju potencijalne soft glukokortikoide sa manje izraženim neželjenim efektima u odnosu na konvencionalne glukokortikoide. Retencija i permeabilnost su važne osobine soft glukokortikoida za primenu na kožu koje mogu značajno uticati na njihovu aktivnost i pojavu neželjenih efekata. Jedna od in vitro metoda koja se najčešće koristi za procenu ovih osobina je PAMPA (Parallel Artificial Membrane Permeability Assay). Cilj ovog rada je procena permeabilnosti i retencije u koži pet amida (MPMA, MPMAB, MPMAIB, MPMGB i MPPA) i jednog tioestra (MPEMP) kortienske kiseline metilprednizolona primenom PAMPA testa.

Procena permeabilnosti i retencije u koži izvršena je na hidrofobnoj Millipore PVDF PAMPA mikrotitarskoj ploči sa 96 odeljaka. Praćena je difuzija ispitivanih jedinjenja kroz membranu koju čine 70 % silikonsko ulje i 30 % izopropilmiristat. Koncentracije u polaznim rastvorima, donorskim i akceptorskim odeljcima određene su primenom LC-MS/MS metode.

Primenom PAMPA testa određeni su koeficijenti permeabilnosti (logPe) i retencije (R). Vrednosti logPe su u opsegu od -6,81 do -5,09, dok su vrednosti R u opsegu 1,52 - 65,25. Jedinjenje sa najnižom vrednošću logPe je MPMGB, dok su za MPEMP određene najviše vrednosti parametara logPe i R, te se od ovog derivata mogu očekivati najveća permeabilnost i retencija u koži.

Permeabilnost i retencija u koži pet amida (MPMA, MPMAB, MPMAIB, MPMGB i MPPA) i jednog tioestra (MPEMP) kortienske kiseline metilprednizolona procenjeni su primenom PAMPA testa. Najviše vrednosti parametara logPe i R su dobijene za MPEMP, te se ovaj derivat izdvaja kao najpovoljniji za primenu na kožu.

THE USE OF PAMPA FOR SKIN PERMEABILITY AND RETENTION EVALUATION OF METILPREDNISOLONE – DERIVED CORTIENIC ACID DERIVATIVES AS POTENTIAL SOFT GLUCOCORTICOIDS

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Amides or esters of methylprednisolone-derived cortienic acid are potential soft glucocorticoids with fewer side effects in comparison to traditional glucocorticoids. Retention and permeability are important properties of soft glucocorticoids for local skin application which could significantly influence their activity and occurrence of side effects. PAMPA (Parallel Artificial Membrane Permeability Assay) is one of the mostly used in vitro methods for the estimation of these properties. The aim of this study was estimation of skin permeability and retention of five amides (MPMA, MPMAB, MPMAIB, MPMGB and MPPA) and one thioester (MPEMP) of metilprednisolone - derived cortienic acid using PAMPA.

Estimation of skin permeability and retention was performed on hydrophobic Millipore PVDF PAMPA microtiter 96-well plate. Diffusion of tested compounds through membrane, consisting of 70% silicon oil and 30% isopropyl myristate, was tested. Concentrations in starting solutions, as well as in donor and acceptor compartments were determined using LC-MS/MS method.

PAMPA permeability coefficients (logPe) and retention (R) were determined. logPe values ranged from -6.81 to -5.09, whereas R values ranged from 1.52 to 65.25. Derivative with the lowest value of logPe was MPMGB, whereas the highest values of logPe and R were determined for MPEMP. Therefore, highest skin permeability and retention could be expected from MPEMP.

Skin permeability and retention of five amides (MPMA, MPMAB, MPMAIB, MPMGB and MPPA) and one thioester (MPEMP) of metilprednisolone - derived cortienic acid was estimated by use of PAMPA. The highest values of logPe and R were calculated for MPEMP, which could be considered the best candidate for skin application.

KOMPJUTERSKI DIZAJN AGONISTA I ANTOGNISTA 5-HT2A RECEPTORA

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Serotoninski 5-HT_{2A} receptori su uključeni u mnogobrojne fiziološke i patofiziološke procese. Strukturno različiti ligandi (agonisti, antagonisti i inverzni agonisti) dovode do različitih konformacionih promena ovih receptora, izazivajući brojne biološke odgovore.

Da bi se molekul ponašao kao agonista/antagonista potrebno je da poseduje različite funkcionalne grupe i specifične interakcije sa određenim aminokiselinama u vezujućem mestu receptora. Razumevanje i objašnjavanje različitosti u strukturi i vezivanju za receptor, kod agonista i antagonista, može biti od značaja za racionalni dizajn novih lekova.

Za razumevanje strukturnih razlika u farmakoforama, kao i kinetici vezivanja između agonista i antagonista korišćeni su *ligand-based* i *structure-based* pristupi. 3D-QSAR (*3D-quantitative structure activity relationship*) studije su izvođene na grupama od 79 agonista i 90 antagonista. Uporedo su odrađene četiri simulacije molekularne dinamike: serotoninski 5-HT_{2A} receptor u kompleksu sa agonistima (serotonin, lorkaserin) i antagonistima (klozapin, ziprazidon).

Dobijeni statistički i validacioni parametri za modele agonista i antagonista ukazuju na pouzdanost i dobru predviđajuću moć 3D-QSAR modela. Najznačajnije varijable formiranih modela daju nam uvid u najvažnije strukturne razlike između njih. Rezultati MD simulacije otkrivaju najvažnije razlike u konformacionim promenama uzrokovane vezivanjem agoniste/antagoniste, kao i interakcije liganada sa ključnim aminokiselinama, odgovornim za vezivanje. Pomoću trajektorije iz MD simulacije izvučeni su modeli, 3D strukture 5-HT_{2A} receptora u njegovom aktivnom (agonist-vezujućem) i inaktivnom (antagonist-vezujućem) stanju.

Na osnovu ovih *in silico* rezultata moguće je zaključiti da li je jedinjenje agonista ili antagonista. Formirani modeli će dalje biti korišćeni za *ligand-based* i *structure-based* virtualni skrining i racionalni dizajn novih 5-HT_{2A} liganada.

COMBINED LIGAND AND STRUCTURE-BASED APPROACH IN SEARCH OF 5-HT2A RECEPTOR AGONISTS AND ANTAGONISTS

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The serotonin 5-HT_{2A} receptors have shown a wide range of clinical implications since they are involved in various physiological and pathophysiological processes. Structurally diverse ligands (agonists, antagonists, and inverse agonists) can lead to different biological responses, by provoking different conformational changes of these receptors.

To behave like an agonist/antagonist the molecule should have a set of functional groups and specific interactions with certain amino acids in the binding site. Understanding and explaining dissimilarities in agonist/antagonist structure and receptor binding would be beneficial for future rational drug design.

To understand structural differences in pharmacophores as well as the binding kinetics of agonists and antagonists, we have combined ligand-based and structure-based approaches. 3D-quantitative structure-activity relationship (3D-QSAR) studies were performed on a series of 79 agonists and 90 antagonists. Simultaneously, we run four molecular dynamics (MD) simulations: 5-HT_{2A} in complex with agonists (serotonin, lorcaserin), and antagonists (clozapine and ziprasidone).

Obtained statistical and validation parameters for agonists and antagonists model indicated the reliability and good predictive potential of the 3D-QSAR models. The most influential variables of selected models gave us the insight into major structural dissimilarities between them. Results of MD simulation revealed major differences in conformational changes caused by agonist/antagonist binding, as well as ligand interactions with the key amino acids, responsible for them. Additionally, from MD simulation trajectory, we have extracted 3D structure models of 5-HT_{2A} in its active (agonist-bound) and inactive (antagonist-bound) state.

Using these finding we will be able to discriminate whether a compound is agonist or antagonist, *in silico*. Furthermore, models that we have generated will be further used for ligand-based and structure-based virtual screening and rational drug design of novel 5-HT_{2A} ligands.

ISPITIVANJE CITOTOKSIČNE AKTIVNOSTI AMINOKISELINSKIH ESTARA VITAMINA E NA ĆELIJAMA TUMORA DOJKE I PLUĆA

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U velikom broju studija pokazana je antitumorska aktivnost prirodnih izomera vitamina E, a naročito njihovih polusintetskih derivata. Cilj ove studije je bio ispitivanje citotoksične aktivnosti estara α-tokoferola sa aminokiselinama lizinom, prolinom, glutaminom, asparaginom i estara γ-tokotrienola sa lizinom, prolinom i glutaminom na MCF7 i MDA-MB 231 ćelijskim linijama tumora dojke i A549 ćelijskoj liniji tumora pluća. Sve ćelijske linije tretirane su koncentracijama ispitivanih jedinjenja u opsegu 0,62-50 μM u toku 48 sati. Preživljavanje ćelija nakon tretmana ispitivanim jedinjenjima je određeno MTT-testom. Najveći uticaj na preživljavanje malignih ćelija su imali α-tokoferil lizin, α-tokoferil asparagin u formi nitrila i γ-tokotrienil lizin. α-Tokoferil lizin je ispoljio snažnu antitumorsku aktivnost na A549 (IC_{50}=10,6 μM) i MCF7 (IC_{50}=8,6 μM) ćelijama, dok je γ-tokotrienil lizin je jedini od ispitivanih jedinjenja koji je ispoljio aktivnost na sve tri maligne ćelijske linije, sa IC_{50} vrednostima 20,6 μM (MCF7), 28,6 μM (MDA-MB-231) i 19 μM (A549). Asparaginski estar α-tokoferola u formi nitrila je je doveo do snažne inhibicije preživljavanja MDA-MB-231 ćelija (IC_{50}=9,2 μM) koje se odlikuju višestrukom rezistencijom na lekove koji se koriste u terapiji tumora dojke. Ispitivana jedinjenja nisu ispoljila toksičnost ka MRC-5 zdravoj ćelijskoj liniji fetalnih fibroblasta pluća.

Zahvaljujući pokazanoj *in vitro* citotoksičnoj aktivnosti i selektivnosti za maligne ćelije, aminokiselinski estri α-tokoferola i γ-tokotrienola predstavljaju dobre kandidate za buduća *in vivo* ispitivanja.

CYTOTOXIC ACTIVITY OF AMINO ACID ESTERS OF VITAMIN E AGAINST BREAST AND LUNG CANCER CELL LINES

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In recent studies, the antitumor activity of vitamin E derivatives has been demonstrated. The aim of this study was to investigate the cytotoxic activity of α-tocopherol esters with amino acids lysine, proline, glutamine, asparagine and γ-tocotrienol esters with lysine, proline and glutamine on MCF7 and MDA-MB 231 breast cancer cell lines and A549 lung cancer cell line. All cell lines were treated with concentrations of the test compounds in the range of 0.62-50 μM for 48 hours. Cell survival after treatment with the investigated compounds was determined by MTT test.

The greatest influence on the survival of malignant cells was observed with α-tocopheryl lysine, α-tocopheryl asparagine in the form of nitrile and γ-tocotrienyl lysine. α-Tocopheryl lysine exhibited strong cytotoxic activity on A549 (IC_{50} = 10.6 μM) and MCF7 (IC_{50} = 8.6 μM) cells, while γ-tocotrienyl lysine is the only compound that exhibited activity on all three cancer cell lines, with IC_{50} values of 20.6 μM (MCF7), 28.6μM (MDA-MB-231) and 19 μM (A549). The α-tocopheryl asparagine nitrile led to a strong inhibition of the survival of MDA-MB-231 cells (IC_{50} =9.2 μM) that are characterized by multiple resistance to drugs used for treatment of breast cancer. All investigated compounds did not exhibit toxicity to normal MRC-5 cell line of the fetal fibroblasts of the lungs.

Based on the shown *in vitro* cytotoxic activity and selectivity for tumor cells, α-tocopherol and γ-tocotrienol amino acid esters represent promising candidates for future *in vivo* studies.

OPTIMIZACIJA HPLC METODE ZA ODREĐIVANJE METILPARABENA I PROPILPARABENA IZ HIDROGELA

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Parabeni su supstance koje se u farmaciji koriste kao konzervansi. Kako je dokazano njihovo estrogensko delovanje, kontrola njihove upotrebe dobija na značaju. Metilparaben i propilparaben su supstance slične strukture i polarnosti, pa je njihovo određivanje HPLC metodom zahtevno. Prisustvo ekscipijenasa i aktivnih supstanci u formulacijama dodatno otežava određivanje parabena. Cilj ovog rada je bila optimizacija HPLC metode za određivanje metilparabena i propilparabena iz hidrogela.

Rastvori standardnih supstanci i pripremljeni uzorci su podvrgnuti HPLC analizi na Zorbax Eclipse Plus C8 (3,0x150mm, 3,5mm) i Zorbax Eclipse Plus C18 (3,0x150mm, 3,5mm) kolonama. Kao mobilna faza. ispitivana je smeša metanola i fosfatnog pufera (pH=2,5) i smeša metanola i vode u zapreminskim odnosima 50:50, 60:40, 70:30, 80:20, 90:10 kao i 100% metanola. Ispitivan i gradijentni režim rada. Temperatura je održavana na konstantnih 35°C, a detekcija je vršena u UV oblasti na 254 nm. Brzina protoka mobilne faze je ispitivana u rasponu od 0,3 do 0,8 ml/min. Upoređivani su simetrija i širina pikova, rezolucija, retenciona vremena, selektivnost i broj teorijskih platoa za odabrane hromatografske uslove.

Najbolji izgled pikova (simetrija i širina), kao i rezolucija i broj teorijskih platoa, dobijeni su korišćenjem kolone Zorbax Eclipse Plus C18, mobilne faze procentualnog sastava metanola i fosfatnog pufera (pH=2,5) 70 prema 30 i brzine protoka mobilne faze 0,5 ml/min. Gradijentni režim rada se pokazao dobrim za analizu smeše standarda metilparabena i propilparabena, međutim kod realnih uzoraka nisu dobijeni bolji rezultati u odnosu na izokratni režim. Prisustvo fosfatnog pufera u mobilnoj fazi značajno povećava kvalitet hromatograma.

S obzirom na malu razliku u hemijskoj strukturi i polarnosti, HPLC analiza parabena iz farmaceutskih uzoraka predstavlja izazov. U radu smo uspešno optimizovali HPLC metodu za simultano određivanje metilparabena i propilparabena iz uzoraka hidrogela.

OPTIMIZATION OF THE HPLC METHOD FOR THE DETERMINATION OF METHYLPARABEN AND PROPYLPARABEN IN HYDROGELS

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Parabens are used as preservatives. Since their estrogenic activity has been demonstrated, control over their use is gaining in importance. Methylparaben and propylparaben are of similar structure and polarity, so their HPLC determination is difficult. The presence of interfering substances in formulations further complicates the determination. The aim of this paper was optimization of the HPLC conditions for the determination of methylparaben and propylparaben from hydrogels.

Standard substances and prepared samples were subjected to HPLC analysis on Zorbax Eclipse Plus C8 (3.0x150mm, 3.5mm) and Zorbax Eclipse Plus C18 (3.0x150mm, 3.5mm) columns. As a mobile phase mixtures of methanol and phosphate buffer (pH=2.5) and mixtures of methanol and water (50:50, 60:40, 70:30, 80:20, 90:10, 100:0, respectively) were investigated. Gradient elution was also examined. The temperature was kept at 35°C and the detection was carried out at 254 nm. The flow rate was tested in the range of 0.3 to 0.8 ml/min. The symmetry and width of the peaks, resolution, retention time, selectivity and the number of theoretical plates were compared.

The best peak parameters, resolution and the number of theoretical plates were obtained using Zorbax Eclipse Plus C18 column, a mixture of methanol and phosphate buffer (pH=2.5) in 70:30 volume ratio and the flow rate of 0.5 ml/min. The gradient elution was efficient for the analysis of standards, however, it was not adequate for the analysis of the samples. The phosphate buffer significantly increased the quality of the chromatograms.

Considering the small difference in chemical structure and polarity, HPLC analysis of the parabens from pharmaceutical samples is a challenge. In this paper, HPLC conditions for simultaneous determination of methylparaben and propylparaben from hydrogels were successfully optimized.

RAZVOJ HPLC METODE ZA ODREĐIVANJE SADRŽAJA NEORGANSKIH NITRATA U IZOSORBID-MONONITRAT TABLETAMA PRIMENOM AQBD PRISTUPA

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Na kvalitet farmaceutskih supstanci izuzetno značajan uticaj može imati količina prisutnih neorganskih nečistoća naročito što neke od njih (npr. nitrati i nitriti) mogu biti veoma reaktivne. Cilj ovog rada bio je razvoj pouzdane, robusne, selektivne i osetljive HPLC metode za određivanje sadržaja neorganskih nitrata kao nečistoća u tabletama izosorbid-mononitrata primenom AQbD pristupa.

Uticaj CMPs (sadržaj metanola u mobilnoj fazi, koncentracija CH_3COONH_4 u vodenoj fazi, temperatura kolone) na odabrane CMAs (retencioni faktor neorganskog nitrata, visina pika i broj teoretskih platoa) ispitan je primenom Boks-Benken dizajna. Za definisanje *Design Space*-a (DS), unutar koga je verovatnoća dostizanja željenih performansi metode $\pi \geq 95\%$, primenjene su Monte Karlo simulacije. Zbog prisustva neorganskih nitrata u placebu, što je potvrđeno limit testom za identifikaciju nitrata u vodi (EP 9) i HPLC analizom sa CAD detekcijom, definisana je radna koncentracija rastvora tako da nivo nitrata u placebu bude ispod limita detekcije. Ispitivani rastvori su filtrirani kroz hidrofilne PTFE špric filtre, a eksperimenti su izvedeni na koloni *Nucleodur® PolarTec* 150 mm×4,6 mm, 5 µm, uz UV detekciju (λ=220 nm).

Veza između ispitivanih CMPs i CMAs opisana je kvadratnim matematičkim modelom, čija je validnost potvrđena statističkim testovima. Primenom Monte Karlo simulacija izvršeno je propagiranje greške koja potiče od izračunatih koeficijenata modela na odabrane odgovore, dobijena je njihova prediktivna distribucija i definisan DS. Radna tačka je odabrana iz centralnog dela DS: mobilna faza metanol-11 mM CH_3COONH_4 (32:68, V/V), pH vodene faze 4,9; T=38°C. Robusnost kvantitativnih performansi metode procenjena je Plaket-Burmanovim dizajnom, a pogodnost metode za definisanu namenu potvrđena je sprovođenjem validacije.

Kako su svi dobijeni rezultati u skladu sa definisanim zahtevima, potvrđeno je da je razvijena metoda pouzdana i pogodna za rutinsku kontrolu neorganskih nitrata u izosorbid-mononitrat tabletama.

DEVELOPMENT OF HPLC METHOD FOR THE DETERMINATION OF INORGANIC NITRATE IMPURITY IN ISOSORBIDE-MONONITRATE TABLETS BY AQBD APPROACH

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The quality of pharmaceutical substances can significantly be affected by present inorganic impurities, especially if these are highly reactive (e.g. nitrates and nitrites). The aim of present study was development of reliable, robust, selective and sensitive analytical method for inorganic nitrate impurity assay in isosorbide-mononitrate tablets with the aid of AQbD approach.

The influence of CMPs (methanol content in mobile phase, concentration of CH_3COONH_4 in water phase, column temperature) on the set of selected CMAs (retention factor of inorganic nitrate impurity, peak height, number of theoretical plate) was studied by Box-Behnken design. Design space (DS) was defined by Monte Carlo simulations as a zone where CMAs fulfill predefined acceptance limits with a high level of probability $\pi \geq 95\%$. Due to the inorganic nitrates presence in placebo, confirmed by the nitrate limit test (EP 9) and HPLC analysis with CAD detection, the working solutions concentration was adjusted so that the placebo nitrate level is below detection limit. Test solutions were filtered through hydrophilic PTFE syringe filters, and the experiments were performed on *Nucleodur® PolarTec* column 150 mm×4.6 mm, 5 μm, with UV detection (λ=220 nm).

Relationships between CMPs and CMAs were described by quadratic mathematical models, their validity confirmed statistically. Monte Carlo simulations were used to propagate the error originating from the calculated model coefficients to selected responses in order to obtain their predictive distribution and to define DS. Working point was selected from the central part of DS: mobile phase methanol–11 mM CH_3COONH_4 (32:68, V/V), aqueous phase pH=4.9; T=38°C. Robustness of quantitative method performance was assessed by Plackett-Burman design, while its suitability for intended purpose was confirmed by validation.

Since the obtained results comply with defined requirements, reliability and suitability of developed method for routine inorganic nitrate control in isosorbide-mononitrate tablets has been confirmed.

MICELIZACIJA BINARNIH SMEŠA ŽUČNIH SOLI NATRIJUM-DEOKSIHOLATA I NATRIJUM-HIODEOKSIHOLATA

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Žučne soli su prirodni surfaktanti, koji imaju fiziološku ulogu u emulzifikaciji masti u digestivnom lumenu. Ispoljavaju promotornu aktivnost u transportu različitih lekova kroz biološke membrane, čime povećavaju njihovu bioraspoloživost. U ovom radu je ispitana micelizacija binarnih smeša žučnih soli natrijum-deoksiholata i natrijum-hiodeoksiholata, sa aspekta termodinamičke stabilnosti njihovih mešovitih micela.

Napravljeni su rastvori smeša natrijum-deoksiholata i natrijum-hiodeoksiholata u različitim molskim odnosima (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), pri čemu je za izradu svih rastvora korišćena dejonizovana voda zasićena pirenom, a jonska jačina rastvora podešena je na 300 mM natrijum-hloridom. Kritične micelarne koncentracije rastvora surfaktanata određivane su spektrofluorimetrijski, praćenjem zavisnosti intenziteta prve i treće emisione trake pirena, u odnosu na ukupnu koncentraciju surfaktanata, na temperaturama od 10°C, 25°C, 35°C i 50°C.

Eksperimentalno određene vrednosti kritičnih micelarnih koncentracija binarnih mešovitih micela natrijum-deoksiholata i natrijum-hiodeoksiholata imaju niže vrednosti od idealnih kritičnih micelarnih koncentracija, izračunatih po Clint-u, što ukazuje na postojanje interakcija između različitih komponenti mešovitih micela. Koeficijent interakcije i dodatna Gibbs-ova energija, određeni na osnovu teorije regularnih smeša, imaju negativne vrednosti na svim ispitivanim temperaturama, što ukazuje na sinergističke interakcije između različitih komponenti u micelama, odnosno realne mešovite micele su termodinamički stabilnije od idealnih mešovitih micela.

Ispitivane žučne soli u binarnoj smeši stupaju u međusobne inerakcije koje favorizuju nastanak micela u vodenom rastvoru, što za posledicu ima sniženje vrednosti kritičnih micelarnih koncentracija i termodinamičku stabilizaciju mešovitih micela. S povećanjem temperature vrednosti kritičnih micelarnih koncentracija rastu, što se objašnjava povećanom pokretljivošću gradivnih jedinica micela na višim temperaturama.

MICELLIZATION OF THE BINARY MIXTURES OF BILIARY SALTS SODIUM-DEOXYCHOLATE AND SODIUM-HYODEOXYCHOLATE

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Bile salts are natural surfactants and have a physiological role in emulsification of fats in digestive lumen. They promote transport of various drugs through biological membranes, which results in the enhanced drug bioavailability. In this work, the micellization of the binary mixture of sodium-deoxycholate and sodium-hyodeoxycholate is examined, from the aspect of thermodynamic stability of their mixed micelles.

The solutions of the binary mixtures of sodium-deoxycholate and sodium-hyodeoxycholate in different molar rations (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) are prepared with deionized water, saturated with pyrene, and the ionic strength of the solutions is adjusted with 300 mM sodium-chloride. Critical micellar concentrations of these mixtures are determined spectrofluorometrically, by monitoring the intensities of the first and the third emission bands in relation to the total concentration of surfactants, at different temperatures: 10°C, 25°C, 35°C and 50°C.

Experimentally determined critical micellar concentrations of the binary mixed micelles of sodium-deoxycholate and sodium-hyodeoxycholate are lower than the ideal critical micellar concentrations, calculated according to Clint, which indicates the existence of interactions between the different components of the mixed micelles. The coefficient of interaction and the excess Gibbs energy, determined using the regular solution theory, have negative values at all temperatures, indicating synergistic interactions between the different components in micelles, that is, the real mixed micelles are thermodynamically more stable than the ideal mixed micelles.

The examined bile salts in aqueous solution are involved in the interactions that favour the formation of micelles, which results in the decrease of the values of critical micellar concentrations and the thermodynamic stabilization of the mixed micelles. By increasing the temperature, the values of critical micellar concentrations increase, which is explained by greater mobility of the micelle's building units at higher temperatures.

ISPITIVANJE USLOVA ZA ENANTIOSEPARACIJU MOKSIFLOKSACINA I POTENCIJALNE HIRALNE NEČISTOĆE (R,R)-IZOMERA

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Moksifloksacin-hidrohlorid u strukturi poseduje dva hiralna centra, i od moguća četiri izomerna oblika farmakološku aktivnost poseduje jedino (*S,S*)-izomer, dok se ostali izomeri smatraju potencijalnim nečistoćama. Cilj ovog rada bio je ispitivanje uslova za razdvajanje enantiomernog para, moksifloksacina i njegovog (*R,R*)-izomera, na ciklodekstrinskom tipu hiralne stacionarne faze.

Hromatografska analiza je izvedena na *Dionex UltiMate* 3000 sistemu, upotrebom *Chiral* CD-Ph kolone (4,6x250mm, 5μm). Mobilna faza je pripremana mešanjem acetonitrila i vodenog rastvora pufera trietilamonijum-acetata. Protok mobilne faze bio je 1 mL min^{-1}, talasna dužina detekcije 293 nm, volumen injektovanja 10 μL. Skrining uslova za razdvajanje enantiomera moksifloksacina i (*R,R*)-izomera sproveden je variranjem sadržaja acetonitrila u mobilnoj fazi (32,5-47,5% V/V), sadržaja trietilamina (0,5-1,5% V/V), pH vrednosti vodene faze (3,5-4,5 podešena glacijalnom sirćetnom kiselinom) i temperature kolone (20-30°C) prema eksperimentalnom planu formiranom upotrebom 2^4 Punog faktorskog dizajna. Kao odgovori sistema praćeni su faktor rezolucije, faktor enantioselektivnosti i asimetrija pikova. U programu *Design-Expert*® 7.0.0 izvršena je grafička obrada dobijenih rezultata korišćenjem *half-normal probability* grafikona i *Pareto* dijagrama.

Kolona sa hiralnim selektorom fenilkarbamat β-ciklodekstrinom, na kojoj je sprovedeno ispitivanje, pokazala je stereoselektivno molekulsko prepoznavanje enantiomernog para. Tokom eksperimentalnog rada zapaženo je da povećanje sadržaja trietilamina utiče na parametre razdvajanja, dovodeći do smanjenja faktora rezolucije i enantioselektivnosti. Primećeno je da promena sadržaja acetonitrila dovodi do smanjenja faktora rezolucije, dok istovremeno dolazi do poboljšanja asimetrije pika (*R,R*)-izomera i povećanja faktora enantioselektivnosti. Grafičkom obradom rezultata potvrđen je značajan uticaj svih ispitivanih faktora na posmatrane odgovore sistema. Daljim ispitivanjem dodatno je uočeno da trietilamin i pH vrednost vodene faze imaju različit uticaj na asimetriju pikova. Pored toga, na asimetriju (*R,R*)-izomera i faktor rezolucije značajan uticaj ima i interakcija ova dva faktora.

Tokom skrininga zapaženi značajni uticaji ispitivanih faktora i njihovih interakcija na odabrane odgovore sistema, ukazuju na potrebu detaljnijih ispitivanja posmatranih faktora u fazi optimizacije.

INVESTIGATION OF CONDITIONS FOR ENANTIOSEPARATION OF MOXIFLOXACIN AND ITS POTENTIAL CHIRAL IMPURITY (R,R)-ISOMER

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Moxifloxacin hydrochloride contains two chiral centres which pharmacologically active form is (*S,S*)-isomer, while other isomers are considered as potential impurities. The aim of this study was to investigate separation conditions of moxifloxacin and its enantiomer (*R,R*)-isomer, using cyclodextrin CSP.

Chromatographic analysis were performed on *Dionex UltiMate* 3000 system using *Chiral* CD-Ph column (4.6x250mm, 5μm). The mobile phase was prepared by mixing acetonitrile and triethylammonium acetate buffer solution. The mobile phase flow rate was 1 mL min^{-1}, detection wavelength 293 nm, injection volume 10 μL. Screening approach for separation of moxifloxacin and its (*R,R*)-isomer, were monitored by variations of acetonitrile content in mobile phase (32.5-47.5% v/v), content of triethylamine (0.5-1.5% v/v), water phase pH value (3.5-4.5 set with glacial acetic acid) and column temperature (20-30°C) following the experimental plan according to 2^4 FFD. Monitored system responses were: resolution factor, enantioselectivity factor and assimetry of peaks. Graphical interpretation (half-normal probability plots, Pareto charts) of results was done using *Design-Expert*® 7.0.0 software.

Used column with phenylcarbamated β-cyclodextrin as chiral selector, showed enantiorecognition of enantiomers. During experimental work it was observed that the increase of triethylamine content has effect on separation parameters, leading to decrease of resolution and enantioselectivity factor. It was noticed that change of acetonitrile content leads to decrease of resolution factor, while simultaneously there is an improvement in asymmetry of (*R,R*)-isomer and increase in enantioselectivity. Graphical interpretation of results, confirmed significant effect of all investigated factors on system responses. Further investigation showed that triethylamine and water phase pH value have different influence on peaks assimetry. Besides that, the interaction of this two factors have significant impact on assimetry of (*R,R*)-isomer and resolution.

During the screening, significant effects of the investigated factors and their interactions on monitored system responses, indicates the need for detailed examination of this factors through optimization phase.

ISPITIVANJE RETENCIONIH MEHANIZAMA AMLODIPIN BESILATA, BISOPROLOL FUMARATA I NJIHOVIH NEČISTOĆA UPOTREBOM TRI RAZLIČITE HILIC KOLONE

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Mehanizmi razdvajanja u HILIC sistemu baziraju se na specifičnim i nespecifičnim mehanizmima, te ne postoji kvantitativan model koji može sigurno predvidjeti ponašanje nekog jedinjenja u HILIC metodi. Vremenom je zaključeno da brojne interakcije mogu imati važnu ulogu prilikom razdvajanja ispitivanih jedinjenja: raspodjela/particija, adsorpcija i proces jonske izmjene. HILIC sistem je vrlo specifičan, razdvajanje analita uvijek se vrši sa više različitih mehanizama koji su istovremeno prisutni.Cilj ovog rada bio je da se odrede mehanizmi razdvajanja ispitivanih jedinjenja korišćenjem particionih i adsorpcionih teorijskih modela.

Kao hromatografski sistem korišćen je Agilent 1200 sa DAD detektorom. Razdvajanje je izvršeno na tri različite HILIC kolone: silika, amino i diolna, s mobilnom fazom sastava ACN–50 mmol L^{-1} vodeni rastvor pufera (pH 4,0 podešena sa koncentrovanom sirćetnom kiselinom), u odnosu koji je varirao od 84:16 do 96:4 (V/V). UV detekcija vršena je na 230 nm, zapremina injekotovanja bila je 20 μL, brzina protoka mobilne faze 1 mL min^{-1} i temperatura kolone 30◦C.

Izračunati su regresioni koeficijenti particionih i adsorpcionih modela i odgovarajući koeficijenti determinacije (R^2). Veće R^2 vrijednosti imali su particioni modeli za sva ispitivana jedinjenja na amino koloni – bilo je neophodno obezbjediti veću koncentraciju soli pufera (CH_3COO^-), te na taj način suzbiti elektrostatsko odbijanje ispitivanjih jedinjenja. Veće R^2 vrijednosti imali su adsorpcioni modeli za sva ispitivana jedinjenja na silika i diolnoj koloni. Na sve tri kolone zaključeno je da sa povećavanjem sadržaja acetonitrila u mobilnoj fazi produžava se retenciono vrijeme svih ispitivanih jedinjenja, usljed smanjenja njene eluacione moći. Zaključeno je da se na amino koloni retencioni mehanizmi svih ispitivanih jedinjenja provode postupkom razdvajanja/particije, dok na diolnoj i silika koloni mehanizmom adsorpcije.

INVESTIGATION OF THE RETENTION MECHANISMS OF AMLODIPINE BESYLATE, BISOPROLOL FUMARATE AND THEIR IMPURITIES ON THREE DIFFERENT HILIC COLUMNS

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Mechanisms of separation in HILIC are based on specific and non-specific mechanisms. There is no quantitative model that can safely predict the behavior of a compound in HILIC method. It was concluded that many interactions can have a very important role during separation: partition, adsorption and process of ion exchange. HILIC system is specific, separation is simultaneously done by multiple mechanisms.The aim of this artical was to determine retention mechanisms of tested compounds using partitioning and adsorption retention theoretical models.

It's used chromatographic system Agilent 1200–DAD detector. Separations were performed under HILIC mode on three different columns: silica, amino and diol. Mobile Phase: ACN–50 mmol L^{-1} aqueous buffer solution mixture (pH 4.0 was adjusted with glacial acetic acid) in ratio from 84:16 to 96:4 (V/V). UV detection was performed at 230 nm, injection volume 20 μL, flow rate 1 mL min^{-1}, column temperature 30°C.

Calculated the regression coefficients of partitioning and adsorption retention models and corresponding coefficient of determination (R^2). Higher values of R^2 had partitioning models for all compounds at amino column – it was necessary to provide a higher concentration of buffer salt ($CH3COO^-$) and to prevent the process of electrostatic repulsion with tested compounds. The higher value R^2 had adsorption models for all compounds at silica and diol column. On all three columns, it was shown that with increasing acetonitrile content in mobile phase, the retention time of tested compounds extends, due to decline of its eluting power.It was concluded that on amino column, retention mechanisms of all tested compounds were carried out by partitioning process, while on diol and silica column, retention mechanisms are taking place by adsorption process, respectively.

QbD PRISTUP U RAZVOJU I VALIDACIJI HILIC METODE ZA ANALIZU AMITRIPTILIN HIDROHLORIDA I NJEGOVIH NEČISTOĆA

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Quality by Design (QbD) koncept je naučno zasnovan pristup koji omogućava definisanje *prostora dizajna* (DS) kao robusnog regiona u toku razvoja analitičke metode. Cilj ovog rada bio je da se primjenom QbD koncepta razvije HILIC metoda za kontrolu kvaliteta tableta sa amitriptilinom.

Program MODDE® Pro 12.0.V. (Umetrics Sartorius-Stedim, Švedska) korišćen je za kreiranje plana eksperimenta, statističku obradu rezultata, te za kreiranje *prostora znanja* i *prostora dizajna*. Kao tečni hromatogram korišćen je Agilent 1200 sa DAD detektorom, kolona ZORBAX-NH2 (250 mm x 4,6 mm, 5 μm veličina čestica), mobilnafaza: acetonitril–60 mM rastvor amonijum acetata (pH 4,5 podešena koncentrovanom sirćetnom kiselinom) u odnosu 92,5:7,5 (V/V). Temperatura kolone 30 °C, protok mobilne faze 1 mL min^{-1}, talasna dužina određivanja 254 nm.

Kroz nekoliko dobro definisanih koraka, QbD koncept korišćen je za razvoj prve HILIC metode za analizu amitriptilina i njegovih nečistoća. S ciljem da se postigne dobro razdvajanje spitivanih jedinjenja sa što kraćim vremenom trajanja analize, za proces optimizacije korišćen je *Box-Behnken dizajn*, a kao kritični parametri korišćeni su: sadržaj acetonitrila u mobilnoj fazi, pH vrijednost vodenog rastvora pufera i koncentracija amonijum-acetata. Procjena robusnosti metode izvršena je upotrebom *frakcionog faktorskog dizajna* (FFD 2^{4-1}), a zatim su ispitani i ostali parametri validacije metode: selektivnost, linearnost, tačnost, preciznost i određeni su limit detekcije (LOD) i limit kvantifikacije (LOQ).

Upotrebom QbD koncepta razvijena je i validirana metoda za određivanje amitriptilin hidrohlorida i njegovih nečistoća A, B, C i F u tabletama. Ovakav pristup u razvoju metode omogućio je ugrađivanje kvaliteta u toku procesa razvoja metode, te je razvijena robusna metoda. Metoda je validirana i primjenjena za analizu amitriptilina i njegovih nečistoća u tabletama.

QbD ORIENTED DEVELOPMENT AND VALIDATION OF THE HILIC METHOD FOR THE ANALYSIS OF AMITRIPTYLINE HYDROCHLORIDE AND ITS IMPURITIES

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A Quality by Design (QbD) approach is a scientific based concept which can be used to define Design Space (DS) for analytical methods. The aim of this paper was to develop the HILIC method for the quality control of amitriptyline tablets using a QbD concept.

MODDE® Pro 12.0.V. (Umetrics Sartorius-Stedim, Sweden) was used to create an experiment plan, statistical analysis of results and constructing a knowledge space and design space. Liquid chromatograph Agilent 1200 series with DAD detector, ZORBAX-NH2 column (250 mm x 4.6 mm, 5 μm particle size), mobile phase: acetonitrile-60 mM aqueous ammonium acetate solution, pH 4.5 adjusted with concentrated acetic acid (92.5:7.5 V/V). Column temperature: 30°C, mobile phase flow 1 mL min^{-1}, wavelength of detection 254 nm.

Through several well defined steps, a QbD concept was implemented for development of the first HILIC method for aminotriptiline and its impurities analysis. To reach the desired chromatographic resolution with a limited number of experiments in a minimum amount of time, *Box-Behnken design* was used to simultaneously optimize for important chromatographic parameters: the acetonitrile content in the mobile phase, pH of the aqueous phase and the concentration of ammonium acetate in aqueous phase. Using the *fractional factorial design* (FFD 2^{4-1}), the robustness of the method was estimated, and then other parameters of the validation of the method were determined: selectivity, linearity, accuracy, precision, LOD and LOQ.

Using the QbD concept, a HILIC method for the analysis of amitriptyline and its impurities A, B, C and F in tablets was developed and validated. This approach in the development of the method has enabled the embedding of quality during the development of the method and a robust method has been developed. The method was validated and applied to the analysis of amitriptyline hydrochloride and its impurities in tablets.

ODREĐIVANJE TELMISARTANA I NJEGOVIH NEČISTOĆA U TABLETAMA RP-HPLC METODOM S GRADIJENTNIM ELUIRANJEM

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Telmisartan je nepeptidni antagonista angiotenzin II receptora koji se koristi u terapiji hipertenzije. Cilj ovog rada je razvoj i validacija nove RP-HPLC metode za kvalitativnu i kvantitativnu analizu telmisartana i njegovih nečistoća A i C u tabletama.

Optimalno razdvajanje između ispitivanih supstanci sa sličnim pKa vrednostima postignuto je pomoću Chromolith C18 kolone (100 mm x 4,6 mm, 5 μm veličina čestica), na temperaturi od 25°C i uz gradijentno eluiranje. Kao eluent A upotrebljen je 0,1% vodeni rastvor trifluorosirćetne kiseline, dok je kao eluent B korišćen acetonitril. Volumen injektovanja bio je 20 μL, a UV detekcija vršena je na 230 nm. Metoda je validirana i ispitani su sledeći parametri validacije: selektivnost, linearnost, preciznost, tačnost, limit detekcije (LOD) i limit kvantifikacije (LOQ). Predložena RP-HPLC metoda je primenjena za kvalitativnu i kvantitativnu analizu telmisartana i nečistoća A i C u film tabletama.

Na retencionim vremenima koja odgovaraju telmisartanu i njegovim nečistoćama nisu uočene komponente koje interferiraju. Lineranost metode je određena u opsegu koncentracija 1-15 μg mL^{-1} za telmisartan (r = 0,9999), 0,12-1,50 μgmL^{-1} za nečistoću A (r = 0,9998), odnosno 0,11-1,50 μg mL^{-1}za nečistoću C (r = 0,9997). Preciznost metode dokazana je na osnovu izračunatih RSD vrednosti za telmisartan (0,99%), nečistoću A (1,22%) i nečistoću C (1,37%). Tačnost metode potvrđena je *Recovery* vrednostima za telmisartan (99,8%), nečistoću A (99,3%) i nečistoću C (99,4%). Osetljivost metode pokazana je definisanjem LOD i LOQ vrednosti za nečistoće. Rezultati ispitivanja u film tabletama odgovaraju zahtevima specifikacije, sadržaj telmisartana je 99,35%, sadržaj nečistoće A je manji od LOQ, a nečistoće C manji od LOD vrednosti.

Predložena RP-HPLC metoda pod datim eksperimentalnim uslovima predstavlja brz, precizan, tačan i selektivan postupak za istovremenu kvalitativnu i kvantitativnu analizu telmisartana i nečistoća u tabletama.

DETERMINATION OF TELMISARTAN AND ITS IMPURITES IN TABLETS BY RP-HPLC METHOD WITH GRADIENT ELUTION

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Telmisartan is a non-peptide angiotensin II receptor antagonist with antihypertensive property. The aim of this work was development and validation of a new RP-HPLC method for qualitative and quantitative analysis of telmisartan and its impurities A and C in tablets.

The optimal separation among investigated substances with similar pKa values was achieved using Chromolith C18 column (100 mm x 4.6 mm, 5 μm particle size), at temperature of 25°C and gradient elution. As the eluent A, 0.1% aqueous solution of trifluoroacetic acid was used, whereas acetonitrile was used as the eluent B. The injection volume was 20 μl and UV detection was performed at 230 nm. The method was validated in terms of selectivity, linearity, precision, accuracy, limit of detection and limit of quantification. The proposed RP-HPLC method was applied in qualitative and quantitative analysis of telmisartan and impurities in film coating tablets.

At retention times corresponding to telmisartan and its impurities no interferences were observed. Linearity was proved in concentration ranges 1-15 μg $mL^{-1}$for telmisartan (r = 0.9999), 0.12-1.50 μg mL^{-1} for impurity A (r =9998) and 0.11-1.50 μg mL^{-1} for impurity C (r = 0.9997). The precision of the method was proved according to calculated RSD values for telmisartan (0.99%), impurity A (1.22%) and impurity C (1.37%). The accuracy of the method was confirmed by Recovery values obtained for telmisartan (99.8%), impurity A (99.3%) and impurity C (99.4%). The sensitivity of the method was shown by defining LOD and LOQ values of impurities. The results of analysis of film coating tablets meet the requirements of the specification. The content of telmisartan was 99.35%, imputity A was below LOQ, while the content of impurity C was below LOD value.

The established RP-HPLC method was found to be rapid, precise, accurate and selective for qualitative and quantitative analysis of telmisartan and its impurities in film coating tablets.

KARAKTERIZACIJA BIOMOLEKULA SA ANTIBIOTIČKIM DEJSTVOM IZ ENDOFITA PHOMOPSIS SPECIES

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Evolucionarno, biljke su razvile određene odbrambene sposobnosti kao što je proizvodnja specifičnih sekundarnih metabolita kako bi se zaštitile od insekata i/ili životinja koje se hrane njima. Povrh toga, tkiva biljaka sadrže mnoge vrste mikroorganizama koje se nazivaju endofiti. Endofiti su specifični za svakog domaćina i karakteristični su za klimatske uslove i geografsku oblast u kojoj se biljka domaćin razvija. Postoje brojni izveštaji o različitim biološkim aktivnostima endofitnih gljiva. Odrediti antibiotički potencijal endofita Phomopsis species koja raste na četinarima u Sloveniji.

Kultura endofita je zasejana na agar pločama i ostavljena na sobnoj temperaturi za proces rasta, nakon čega je metanolni ekstrakt endofita (50%, v/v) podvrgnut mikrodilucijskom testu. Zapažena je značajna inhibicija rasta Gram (-) bakterije Escherichia Coli. Ekstrakt je dodatno analiziran pomoću semipreparativne HPLC metode s ciljem otkrivanja, razdvajanja i sakupljanja frakcija vrhova pripisanih specifičnim biomolekulama. Dodatno je određena njihova hemijska struktura uz pomoć masne spektroskopije visoke definicije (LC-QTOF analiza) i spektroskopije nuklearne magnetne rezonance.

Dva glavna vrha koja se eluiraju na retencionom vremenu od 3,34 i 3,89 minuta, pokazale su molekularne jone u režimu pozitivne elektrospejne jonizacije sa m/z vrednostima od 318 i 335, respektivno. Njihov identitet je konačno opisan kao (Z)-(Z) -2-acetoksiprop-1-en-1-il-3-(3-((E)-3,4-dihidroksipent-1-en-1-il)-oksiran-2-il)-akrilat i (Z)-(Z)-2-acetoksiprop-1-en-1-il-3-(3-((E)-4-hidroksi-3-oksipent-1-en-l-il)oksiran-2-il)-akrilat). Predložena jedinjenja kao potencijalni terapeutski agensi se mogu koristiti kao vodeći molekuli za otkrivanje novih antibiotika. Takođe, kombinovane analitičke tehnike korišćene u ovom istraživanju mogle bi se koristiti kao analitička platforma za karakterizaciju i određivanje novih molekulskih entiteta u ekstraktima endofita.

CHARACTERIZATION OF BIOMOLECULES WITH ANTIBIOTIC ACTIVITY FROM ENDOPHYTE PHOMOPSIS SPECIES

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Evolutionally, plants have developed certain defending capabilities such as producing of specific secondary metabolites in order to repell insects and/or animals that are feeding on them. Moreover, tissues of plants contain many types of microorganisms which are reffered to as endophytes. Endophytes are specific for each host and are characteristic for the conditions and geographical area in which the host develops. At the same time, there are numerous reports on different biological activities of endophytic funghi. Antibiotic potential of endophyte Phomopsis species growing on conifers in Slovenia has been tested.

Crude matherial was sawed on agar plates and left on room temperature for growing process upon which its methanol extract (50%, v/v) was subjected to microdilution assay. Considerable inhibition of the growth of Gram(-) bacteria Escherichia Coli was noticed. Prepared sample was further investigated using semipreparative HPLC in order to detect, separate and collect fractions of peaks attributed to specific biomolecules and consequently elucidate their chemical structure with the assistance of high definition mass spectroscopy (LC-QTOF analysis) and nuclear magnetic resonance spectroscopy.

Two major peaks which eluted at retention times at 3.34 min and 3.89 min showed molecular ions in positive electrospay ionisation mode with m/z values 318 and 335, respectively. Their identity was finaly characterized as (Z)-(Z)-2-acetoxyprop-1-en-1-yl-3-(3-((E)-3,4-dihydroxypent-1-en-1-yl)oxiran-2-yl)-acrilate and (Z)-(Z)-2-acetoxyprop-1-en-1-yl-3-(3-((E)-4-hydroxy-3-oxopent-1-en-1-yl)oxiran-2-yl)-acrylate).

Beside proposition of presented compounds as potential therapeutic agents, these structures could even be used as leading molecules for novel antibiotics discovery. Moreover, combined analytical techninques used in this research could be employed as analytical plathform for characterization and determination of molecular entities in endophyte extracts.

A DESIRABILITY BASED MULTIOBJECTIVE APPROACH FOR MODELING, PREDICTING AND OPTIMIZATION OF EXPERIMENTAL CONDITION FOR FORCED DEGRADATION OF ROSUVASTATIN

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Forced degradation studies provide data to support identification of possible degradants, degradation path ways, intrinsic stability and validation of stability indicating methods. Generally, a trial and error approach (adopted to select the strength, temperature and time of exposure to achieve desired degradation) involves considerable cost, time consumption and scientific expertise with high incidence of random results. The aim of this research was to use desirability function as a systematic approach for multiobjective optimization of the experimental condition for conducting forced degradation on rosuvastatin as model drug.

The degradation behavior of rosuvastatin was estimated based on 2^3 full factorial designs, where strength of HCL/NaOH/H_2O_2, temperature and time of exposure were considered as independent and % total impurities (determined by validated RP-HPLC method) as dependent variable. The experimental results were evaluated using the proposed predictive model which included desirability function to find set of coordinates with desirability value close to 1.

Statistical evaluation, using ANOVA, showed that proposed model fit the data well and have high predictive power for new observations. Selections of optimal conditions were performed using desirability function, where targeted degradation was set in the range 5-20% and % of major unknown impurity to 0.1 %. The predicted conditions by the model with high desirability value were 0,1M HCl/25^{o}C/45 minutes and 0.1 M NaOH/25^{o}C /45 minutes, respectably. These conditions were selected in the verification study, and the obtained results demonstrated good agreement between the experimental data and predicted values.

The proposed model presents an efficient and easily accomplishable approach for searching optimum degradation conditions which can replace the trial and error approach, providing information rich presentation of results with less laboratory work.

DOE APPROACH FOR OPTIMIZATION OF A GENERIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF UNDECLARED COMMON COUGH AND COLD INGREDIENTS IN NATURAL PRODUCTS

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In a need of addressing the current worrying problem of falsified medicines, an all-encompassing method that can be used for screening and quantifying components of dubious samples of medicines and detecting undeclared active substances in natural products can be of great use.

The aim of this work was to develop a fast and simple, generic HPLC-DAD method for simultaneous determination of the most frequently used active substances in cough and cold preparations, which would be applicable for detecting undeclared ingredients in natural products for cough and cold treatment.

The chromatographic separation was achieved on an Agilent 1260 Infinity system, on a Poroshell 120 EC-C8 50 mm×4.6 mm, 2,7 μm column, using gradient elution consisted of solvent A (0,1% formic acid in water) and solvent B (0,1% formic acid in acetonitrile).

Eleven common cough and cold active ingredients with broad range of polarities were successfully separated on a reversed phase core-shell HPLC column using gradient elution with a very simple mobile phase in just 14 minutes with excellent sensitivity and good resolution. The optimisation was performed by the design of experiments approach using the Central Composite Face Centered (CCF) model in two sets of experiments. The summed results of both sets of experiments suggest the proposed method conditions providing satisfactory resolution between all of the critical peak pairs. The proposed method has been validated according to ICH guidelines and proved to be suitable for the simultaneous qualitative and quantitative determination of the selected compounds.

The optimized efficient and fast HPLC method for simultaneous determination of the most frequently used cough and cold active substances is generally applicable for a number of possible formulation compositions of cough and cold medicines, including preparations where they should not be present.

PRIMENA METODA MULTIVARIJANTNIH ANALIZA GLAVNIH KOMPONENTI I HIJERARHIJSKOG GRUPISANJA U ISPITIVANJU RAZDVAJANJA JEDINJENJA ZIPRASIDONA TEČNOM HROMATOGRAFIJOM

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Ziprasidon je novi atipični antipsihotik druge generacije, koji deluje kao antagonista na serotoninskim i dopaminskim receptorima, i inhibira preuzimanje norepinefrina. Glavni cilj hemometrijske studije je ispitivanje selektivnosti 20 reverzno-faznih (RP) stacionarnih faza u odnosu na ziprazidon i šest nečistoća ((I-V) i nepoznata). Velika strukturna sličnost ziprasidona i nečistoće II bila je ključni problem i razdvajanje kritičnog para je bilo odlučujuće za odabir RP stacionarne faze.

Za analizu glavnih komponenti (PCA) korišćen je matematički program Soft Independent Modeling of Class Analogy SIMCA P+ 12.0, a za analizu hijerarhijskog grupisanja (HCA) korišćen je program MATLAB ver. 6.5.

Ispitivanje selektivnosti 20 RP stacionarnih faza je vršeno pri dobijenim optimalnim eksperimentalnim uslovima (25 ºC; pH: 2,5; cTEA: 1% i cKH2PO4: 50mM) u odnosu na ispitivana jedinjenja. Eksperimentalno dobijeni hromatografski parametri (broj teoretskih platoa-N, faktor simetrije pika -SF, rezolucija -Rs i faktor selektivnosti - α između jedinjenja koja se blizu eluiraju) na 20 RP stacionarnih faza analizirani su primenom PCA i HCA analiza.

Grafikoni rezultata i PCA koeficijenata variabli za PC1(osnovna komponeta 1) i PC2 (osnovna komponeta 2) pokazuju glavne razlike između svih 20 RP-kolona i glavne sličnosti između hromatografskih parametara. Rs i α su odabrani kao najznačajniji parametri za izbor stacionarne faze. Dobijeni dendogrami pokazali su tri glavne grupe za stacionarne faze i četiri grupe za hromatografske parametre. Dendogrami RP kolona i hromatografskih parametara su prikazani i obojenom mapom što je jednostavnija metoda vizuelizacije.

Određene su RP stacionarne faze posebno selektivne za efikasno razdvajanje ziprasidona i strukturno sličnih jedinjenja primenom PCA i HCA (grupa 1 PCA i grupa C AHC):Waters Spherisorb® ODS1, Waters Spherisorb® ODS2 i Nucleosil® 100-5 C18. Dobijeni podaci su u skladu sa eksperimentalnim rezultatima. Odabrana je Waters Spherisorb® ODS 1 kolona za HPLC metodu u odnosu na najbolju SF i faktor retencije (k').

MODELING OF LIQUID CHROMATOGRAPHY SEPARATION OF ZIPRASIDONE COMPOUNDS USING MULTIVARIATE METHODS OF PRINCIPAL COMPONENT AND HIERARCHICAL CLUSTERING ANALYSIS

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Ziprasidone is a novel „atypical" or „second generation" antipsychotic drug which acts primarily through serotonergic and dopaminergic receptor antagonism, and as an inhibitor of the norepinephrine reuptake. The main aims of the presented chemometric study was to test selectivity of the set of 20 Reversed-phase (RP) - columns towards ziprasidone and its six impurities ((I-V) and one unknown). Separation of structurally similar pair of ziprasidone/impurity II caused analytical problems and was decisive for the selection of the suitable RP-column.

The Principal Component Analysis (PCA) for column classification was done with use of SIMCA P+ 12.0 program and Hierarchical Clustering Analysis (HCA) with use of MATLAB ver. 6.5. with additional algorithms.

The obtained optimal chromatographic conditions (25C, pH 2,5, cTEA 1% and cKH2PO4 50 mM) were used to test a set of 20 RP-columns. Plate numbers (N), symmetry factors (SF), resolution (Rs) and selectivity (α) of investigated compounds were subjected to PCA and HCA analysis. Score plot and loading plots PC1 (principal component 1) vs PC2 (principal component 2) visualize the main differences between all 20 RP-columns and main similarities between hromatographic parameters, respectively. The Rs and α were selected as the most significant for the column selection. The obtained dendrograms reveal three distinct clusters of RP-columns and four clusters of chromatographic parameters. The color map of data was used as a simpler presentation of the dendrograms of RP-columns and chromatographic parameters.

The RP-columns selective for the efficient separation of ziprasidone and its structurally related compounds were defined by PCA and HCA (group 1 in PCA study and same cluster C in HCA study) : Waters Spherisorb® ODS1, Waters Spherisorb® ODS2 and Nucleosil® 100-5 C18. The results were in accordance with experimentally obtained results. Finally, the column Waters Spherisorb® ODS 1 was selected for the HPLC method due to best SF and retention factor (k').

ELEKTROHEMIJSKA KARAKTERIZACIJA REDOKS PROCESA BRIMONIDINA I VARENIKLINA

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Brimonidin (BRIM), agonist α_2-adrenergičkog receptora, primenjuje se lokalno u terapiji glaukoma oka, a vareniklin (VAR), parcijalni agonist nikotinskih receptora, u terapiji odvikavanja od pušenja. Oba leka su derivati hinoksalina, ali različitog farmakoterapijskog dejstva. Cilj je bio proučiti parametre redoks procesa i okarakterisati elektrohemijsko ponašanje BRIM i VAR.

VAR je proučavan cikličnom voltametrijom (*CV*) uz elektrodu od staklastog ugljenika *(GCE)*. Za elektrohemijsko proučavanje BRIM primenjena je polarografija jednosmerne struje (*DCP*) i kapljuća živina elektroda (*DME*). Korišćen je potenciostat/galvanostat μAUTOLAB (*Ecochemie,* Holandija), Metrohm 663 VA Stand i interfejs IME 663 (*Metrohm,* Švajcarska) podržani GPES 4.9 programom, i troelektrodna ćelija.

Redukcija BRIM proučavana je: primenom *DCP*/*DME*, pri pH=2,0-12,0 (Briton-Robinsonov pufer); a predstavljena je polarografskim talasom (I_c) sa pomerajem polutalasnih potencijala ka negativnijim vrednostima ($E_{1/2}$=-0,060*pH*-0,167; R=0,9993). Pri pH≤3,0 uočen je slabo izražen drugi redukcioni talas (II_c). Logaritamska analiza BRIM talasa (I_c) u kiseloj sredini dala je vrednosti m (broja protona) 1,76-1,84.

Primenom *CV/GCE,* u različitim puferima pH 2,15-9,20 i pri potencijalu -1,5 V do +1,5 V, zabeleženi su VAR pikovi: katodni (I_c) u direktnom i dva anodna pika (I_a, II_a) u povratnom smeru. Zabeleženo je pomeranje potencijala I_c i I_a pika ka negativnijim vrednostima sa porastom pH ($E_{p,Ic}$=-0,052*pH*-0,340; R=0,9976 i $E_{p,Ia}$=-0,053*pH*-0,274; R=0,9972). Kod drugog anodnog pika II_a prisutno je pomeranje potencijala pika ka manje pozitivnim vrednostima ($E_{p,IIa}$= -0,061*pH*+0,533;R=0,9750), što odražava olakšanu oksidaciju. Na osnovu dobijenih rezultata $|E_{p,Ia}-E_{p1/2,Ia}|$ odnosno $|E_{p,IIa}-E_{p1/2,IIa}|$; zatim $I_{p,Ic}/I_{p,Ia}$~1 i ΔE_p~60 mV; utvrđenje broj razmenjenih e^-.

Redukcija BRIM odvija se na C=N vezi hinoksalina do 1,4-dihidro-BRIM putem dvoelektronskog procesa i učešće $2H^+$. U jako kiseloj sredini, uz utrošak još dva elektrona, nagrađeni 1,4-dihidro-BRIM se dalje redukuje do 1,2,3,4-tetrahidro-BRIM. Uz učešće $2e^-/2H^+$, odvija se redukcija VAR na C=N vezi hinoksalina do 1,4-dihidro-VAR, a stepen reverzibilnosti raste sa porastom pH. Drugi anodni pik (II_a) VAR odgovara ireverzibilnoj oksidaciji produkta redukcije (1,4-dihidro-VAR) uz učešće $1e^-/1H^+$,do nastajanja 2-hidroksi-1,4-dihidro-VAR.

ELECTROCHEMICAL CHARACTERISATION OF BRIMONIDINE AND VARENICLINE REDOX PROCESSES

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Brimonidine (BRIM) is an α_2-adrenergic receptor agonist used in local therapy of glaucoma. Varenicline (VAR), a partial agonist of nicotinic receptors, is applied for smoking cessation. Both drugs are quinoxaline derivatives with different pharmacotherapeutic effects. The aim was to point out parameters of redox process and desribe electrochemical behaviour of BRIM and VAR.

VAR was examined by Cyclic Voltammetry (CV)/Glassy Carbon Electrode (GCE), while Direct Current Polarography (DCP)/Dropping Mercury Electrode (DME) were used for BRIM electrochemical study. Potentiostat/galvanostat μAUTOLAB (*Ecochemie,* Netherlands), Metrohm 663 VA Stand and IME 663 (*Metrohm,*Switzerland) supported by GPES 4.9, and system of three electrodes were employed.

Reduction of BRIM was investigated by DCP/DME in Britton-Robinson buffer pH=2.0-12.0 and polarographic wave (I_c) with changes of halfwave potential towards more negative values ($E_{1/2}$=-0.060*pH*-0.167; R=0.9993) was obtained. At pH≤3.0, poorly differentiated the second reduction wave (II_c) was noticed. Logarithmic analysis of I_c wave in acidic media resulted in experimental values of m (H^+-number)=1.76-1.84.

Application of CV/GCE and potential range -1.5 V to +1.5 V (indifferent buffers pH=2.15-9.20) for VAR study gave: cathodic peak (I_c)(direct scan) and two anodic peaks (I_a, II_a) (reverse scan). Movement of I_c and I_a peak potential with pH increase towards more negative values was recorded ($E_{p,Ic}$= -0,052*pH*-0,340;R=0,9976 and $E_{p,Ia}$= -0,053*pH*-0,274;R=0,9972). Moving of potential anodic II_a peak towards less positive values ($E_{p,IIa}$= -0,061*pH*+0,533;R=0,9750) reflects facilitated oxidation. Based on obtained results for $\left|E_{p,Ia}-E_{p1/2,Ia}\right|$ and $\left|E_{p,IIa}-E_{p1/2,IIa}\right|$;also $I_{p,Ic}/I_{p,Ia}$ ~1 and ΔE_p~60 mV; number of transferred e^- was established.

BRIM reduction appears at C=N bond of quinoxaline by participation of $2e^-/2H^+$, forming 1,4-dihydro-BRIM. In strong acidic media, additional $2e^-$ reduction step of formed 1,4-dihydro-BRIM is registered giving 1,2,3,4-tetrahydro-BRIM.VAR reduction happens with $2e^-/2H^+$ participation, at C=N bond of quinoxaline, generating 1,4-dihydro-VAR. Reversibility increases with increasing pH. The second anodic (II_a) VAR peak corresponds to irreversible$1e^-/1H^+$ oxidation of reduction product (1,4-dihydro-VAR) forming 2-hydroxy-1,4-dihydro-VAR.

ODREĐIVANJE SADRŽAJA EFEDRIN-HIDROHLORIDA U FARMACEUTSKIM PREPARATIMA PRIMENOM RP HPLC METODE

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Efedrin spada u grupu „mešovitih” simpatomimetika koji svoj efekat ostvaruju *direktno* preko adrenergičkih receptora i *indirektno* preko transportera za ponovno preuzimanje kateholamina. U terapiji se koristi kao vazokonstriktor u kapima za nos. U ovom radu razvijena je i validirana RP-HPLC metodaza određivanje sadržaja efedrin-hidrohlorida u farmaceutskim doziranim oblicima. Hromatografsko razdvajanje je postignuto na ZORBAX Extend C18, 250 x 4,6 mm, 5µm koloni sa mobilnom fazom koju čini smeša 0,1% H_3PO_4 i acetonitrila (90:10, v/v), pri brzini protoka od 1 ml/mini detekciji na 210 nm. Validacija je izvedena u skladu sa ICH smernicama. Metoda je linearna u opsegu koncentracija od 20 do 80 µg/mL (r=0,9995). Preciznost metode je ispitana na dva nivoa: ponovljivost metode i srednja preciznost. Na osnovu dobijenih vrednosti relativne standardne devijacije za ponovljivost (1,22 % < 2%) i srednju preciznost (1,09% < 3%) može se zaključiti da je metoda precizna. Tačnost metode je ispitana za tri koncentracije (80, 100 i 120%u odnosu na radnu koncentraciju). Dobijene *Recovery* vrednosti za sve tri ispitivane koncentracije su u intervalu od 98,17% do 101,38% što zadovoljava zahteve za tačnost metode (98-102%). Male promene hromatografskih parametara (radne temperature kolone i sastava mobilne faze) nisu značajno uticale na ispitivane hromatografske parametre čime je potvrđena robusnost metode. Validirana RP-HPLC metoda je primenjena za određivanje sadržaja efedrin-hidrohlorida u kapima za nazalnu primenu Dobijeni rezultati za sadržaj efedrina (99,02 do 99,36%)zadovoljavaju zahteve od 90% do 110%. Validirana metoda je ekonomična, efikasna, tačna i precizna pa se može primenjivati u rutinskoj kontroli preparata koji sadrže efedrin.

DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR DETERMINATION OF EPHEDRINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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In this paper development of new RP HPLC method for determination of ephedrine hydrochloride in pharmaceutical dosage forms was described. HPLC separation was performed on ZORBAX Extend C18, 250 x 4,6 mm, 5µm chromatographic column. Column temperature was set on 25 °C. Mobile phase consisted of 0.1 % H_3PO_4 and acetonitril (90:10, v/v). Flow rate of mobile phase was 1.0 mL/min and detection wavelength 210 nm. In equilibrated chromatographic system 5 µL od prepared solutions was injected. The developed method was validated according to ICH guidelines. Method is linear over the concentrations range from 19,7 to 78.9 µg/mL (r=0.9995). Precision was tested at two levels: intra-assay precision and intermediate precision. Calculated relative standard deviations (1.22 % < 2% for intra-assay precision and 1.09% < 3% for intermediate precision) confirmed that method was precise. Accuracy was tested at three concentrations levels (80, 100 and 120%) and confirmed by calculated recovery values (98.17% to 101.38% which is in accordance with the requirements 98% to 102%). Small variations of mobile phase composition and column temperature did not affect qualitative and quantitative system responses significantly, which proved method's robustness. Validated RP-HPLC method was applied for assay determination of ephedrine hydrochloride in 0.5% and 1.0% nose drops. Assay was calculated by calibration curve method and working standard method. Results obtained (99.36% in 0.5% nose drops and 99.02% in 1.0% nose drops by calibration curve method and 99.40% in 0,5% nose drops and 99.00% in 1.0% nose drops by working standard method) were in accordance with requirements of manufacturer.

ODREĐIVANJE SADRŽAJA PARTENOLIDA U FARMACEUTSKIM PREPARATIMA PRIMENOM RP HPLC METODE

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Partenolid je seskviterpenski lakton germakranolidnog tipa. Predstavlja modifikaciju germakranolida, jedinjenja koje nastaje iz ciklodekadienskog katjona, germakradiena. Izolovan je iz biljke *Tanacetum parthenium* (povratić). Elektrofilni α-metilen-γ-laktonski prsten lako interaguje sa nukleofilnim (tiolne i amino grupe) delovima bioloških molekula, a prisustvo epoksidne grupe u molekulu intenzivira aktivnost laktona. Najnovija istraživanja pokazuju da partenolid pored antimikrobne, antiinflamatorne i antiproliferativne pokazuje i antimigrenoznu aktivnost. Pokazano je da fitopreparati povratića smanjuju: frekvencu migrenoznih bolova,jačinu migrenoznih bolova, stepen mučnine i povraćanja. Imajući u vidu navedene podatke razvijena je i validirana RP-HPLC metodaza određivanje sadržaja partenolida u farmaceutskim doziranim oblicima koji sadrže ekstrakt povratića. Kao stacionarna faza korišćena je ZORBAX Extend C18, 250 x 4,6 mm, 5μm kolona, a mobilnu fazu činila je smeša vode i acetonitrila čiji se odnos menja tokom vremena pri konstantnoj brzini protoka mobilne faze od 1 ml/min. Detekcija je vršena na 210 nm. Metoda je linearna u opsegu koncentracija od 0,030 do 1,20mg/mL (r=1,0000). Preciznost metode je ispitana na dva nivoa: ponovljivost metode i srednja preciznost. Na osnovu dobijenih vrednosti RSD za ponovljivost (0,9997 % < 2%) i srednju preciznost (1,0358% < 3%) može se zaključiti da metoda ispunjava zahteve za preciznost. Tačnost je ispitana za tri koncentracije (80%, 100% i 120%). Dobijene *Recovery*vrednosti za sve tri ispitivane koncentracije su u intervalu od 99,84% do 100,47%što ispunjava zahteve tačnosti metode (98-102%). Validirana RP-HPLC metoda je primenjena za određivanje sadržaja partenolida u kapsulama koje sadrže ekstrakt povratića. Dobijeni rezultati za sadržaj partenolida (96%) ispunili su zahteve od 90 do 110%.

DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR DETERMINATION OF PARTENOLIDE IN PHARMACEUTICAL DOSAGE FORM

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Partenolid is a sesquiterpenic lactone of the germacranolid type. It represents the modification of germacranolide, a compound formed from cyclodecadien germacradiene which is isolated from the plant *Tanacetum parthenium*. The electrophilic α-methylene-γ-lactone ring easily interacts with nucleophilic (thiol and amino groups) parts of biological molecules, and the presence of the epoxy group in the molecule intensifies the activity of the lactone. Recent studies have shown that partenolide, in addition to antimicrobial, antiinflammatory and antiproliferative agents, shows antimigrenous activity. It has been shown that preparations of this plant reduce: the frequency of migraine pain, the severity of migraine pain, the degree of vomitting and sickness. Development and validation of new RP HPLC method for determination of partenolide in pharmaceutical dosage forms has been described. Chromatographic column ZORBAX Extend C18, 250 x 4.6 mm, 5μm was used for analysis of investigated compound. Analysis was performed at room temperature which means that column temperature was not controled. Gradient elution was used. Ratio of water and acetonitrile, which were components of mobile phase, changed over time (0 to 5 minutes – 10% of acetonitrile, 5 do 20 minutes- share of acetonitrile has grown to 35%, 20 do 30 min – isocratic elution with 35% of acetonitrile, 30 to 40 min - share of acetonitrile has grown to 50%). Flow rate of mobile phase was 1.0 mL/min and detectionwas performed at 210 nm. Injected volume of prepared solution was 5 μL. The developed method was validated according to ICH guidelines. Method is linear over the concentrations range from 0.030 to 1.20 mg/mL (r=1,0000). Precision was tested at two levels: intra-assay precision and intermediate precision. Calculated relative standard deviations (0.9997 % < 2% for intra-assay precision and 1.0358% < 3% for intermediate precision) confirmed precision of method. Accuracy was tested at three concentrations levels (80, 100 and 120%) and confirmed by calculated recovery values (99.84% to 100.47%) which corresponds with the requirements (98% to 102%). Validated RP-HPLC method was applied for determination of partenolide in capsules. Assay was calculated by calibration curve method and working standard method. Results obtained (96.26% by calibration curve method and 96.02% by working standard method) were in accordance with requirements of manufacturer.

EQUIVALENCE TESTS AS A NEW STATISTICAL APPROACH IN ANALYTICAL METHOD TRANSFERS

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The transfer of an analytical method from a laboratory, where it was originally developed and validated, to another laboratory, which is close to an additional production site as well as the transfer for outsourcing purposes became an important issue during the life cycle of a product. With respect to the method performance criteria, all guidelines agree to evaluate accuracy and precision (variability) during analytical method transfer. For both, statistical equivalence tests are preferred over significance tests such as t-test or F-test, recommended by ISPE concept and USP<1010>General chapter. For the purposes of our study, we have implemented the method described by Wätzig in order to compare the means obtained in the two laboratories using an equivalence test. First, we have denoted tested parameter (μ_R-μ_S) by θ, and the nominal reference value by θ_o. We have defined symmetrical tolerance interval around θ_o, as [θ_o-ε, θ_o+ε]. 100·(1-2α) confidence interval $\delta_{L,CL}$ and $\delta_{H,CL}$ for μ_R-$\mu_S$are calculated with α=0.05.Equivalence can be proved if the whole confidence interval is included within interval of tolerance ($\delta_{L,TOL} \leq \delta_{L,CL}$)∧($\delta_{H,CL} \leq \delta_{H,TOL}$), so null hypothesis ($H_0=\theta \leq \theta_o-\varepsilon$)∧($\theta \geq \theta_o+\varepsilon$) is rejected and alternative hypothesis ($H_1=\theta_O-\varepsilon < \theta < \theta_O+\varepsilon$) is accepted.

Analytical method transfers on several pharmaceutical forms were investigated in this study. The obtained results were compared by using F-test and equivalence test by Wätzig. Set up of tolerance intervals depends mostly form method parameter, specification limit, defined bias between two labs, etc. From the data obtained, it has been confirmed that F-test is not suitablefor comparison, mainly wherethe values for variances were small. Regarding the equivalence test applied, it was proved that there is no difference between two laboratories. Using equivalence tests is the approach of choice, because systematic and random error can be followed by both laboratories.

ODREĐIVANJE SADRŽAJA KOFEINA U KOZMETIČKIM PREPARATIMA ZA KOSU PRIMENOM EKSTRAKCIJE NA ČVRSTOJ FAZI I HPLC METODE

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U novije vreme, na našem tržištu su sve prisutniji kozmetički preparati za kosu sa kofeinom namenjeni prevenciji i tretmanu alopecije. Kofein je često aktivna komponenta šampona, jer stimuliše rast kose i poboljšava mikrocirkulaciju kože. On inhibira enzimsku aktivnost 5-*α*-reduktaze i fosfodiesteraze i omogućava ponovno uspostavljanje ciklusa rasta kose. Preporučena koncentracija kofeina u šamponima je u opsegu 1-3%. Kozmetički preparati tipa šampona su uzorci složenog hemijskog sastava i ne mogu se direktno analizirati bez prethodno izvršene adekvatne pripreme uzoraka. Cilj ovog rada bio je primena metode ekstrakcije na čvrstoj fazi za pripremu šampona za HPLC analizu kofeina. Pored toga, proveravan je i sadržaj kofeina u kozmetičkim preparatima za kosu na našem tržištu.

Šamponi sa deklarisanim kofeinoma nabavljeni su u lokalnim apotekama. Tačna koncentracija kofeina nije navedena na deklaraciji. Analizirana su dva preparata: *Balea* šampon (*Dm-drogerie markt, Karlsruhe, Germany*) i *Alpecin*®C1 (*Dr Kurt Wolff, Bielefeld, Germany*). Uzorci su rastvoreni u 10 mL destilovane vode i pripremljeni korišćenjem ekstrakcije na čvrstoj fazi. Najbolji rezultati dobijeni su prečišćavanjem uzorka i ekstrakcijom kofeina iz šampona primenom *Chromabond*®HR-X (100 mg, 1 mL *volume*, *Macherey-Nagel, Germany*). Reverzno-fazna HPLC analiza izvršena je na koloni *Restek Ultra* IBD C18 (3 μm, 150×3 mm), a mobilna faza sastojala se od metanola i vode (40:60, V/V). Sadržaj kofeina u šamponu *Balea* je (1,016 ± 0,08) g/100g (1,016%), dok je u preparatu *Alpecin*®C1 bio (1,08 ± 0,2) g/100g (1,08%), što je u skladu sa očekivanim vrednostima i literaturnim podacima.

Rezultati pokazuju da su analizirani kozmetički preparati dobrog kvaliteta u pogledu sadržaja kozmetički aktivne supstance. Samim tim, pokazano je i da se predloženi postupak pripreme uzoraka i uslovi ekstrakcije na čvrstoj fazi za ekstrakciju kofeina iz šampona kao i primenjeni uslovi HPLC analize, mogu koristiti u kontroli kvaliteta ovih komercijalnih preparata.

ASSESSMENT OF CAFFEINE CONTENT IN HAIR CARE PRODUCTS BY SOLID PHASE EXTRACTION AND HPLC METHOD

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On our market, hair products containing caffeine have recently been used to prevent and treat alopecia. Caffeine is an active ingredient in shampoos, because it stimulates hair growth and improves skin microcirculation. It inhibits 5-α-reductase and phosphodiesterases and allows a restoration of the hair growth cycle. The recommended caffeine concentration in shampoos is in the range from 1-3%. Given the complexity of the shampoo as an extraction medium, in order to further analyze it, an appropriate sample preparation has to be performed previously. The goal of this paper was to develop the solid phase extraction method for HPLC determination of caffeine in shampoos. In addition, the caffeine content in hair care preparations was also checked.

Caffeine-enriched shampoos were purchased at local pharmacies. The precise caffeine content was not declared. Two products were analyzed: Balea Shampoo (Dm-drogerie markt, Karlsruhe, Germany) and Alpecin® C1 (Dr Kurt Volff, Bielefeld, Germany). Samples were dissolved in 10 mL of distilled water. Further sample preparation was done using solid-phase extraction Chromabond® HR-X cartridges (100 mg, 1 mL volume, Macherei-Nagel, Germany) which gave the best results regarding sample clean-up and caffeine extraction. The reverse-phase HPLC analysis was performed using Restek Ultra IBD C18 column (3 μm, 150×3 mm), and methanol-water solution (40:60, v/v), as a mobile phase. Found content of caffeine in Balea shampoo and Alpecin® C1 was (1.016 ± 0.08) g/100 g (1.016%) and (1.08 ± 0.2) g/100 g (1.08%), respectively. These results agreed with the expected values and literature data.

Results of this work show that examined products have good quality in terms of active ingredient content. The proposed extraction method and applied HPLC determination was suitable for the analysis of caffeine in shampoos, therefore they could be used in quality control of these commercial preparations.

VALIDACIJA HPLC-UV METODE ZA ODREĐIVANJE MOKSIFLOKSACINA I CIPROFLOKSACINA U PERITONEALNOJ TEČNOSTI KOD PACIJENATA NA PERITONEALNOJ DIJALIZI

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Nova HPLC-UV metoda, kombinovana sa precipitacijom proteina, je namenjena za kvantifikaciju moksifloksacina i ciprofloksacina u peritonealnoj tečnosti pacijenata sa peritonitisom, koji su na lečenju kontinuiranom ambulatornom peritonealnom dijalizom (CAPD).Precipitacija proteina iz uzoraka peritonealne tečnosti je izvršena pomoću ledene 0,1% trifluorosirćetne kiseline u metanolu (v/v). U analizi je korišćena Agilent Zorbax SB-C18 analitička kolona (150 mm x 4,6mm;3,5 μm). Optimalni uslovi za hromatografsko razdvajanje su: mobilna faza metanol – 0,1% trifluorosirćetna kiselina (34:66, v/v), protok od 1 ml/min itemperatura kolone 35°C, uz UV detekciju na 285 nm. Ukupno trajanje hromatografskog rana iznosi oko 10 min. Tokom validacije metode, linearnost je potvrđena u koncentracionom opsegu 0,2-50 μg/ml, sa koeficijentom korelacije većim od 0,9857 za oba analita. Preciznost metode u toku jednog i u toku više dana je zadovoljavajuća, sa relativnom standardnom devijacijom nižom od 13,92%, dok tačnost metode obuhvata vrednosti u opsegu 87,76-111,74%, za oba analita. Dobijeni rezultati ukazuju da je primenjena metoda jednostavna, precizna, brza i pogodna za razdvajanje i kvantifikaciju moksifloksacina i ciprofloksacina u peritonealnoj tečnosti kod pacijenata na CAPD. Stoga, metoda može da se primeni u optimizaciji doze moksifloksacina ili ciprofloksacina u terapiji peritonitisa.

VALIDATION OF HPLC-UV METHOD FOR DETERMINATION OF MOXIFLOXACIN AND CIPROFLOXACIN IN PERITONEAL FLUID OF PATIENTS ON PERITONEAL DIALYSIS

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New HPLC-UV method, combined with protein precipitation, is developed for quantification of moxifloxacin and ciprofloxacin in peritoneal fluid of patients with peritonitis, who are on continuous ambulatory peritoneal dialysis (CAPD) treatment.Precipitation of proteins from peritoneal fluid samples was performed with ice-cold 0.1% trifluoroacetic acid in methanol (v/v). Agilent Zorbax SB-C18 analytical column (150 mm x 4.6 mm; 3.5 μm) was used. The optimal conditions for chromatographic separation were: mobile phase methanol – 0.1% trifluoroacetic acid (34:66, v/v), flow rate of 1 ml/min, and column temperature of 35°C, with UV detection wavelength at 285 nm. The total run time was around 10 min. During validation, linearity was confirmed over the concentration range 0.2-50 μg/ml, with correlation coefficients higher than 0,9857 for both analytes. The assay exhibited adequate intra-day and inter-day precision, with relative standard deviation less than 13.92%, while the accuracy ranged 87.76-111.74%, for both analytes.The obtained results indicate that the method is simple, precise, fast and suitable for the separation and quantification of moxifloxacin and ciprofloxacin in the peritoneal fluid of patients on CAPD. Therefore, the proposed method has a potential to be applied for the optimisation of dosage during peritonitis therapy with moxifloxacin or ciprofloxacin.

UTICAJ MICELA RAZLIČITOG NAELEKTRISANJA KAO SIMULIRAJUĆIH SISTEMA BIOMEMBRANA NA JONIZACIJU RUPATADINA

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Rupatadin je selektivni antagonist histaminskih H_1 receptora druge generacije koji se primenjuje u terapiji alergijskog rinitisa i hronične urtikarije. Rupatadin sadrži tri jonizaciona centra, dva aromatična amina i jedan cikličan alkilamin. Dozirani farmaceutski oblici za oralnu primenu kao aktivnu supstancu sadrže rupatadin fumarat. U rastvoru rupatadin fumarata uspostavlja se složen sistem protolitičkih ravnoteža koji uključuje tri bazna centra rupatadina i dve karboksilne grupe fumarne kiseline. Poznavanje pK_a vrednosti lekova neophodno je za procenu farmakokinetičkih osobina i bioraspoloživosti. U fiziološkim uslovima pK_a vrednosti mogu biti promenjene u odnosu na vodeni rastvor usled interakcija sa naelektrisanim i polarnim biomolekulima. Ispitivanje jonizacije u pojednostavljenim sistemima biomembrana, kao što su micelarni rastvori surfaktanata, pruža bolji uvid u ponašanje lekova u fiziološkim uslovima. Ispitan je uticaj micelarnih rastvora, anjonskog natrijum-dodecilsulfata (SDS), katjonskog cetiltrimetilamonijum-bromida (CTAB) i nejonskog 4-oktilfenol polietoksilata (TX-100) na protolitičke ravnoteže rupatadina.

Potenciometrijski su određene pK_a vrednosti bez i u prisustvu 10^{-2} M surfaktanata, na temperaturi 25°C i pri konstantnoj jonskoj sili (0,1 M NaCl)**.** Potenciometrijski podaci analizirani su primenom programa Hyperquad. Nezavisno određene pK_a vrednosti fumarne kiseline korišćene su kao ulazni parametri za određivanje pK_a vrednosti rupatadina.

Određene su pK_a vrednosti u vodenom rastvoru (pK_{a1}=3,34; pK_{a2}=4,72; pK_{a3}=6,75) i uočen je uticaj svih primenjenih surfaktanata, SDS (ΔpK_a do +1,44); CTAB (ΔpK_a od -1,99 do +0,14); TX-100 (ΔpK_a od -0,72 do +0,38), na promenu jonizacije. Jonizujući centri rupatadina uključuju se u elektrostatičke, hidrofobne, dipol interakcije i vodonične veze sa micelama. Dijagram raspodele ravnotežnih oblika u funkciji pH ukazuje da je promena raspodele najizraženija u pH oblasti 4 – 8 koja obuhvata biofarmaceutski značajne pH vrednosti.

Pomeranje protolitičkih ravnoteža rupatadina pod uticajem micela ukazuje da biomolekuli različite polarnosti i naelektrisanja u fiziološkim uslovima mogu izazvati promenu raspodele ravnotežnih oblika od kojih zavise rastvorljivost i permeabilnost.

THE EFFECTS OF DIFFERENTLY CHARGED MICELLES AS BIOMEMBRANE MIMETIC SYSTEMS ON THE IONIZATION OF RUPATADINE

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Rupatadine belongs to selective second-generation histamine H_1 receptors antagonists, used in seasonal allergic rhinitis and chronic urticaria. Rupatadine contains three ionizable basic centers, two aromatic and one cyclic aliphatic amine. Pharmaceutical dosage forms contain rupatadine fumarate as an active substance. Complex system of protolytic equilibria establishes in solution of rupatadine fumarate including three rupatadine basic centers and two carboxylic groups of fumaric acid. The pK_a values are necessary for estimation of pharmacokinetic properties and bioavailability of drugs. Under the physiological conditions protolytic equilibria could be shifted due to interactions with biomolecules. The investigations of ionization in the present of simplified biomembrane systems (micellar solutions of surfactants) give better insight into physiological drug behavior. The effects of surfactants, sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and 4-octylphenol polyethoxylates (TX-100) on rupatadine ionization have been investigated.

The pK_a values were determined potentiometrically in the absence and in the presence of 10^{-2} M surfactants at 25°C and constant ionic strength (0.1 M NaCl). Potentiometric data were analyzed in program Hyperquad. Independently determined pK_a values of fumaric acid were used as input parameters for determination of rupatadine pK_a values.

The ionization in water was defined (pK_{a1}=3.34; pK_{a2}=4.72; pK_{a3}=6.75) and shift in protolytic equilibria in the presence of micelles, SDS (ΔpK_a up to +1.44); CTAB (ΔpK_a from -1.99 to +0.14); TX-100 (ΔpK_a from -0.72 to +0.38), were observed. The ionization centers of rupatadine are involved in electrostatic, hydrophobic, dipole interactions, and hydrogen bonds with micelles. Distribution diagram of equilibrium forms as a function of pH indicates that change in ionization is the most expressed in pH range 4–8 which includes biopharmaceutically important pH values.

The shift in rupatadine protolytic equilibria indicates that biomolecules of different charge and polarity could change distribution of equilibrium forms responsible for solubility and permeability.