## Project summary

The aims of investigations were liquid chromatography, especially new methods in the analysis of newly synthesized pharmaceutical active substances. This project included the retention behavior of newly synthesized substances in various chromatographic systems (classical reversed-phase liquid chromatography, RP–LC method; hydrophilic interaction chromatography – HILIC; micellar liquid chromatography – MLC; high submicellar – HSC; microemulsion liquid chromatography – MELC and haotropic chromatography). Thus, new drug substances and their chromatographic properties were analyzed, using the appropriate descriptors. For this purpose, the quantitative structure retention relationship (QSRR) studies were done. Retention prediction models were developed employing multiple regression analysis (MRA) and artificial neural network (ANN).

In this part of study, all possible degradation pathways were investigated and all impurities were analyzed, i.e. identified and determined.

In order to disclose and avoid possible problems the chemometrics were used and optimized. Method optimized were subjected to method validation and applied to real pharmaceutical samples. Also, for these substances and their metabolites the procedure for the investigation in biological samples (blood, plasma, serum and tears) proposed. Depending on the type of analysis and requirements distinct the appropriate detection included. Mass detector as one of the most sensitive employed for degradation profiling and analysis of biological samples.

This project was focused of on the analysis of new pharmaceutical active substances. Among others, different groups of cardiovascular drugs (inhibitors of angiotenzine converting enzyme, beta blockers, calcium channel antagonists etc.), drugs for treatment of metabolic disorders (statins, antidiabetics, bisphosphonate drugs etc.), drugs for central nervous system diseases (antiparkinsonics, antiepileptics) and many others. As mentioned pharmaceutical substances and theirs dosage forms were analyzed as well as their metabolites from samples of biological material.

In pharmaceutical analysis, chromatographic separation combined with mass spectrometry (MS) is one of the most powerful technique for the monitoring, characterization and identification. The key advantages of using LC/MS methods include: selectivity, peak assignment by chemical fingerprint for the compound of interest in the presence of complex matrices, molecular weight information with the confirmation and identification of known and unknown compounds, structural information by controlled molecular fragmentation, rapid method development, quantitative and qualitative data obtained easily with limited instrument optimization. All the given advantages of MS detector can be furthermore improved by the optimization of its own parameters.

Particular emphasis should be placed on the progress of research with equipment (Waters UPLC/MS/MS), which was secured by a high position on the ranking list. Ranking at the beginning of the project cycle, the project was fifth in 65 projects from the Chemistry group, the researcher rated the highest score of 50. Quality was maintained until the end of the project cycle

The realization of the set goals is best viewed through published scientific papers in important international journals, which is a confirmation of the foreign reviewers of these journals about the actuality, quality and significance of the scientific research of this team.

The research team, consisted of 4 professors (all A1 categories) and 7 PhD researchers (four already defended their doctoral dissertations). The average number of researchers for months was 61.

This research team published 59 scientific papers in journals of international significance, 23 (M21), 5 (M22) and 31 (M23). They published 4 chapters in international scientific monographs (M13) and participated with 60 papers at international scientific conferences (11 M32 and 49 M34).

Keywords: Liquid chromatography, Mass spectrometry, Chemometrics, QSRR study, Pharmaceutical analysis

## Sažetak projekta

Postavljeni ciljevi bili su iz oblasti tečne hromatografije, posebno novih metoda u analizi novosintetisanih farmaceutski aktivnih supstanci. Ovim projektom bila su obuhvaćena retenciona ponašanja novosintetisanih supstanci u različitim hromatografskim sistemima (klasična reverzno-fazna tečna htomatografija, RP–LC; hromatografija zasnovana na hidrofilnim interakcijama, *hydrophilic interaction chromatography* – HILIC; micelarna hromatografija, *micellar liquid chromatography* – MLC; *high submicellar* – HSC; mikroemulziona hromatografija, *microemulsion liquid chromatography* – MELC i haotropna hromatografija, *haotropic chromatography*). U tom cilju primenjena je QSRR studija koja, na osnovu odgovarajućih deskriptora i retencionih parametara, omogućava predviđanje retencionog ponašanja u odabranom sistemu. Modeli za predviđanje formirani su primenom multiple regresione analize (*multiple regression analysis*, MRA) i veštačke neuronske mreže(*artificial neural network*, ANN).

Ispitani su potencijalni mehanizmi degradacije, a nastale nečistoće su identifikovane i određene.

Hemometrijskom evaluacijom, tj. dizajnom eksperimenta definisani su modeli koji u potpunosti opisuju sistem. U tu svrhu ispitane su i optimizovane različite hemometrijske metode. Ovako optimizovane hromatografske metode su validirane, a zatim primenjene na odgovarajuće farmaceutske uzorke. Pored toga, tokom bioanalitičkih istraživanja, za analizirane supstance praćeni su metaboliti i postavljene metode za analizu iz biološkog materijala (krv, serum, plazma i suze). U zavisnosti od tipa i zahteva analize bili su primenjeni i različiti detektori. Maseni detektor, kao jedan od najosetljivijih, primenjen je u definisanju degradacionog profila ispitivanih supstanci, kao i za analizu biološkog materijala.

Istraživanjen su obuhvaćene farmaceutski aktivne supstance, posebno novosintetisane i do sada malo istražene iz različitih farmakoloških grupa: lekovi za kardiovaskularne bolesti (inhibitori angiotenzin-konvertujućeg enzima, beta blokatori, antagonisti kalcijumovih kanala, itd.), lekovi za lečenje različitih metaboličkih poremećaja (statini, antidijabetici, bisfosfonati, itd.), lekovi koji se primenjuju u terapiji bolesti CNS (antiparkinsonici, antiepileptici) i mnogi drugi. Pored jedinjenja i njihovih farmaceutskih oblika, definisani su degradacioni profili, a posebna istraživanja obuhvatila su analizu njihovih metabolita iz uzoraka biološkog materijala.

Kombinovanjem masenog detektora sa hromatografskim metodama dobijena je možda najznačajnija tehnika koja se primenjuje u farmaceutskoj analizi za praćenje, karakterizaciju i identifikaciju analiziranih jedinjenja. Glavne prednosti LC/MS metode su selektivnost, mogućnost identifikacije analiziranog jedinjenja u složenim uzorcima zahvaljujući generisanju karakterističnog masenog spektra, kao i informacije o strukturi koje se mogu dobiti fragmentacijom molekula. U cilju dobijanja potrebnih kvalitativnih i kvantitativnih podataka vršena je i optimizacija parametara masenog detektora.

Posebno treba istaći napredak u istraživanjima zahvaljujući opremi (*Waters* UPLC/MS/MS), a koju je ovaj projekat obezbedio visokom pozicijom na rang listi. Prilikom rangiranja na početku projektnog ciklusa projekat je bio na petom mestu od 65 projekata iz grupe Hemija, a tim istraživača ocenjen najvišom ocenom 50. Kvalitet je održan do kraja projektnog ciklusa.

Realizacija postavljenih ciljeva najbolje se sagledava kroz publikovane radove u vrhunskim međunarodnim časopisima, što predstavlja potvrdu inostranih recenzenata ovih časopisa o aktuelnosti, kvalitetu i značaju naučnih istraživanja ovog tima.

Istraživački tim, sastojao se od 4 profesora (svi kategorije A1) i 7 istraživača-doktoranada (četiri je već odbranilo svoje doktorske disertacije). Prosečan broj istraživač meseci bio je 61.

Ovaj istraživački tim objavio je 59 naučnih radova u časopisima međunarodnog značaja i to 23 (M21), 5 (M22) i 31 (M23). Publikovao je 4 poglavlja u međunarodnim naučnim monografijama (M13) i učestvovao sa 60 radova na međunarodnim naučnim konferencijama (11 M32 i 49 M34).

Ključne reči: tečna hromatografija, masena spektrometrija, hemometrija, QSSR studija, farmaceutska analiza

## Selected results/Odabrani rezultati

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