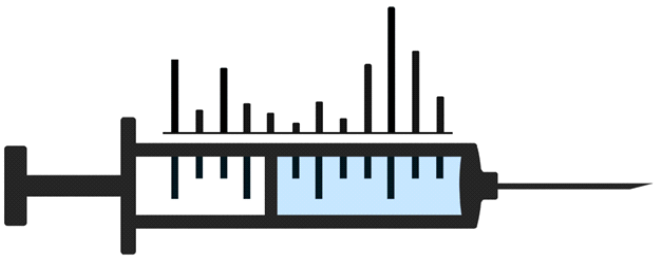
IV Poznańska Konferencja Naukowo - Szkoleniowa 23-24.10.2023 r.



Współczesna analityka farmaceutyczna i biomedyczna w ochronie zdrowia

MODERN PHARMACEUTICAL AND BIOMEDICAL ANALYTICS IN HEALTH CARE

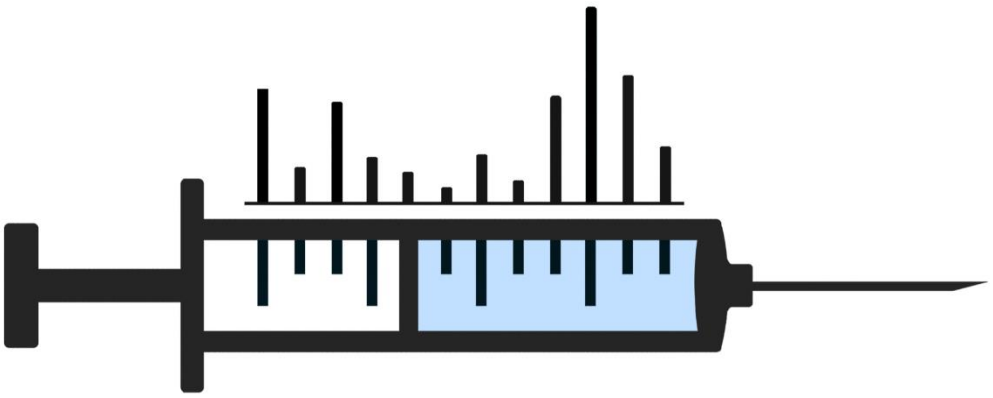
KONFERENCJA HYBRYDOWA

CONFERENCE BOOK

IV Poznańska Konferencja
Naukowo – Szkoleniowa

„Współczesna analityka farmaceutyczna i biomedyczna w ochronie zdrowia”

„Modern pharmaceutical and biomedical analytics in health care”



http://analityka2023.bok-ump.pl/teksty.php

Poznań, konferencja hybrydowa, 23-24.10.2023 r.

Redaktor naukowy: prof. dr hab. Jan Matysiak

Skład komputerowy: dr Szymon Plewa

Projekt okładki: Anna Krzywda

Published by: ZOZ Ośrodek UMEA Shinoda-Kuracejo
31-851 Kraków, os. Albertyńskie 1-2, Poland
office@interrev.com
umea@interia.pl

ISBN: 978-83-959554-9-5

Honorowy Patronat

Prof. dr hab. Andrzej Tykarski

JM Rektor

Uniwersytetu Medycznego

im. Karola Marcinkowskiego w Poznaniu

Prof. dr hab. Judyta Cielecka-Piontek

Kanclerz Kolegium Nauk Farmaceutycznych

Uniwersytetu Medycznego

im. Karola Marcinkowskiego w Poznaniu

Prof. dr hab. Anna Jelińska

Dziekan Wydziału Farmaceutycznego

Uniwersytetu Medycznego

im. Karola Marcinkowskiego w Poznaniu

Komitet Naukowy (alfabetycznie)

Przewodniczący: Prof. dr hab. Jan Matysiak

Honorowy Przewodniczący: Prof. dr hab. Zenon J. Kokot

Członkowie:

Prof. dr hab. Tomasz Bączek
Prof. dr Szabolcs Béni
Prof. dr hab. Agnieszka Bienert
Dr hab. Beata Bystrowska
Prof. Andrea Čalkovská
Prof. dr hab. Michał Ciborowski
Prof. dr hab. Judyta Cielecka-Piontek
Prof. dr Milica Drobac
Prof. dr hab. Zbigniew Fijałek
Prof. dr habil. Judit Forrai DSc
Dr Timothy Garrett
Dr Krzysztof Goryński
Prof. dr hab. Tomasz Gośliński
Dr Marcin Iwanicki
Prof. dr hab. Marta Karaźniewicz-Łada
Prof. dr hab. Bartosz Kempisty
Dr hab. Agnieszka Klupczyńska-Gabryszak
Dr hab. Agata Kryczyk-Poprawa
Prof. AWF dr hab. Krzysztof Kusy
Prof. dr hab. Bogumiła Kupcewicz
Prof. dr Anđelija Malenović
Prof. dr Mirjana Marčetić
Prof. dr hab. Michał Marszałł
Prof. Bozena Michniak-Kohn
Prof. dr hab. Wojciech Miltyk
Prof. dr hab. Ewa Nowak-Markwitz
Prof. dr hab. Bożena Muszyńska
Prof. dr hab. Michał Nowicki
Prof. dr hab. Włodzimierz Opoka
Prof. dr Biljana Otašević
Prof. dr hab. Robert Pietrzak
Prof. dr hab. Ewa Wender-Ożegowska
Prof. dr Ana Protić
Prof. dr hab. Paweł Ramos
Prof. dr hab. Marek Ruchała
Prof. dr hab. Christian Schmelzer
Prof. dr hab. Joanna Sikora
Prof. dr hab. Krystyna Skalicka-Woźniak
Dr hab. Paulina Skupin-Mrugalska
Prof. dr hab. Ryszard Słomski
Prof. dr hab. Nicole Strittmatter
Prof. dr hab. Jarosław Walkowiak
Dr hab. Alicja Warowicka
Prof. dr hab. Przemysław Zalewski

Członkowie Komitetu Naukowego reprezentują następujące jednostki naukowo-badawcze (alfabetycznie)

Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Słowacja

ELTE - Eötvös Loránd University, Węgry

Fraunhofer Institute for Microstructure of Materials and Systems, Niemcy

Gdański Uniwersytet Medyczny

Politechnika Bydgoska im. Jana i Jędrzeja Śniadeckich

Rutgers-The State University of New Jersey, USA

Stevens Institute of Technology, USA

Śląski Uniwersytet Medyczny w Katowicach

Technische Universität München, Niemcy

University of Belgrade, Serbia

University of Florida, USA

Uniwersytet im. Adama Mickiewicza w Poznaniu

Uniwersytet Jagielloński, Collegium Medicum

Uniwersytet Kaliski

Uniwersytet Medyczny im. K. Marcinkowskiego w Poznaniu

Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Uniwersytet Medyczny w Białymstoku

Uniwersytet Medyczny w Lublinie

Uniwersytet Medyczny w Łodzi

Uniwersytet Mikołaja Kopernika w Toruniu, Collegium Medicum w Bydgoszczy

Komitet Organizacyjny (alfabetycznie)

Przewodniczący: Dr Szymon Plewa

Członkowie:
Prof. dr hab. Zbigniew Fijałek
Mgr Monika Gładysz
Dr hab. Agnieszka Klupczyńska-Gabryszak
Mgr Agnieszka Kubiak-Chałupka
Dr Eliza Matuszewska
Prof. dr hab. Jan Matysiak
Prof. dr hab. Włodzimierz Opoka
Mgr Dagmara Pietkiewicz
Mgr inż. Natalia Rzetecka
Mgr inż. Marcin Wysocki
Prof. dr hab. Przemysław Zalewski

Partnerzy Konferencji

Uniwersytet Medyczny

im. K. Marcinkowskiego w Poznaniu

Polskie Towarzystwo Farmaceutyczne

Stowarzyszenie Stop Nielegalnym Farmaceutykom

Diag-Med - autoryzowany dystrybutor firmy Bruker Daltonics


POLYGEN


EFFICIENCY PROGRESS RESEARCH OMICS
epro


AllerGen
CENTRUM MEDYCYNY SPERSONALIZOWANEJ


medson.pl


MERCK


phenomenex

Program Konferencji

Poniedziałek - 23 października, 2023 r.

8:00 – 9:00 Rejestracja uczestników

9:00 – 9:15 Otwarcie Konferencji, przemówienia gości

Sesja e-posterowa – postery dostępne on-line, cały dzień

Sesja VOD (*video on demand*, wideo na życzenie) – wykłady dostępne on-line, cały dzień

<u>Wykłady Plenarne (sesja w języku angielskim)</u>

Miejsce obrad: Centrum Kongresowo Dydaktyczne UMP,

ul. Przybyszewskiego 37a, 60-356 Poznań

Prowadzący: prof. dr hab. Zenon J. Kokot

prof. dr hab. Jan Matysiak

9:15 – 9:45 dr hab. Bartosz Sokół – „*Proteomic changes of cerebrospinal fluid in vascular and neurodegenerative diseases of the brain*"

9:45 – 10:15 prof. dr hab. Christian Schmelzer – „*Elastin Unveiled: Crafting Tomorrow's Healthcare Materials*"

10:15 – 10:45 prof. dr hab. Nicole Strittmatter – „*Using mass spectrometry imaging to increase our understanding of drug delivery mechanisms*"

10:45 – 11:15 Przerwa kawowa

<u>Sesja I</u> – Przestępczość farmaceutyczna (sesja w języku polskim)
Sesja współorganizowana przez Stowarzyszenie Stop Nielegalnym Farmaceutykom

Prowadzący: prof. dr hab. Włodzimierz Opoka

dr Szymon Plewa

11:15 – 11:45 prof. dr hab. Zbigniew Fijałek – *„Przestępczość farmaceutyczna w III dekadzie XXI wieku a rynek pacjenta/konsumenta - pozamedyczne stosowanie leków*"

11:45 – 12:05 prof. dr hab. Włodzimierz Opoka – *„Analityka farmaceutyczna jako jedno z narzędzi w zwalczaniu przestępczości farmaceutycznej*"

12:05 – 12:35 Agata Andrzejewska - Wiceprezes ds. Produktów Leczniczych Weterynaryjnych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych ,,*Bezpieczne leki dla zwierząt warunkiem bezpiecznej żywności*"

12:35 – 13:30 Przerwa lunchowa

13:30 – 14:00 mec. Krzysztof Jop – *„Odpowiedzialność prawna za jakość leku – wybrane zagadnienia"*

14:00 – 14:15 dr Szymon Plewa – *„Wykorzystanie spektrometrii mas do analizy środków odurzających w złożonych matrycach analitycznych i szacowania skali pozamedycznego wykorzystania wybranych leków"*

14:15 – 14:30 komisarz Piotr Woźniak – *„Przestępczość w środowisku farmaceutycznym – studium przypadku"*

14:30 – 14:45 mgr Agnieszka Kalicka – *„Zastosowanie wysokosprawnej chromatografii cieczowej sprzężonej ze spektrometrią mas (LC-MS/MS) do identyfikacji substancji farmakologicznie czynnych w sfałszowanych produktach leczniczych i suplementach diety"*

14:45 – 15:00 Krzysztof Kowalski – *„Spektroskopia i mikrospektroskopia UV-VIS-NIR / FTIR / Raman w badaniu środków leczniczych oraz szybkiej identyfikacji mobilnej, narkotyków, suplementów diety oraz substancji sfałszowanych"*

15:00 – 15:30 Przerwa kawowa

<u>Sesja II</u> – Młodzi naukowcy (sesja w języku angielskim i polskim)

Prowadzący: prof. dr hab. Bogumiła Kupcewicz

dr Magdalena Paczkowska-Walendowska

15:30 – 15:40 Kacper Packi *„Changes in Serum Protein–Peptide Patterns in Atopic Children Allergic to Plant Storage Proteins*"

15:40 – 15:50 Hind Makhloufi *„Apoptotic agents from the Endolichenic fungi against chemoresistant cancers*"

15:50 – 16:00 Cécile Letulle *„Synthesis and vectorization of novel chalcones and derivatives with anticancer activity*"

16:00 – 16:10 Szymon Sip – *„Analysis of amorphous dispersions of natural compounds obtained bySFC"*

16:10 – 16:20 Anna Kaliszewska – "*Development of procedure for the determination of β-estradiol and its metabolites in human plasma samples by LC-MS/MS technique*"

16:20 – 16:30 Kornel Pawlak – "*Development and validation of UPLC-MS/MS method for determination of rivaroxaban in dried blood spot samples*"

16:30 – 16:40 Dobrosława Wiśniewska – "*Selected methods of analysis of triterpene compounds*"

16:40 – 16:50 Katarzyna Dominiak – *„Development and characterization of polymeric micelles with magnolol*"

16:50 – 17:00 Wiktoria Jiers – *„Acute poisonings with antipsychotic and psychotropic drugs – clinical observation and analytical tools*"

17:00 – 17:10 Piotr Ruciński – *„Analysis of poisonings with selected drugs monitored by blood concentration*"

17:10 – 17:15 Ogłoszenie listy e-posterów wybranych do zaprezentowania w sesji najlepszych e-posterów.

19:00 – … Bankiet

Miejsce bankietu: Collegium Pharmaceuticum,

ul. Rokietnicka 3, 60-806 Poznań

Wtorek – 24 października 2023 r.

Miejsce obrad: Centrum Kongresowo Dydaktyczne UMP,

ul. Przybyszewskiego 37a, 60-356 Poznań

Sesja e-posterowa – postery dostępne on-line, cały dzień

Sesja VOD (*video on demand*, wideo na życzenie) – wykłady dostępne on-line, cały dzień

<u>Sesja III</u> – Bioanaliza kliniczna (sesja w języku polskim)

Prowadzący: prof. AWF dr hab. Krzysztof Kusy

prof. dr hab. Wojciech Miltyk

8:30 – 9:00 prof. dr hab. Bogumiła Kupcewicz – „*Comparison of PCA, ASCA, and PLS-DA in experimentally designed lipidomic studies*"

9:00 – 9:15 prof. dr hab. Wojciech Miltyk – „*Recombinant human prolidase induces wound healing in experimental models*"

9:15 – 9:30 prof. AWF dr hab. Krzysztof Kusy – „*Exercise-induced concentration of 42 plasma free amino acids in endurance- and sprint-trained athletes*"

9:30 – 9:45 dr Mikołaj Zaborowski – "*Single-cell image analysis in ovarian cancer patients*"

9:45 – 10:15 dr Katarzyna Krupczyńska Stopa – „*CardioCarePack as a modern solution for therapy monitored by drug concentration*"

10:15 – 10:30 Przerwa kawowa

<u>Sesja IV</u> – Badania produktów pochodzenia naturalnego (sesja w angielskim i w języku polskim)

Prowadzący: prof. Małgorzata Kujawska

prof. Przemysław Zalewski

10:30-10:45 prof. dr hab. Judyta Cielecka-Piontek – „*Paradoksy analityki surowca konopnego*"

10:45-11:00 dr hab. Daniel Załuski, prof. UMK – „*Przeszłość zamknięta w korzeniu - owoc otwierający przyszłość? Farmakologiczna użyteczność owoców Eleutherococcus senticosus*"

11:00-11:15 prof. dr hab. Krystyna Skalicka – Woźniak – „*Współczesne wyzwania w analizie związków pochodzenia naturalnego działających w kierunku centralnego układu nerwowego*"

11:15-11:30 prof. dr Szabolcs Béni – „*Cyclodextrins as versatile scaffolds in pharmacy*"

11:30-11:40 dr Aleksandra Zielińska – „*Metody optymalizacji I analizy nanocząstek lipidowych"*

11:40-11:50 prof. Gökhan Zengin – „*Extractions of aerial parts of Hippomarathrum scabrum with conventional and green methodologies: Chemical profiling, antioxidant, enzyme inhibition and anti-cancer effects*"

11:50-12:00 prof. Everaldo Attard – *„Analysis of honeys from Malta"*

12:00-12:10 dr Ljubos Usjak - „*Investigations of chemical composition and pharmacological activity of nine taxa of the genus Heracleum L. (Apiaceae) from Southeastern Europe"*

12:10-12:20 prof. dr Lijing Ke - "*Incidental Nanoparticles: The Functional Units of Traditional Chinese Medicine"*

12:20-12:30 dr Aleksandra Bazan-Woźniak – „*Analiza biomasy odpadowej wykorzystywanej w syntezie adsorbentów węglowych*"

12:30 – 13:30 Przerwa obiadowa

<u>Sesja V</u> – Bioanaliza w ochronie zdrowia (sesja w języku angielskim)

Prowadzący: dr hab. Agnieszka Klupczyńska-Gabryszak

dr Krzysztof Goryński

13:30 – 13:45 dr hab. Agnieszka Klupczyńska-Gabryszak – *„Influence of smoking status on metabolic profiles of lung cancer patients - from cancer marker discovery perspective*"

13:45 – 14:00 dr Krzysztof Goryński – *„Quantification of prohibited substances in saliva using traditional and alternative microextraction-based sample-preparation methods coupled with LC-MS*"

14:00 – 14:15 prof. Anđelija Malenović – "*Critical issues in quantitative bioanalysis of Dried Blood Spot samples*"

14:15 – 14:30 dr Marcin Iwanicki – *„Taurine activates p53-dependent and independent mechanisms to control cancer cell growth*"

14:30 – 15:00 dr Timothy Garrett – *„Lipidomics and metabolomics with Machine Learning for biomarker identification in Meningioma"*

15:00 – 15:30 Przerwa kawowa

<u>Sesja V</u> – Prezentacje najlepszych e-posterów

Prowadzący/Komisja oceniająca: prof. dr hab. Michał Marszałł

dr Elżbieta Studzińska-Sroka

dr Eliza Matuszewska

15:30 – 16:00 – 3 minutowe prezentacje wybranych przez Komisję oceniającą e-posterów

16:00 – 16:10 – Uhonorowanie najlepszych wystąpień w sesji młodych naukowców oraz sesji e-posterów (wręczenie nagród i wyróżnień).

16:10 – 16:20 – Prof. dr hab. Judyta Cielecka-Piontek, Dyrektor CITF Jacek Wajda, Prof. dr hab. Zenon J. Kokot, Prof. dr hab. Zbigniew Fijałek, Prof. dr hab. Włodzimierz Opoka, Prof. dr hab. Jan Matysiak.

Podsumowanie wszystkich sesji i zakończenie Konferencji.

Streszczenia

Spis treści

„Proteomic changes of cerebrospinal fluid in vascular and neurodegenerative diseases of the brain”

Sokol B.
Associate Professor at the Chair and Department of Neurosurgery, PUMS, Poznan, Poland

Head of the Department of Neurosurgery Szpital Miejski im. J. Strusia w Poznaniu

Cerebrospinal fluid (CSF) proteomic changes play a crucial role in understanding the pathophysiology of various neurological disorders, including subarachnoid hemorrhage (SAH) and normal pressure hydrocephalus (NPH). Both conditions involve disturbances in the cerebrospinal fluid dynamics, leading to distinct protein profiles that can be analyzed for diagnostic and prognostic purposes.

Subarachnoid Hemorrhage (SAH): Subarachnoid hemorrhage is a medical emergency characterized by bleeding into the subarachnoid space, often due to the rupture of an intracranial aneurysm. CSF proteomic analysis in SAH has revealed changes in protein composition that reflect the pathophysiological processes associated with the hemorrhage.

• **Biomarkers:** Identification of specific biomarkers in CSF can aid in early diagnosis and monitoring of SAH. Proteins associated with blood-brain barrier disruption, inflammation, and neuronal injury are often elevated in the CSF after SAH.

• **Inflammatory Response:** Proteomic changes may indicate an inflammatory response in the CNS, involving proteins like HMGB1, TLR 2 and 4, RAGE and many more.

• **Blood-Derived Proteins:** Hemoglobin and its breakdown products are commonly found in the CSF after SAH, reflecting the presence of blood in the cerebrospinal fluid. Identifying these proteins can assist in confirming the diagnosis.

• **Neurodegenerative Markers:** SAH can induce secondary brain injury, leading to neurodegeneration. Proteomic analysis may reveal markers associated with neurodegenerative processes, aiding in the assessment of long-term outcomes.

Normal Pressure Hydrocephalus (NPH): Normal pressure hydrocephalus (NPH) is an abnormal buildup of cerebrospinal fluid (CSF) in the brain's ventricles (cavities). It occurs if the normal flow of CSF throughout the brain and spinal cord is blocked in some way. This causes the ventricles to enlarge, putting pressure on the brain. CSF proteomic changes in NPH can provide insights into the underlying mechanisms and potential diagnostic markers.

• **CSF Dynamics:** Proteomic analysis may reveal alterations in proteins involved in CSF production, circulation, or absorption. Changes in proteins related to the glymphatic system, which facilitates waste clearance from the brain, might be observed.

• **Neuroinflammation:** Inflammation is thought to play a role in NPH. Proteomic profiling may identify inflammatory markers in the CSF, offering clues about the inflammatory processes contributing to the condition.

• **Biomarkers for Diagnosis and Monitoring:** Specific protein markers indicative of NPH could serve as diagnostic and prognostic tools. Proteins associated with ventricular enlargement, alterations in cerebral blood flow, and neuronal damage might be of particular interest.

• **Treatment Response Monitoring:** Proteomic changes in response to treatment interventions can be monitored, providing valuable information about the efficacy of therapies and potential for disease modification.

In both SAH and NPH, ongoing research in proteomics is crucial for refining diagnostic criteria, understanding disease mechanisms, and identifying potential therapeutic targets. The integration of advanced technologies such as mass spectrometry and proteomic profiling holds promise for uncovering novel biomarkers and improving the management of these neurological conditions.

„Elastin Unveiled: Crafting Tomorrow's Healthcare Materials”

Schmelzer Ch.
Fraunhofer Institute for Microstructure of Materials and Systems, Germany

„Using mass spectrometry imaging to increase our understanding of drug delivery mechanisms”

Strittmatter N.
Technical University of Munich (TUM), Germany

Mass spectrometry imaging is a powerful and unique tool to study drug disposition in pharmaceutical development. It offers untargeted, label-free imaging of drugs and drug metabolites at high speed, high specificity and high sensitivity. Simultaneously, it enables the untargeted acquisition of the endogenous metabolome, which can be used to study the effects of drug treatment and for chemical histology to identity the underlying tissue types. MSI is increasingly deployed in multimodal imaging studies to fully harness the benefits of this technique. Additional layers of information comprise traditional microscopy of tissue specimen (such as H&E and IHC) up to highly multiplexed ‘omics approaches such as imaging mass cytometry (IMC) or spatial transcriptomics. Here, we discuss novel multimodal imaging approaches combining MSI and IMC to study the delivery of gemcitabine and its metabolites in pancreatic cancer ductal adenocarcinoma (PDAC), as well as the effect of nanoparticle formulation on the disposition of AYD2811 in three different patient-derived xenograft models of different phenotype regarding the expression of epithelial markers and stromal architecture. Deploying IMC in addition to MSI here enables us to identify the underlying cell types driving drug metabolism and accumulation. In a final example, a novel approach was presented to study size-dependent nanoparticle distribution in the same animal using an integrated imaging approach combining LA-ICP-MS and IMC.

References:

N. Strittmatter & F. Richards et al., Anal. Chem. 2022, 94, 3, 1795

Strittmatter et al, Theranostics 2022, 12, 2162-2174.

„Przestępczość farmaceutyczna w III dekadzie XXI wieku a rynek pacjenta/konsumenta - pozamedyczne stosowanie leków”

Fijałek Z.
Stowarzyszenie Stop Nielegalnym Farmaceutykom

Przestępczość farmaceutyczna jest złożonym zjawiskiem kryminalnym, obejmującym szereg zagrożeń, takich jak: przemyt, nielegalne przekierowanie, kradzieże (kargo, wewnętrzne w firmach farmaceutycznych, hurtowniach i aptekach) oraz fałszowanie aktywnych surowców farmaceutycznych, gotowych produktów leczniczych i wyrobów medycznych. W okresie pandemii COVID-19 zaobserwowano narastanie nowych tendencji w przestępczości farmaceutycznej, które stają się coraz poważniejszymi problemami bezpieczeństwa zdrowia publicznego w Europie. Zjawiskami kryminalnymi ściśle związanymi z przestępczością farmaceutyczną są również: pranie „brudnych” pieniędzy, korupcja, cyberprzestępczość, terroryzm i przestępczość korporacyjna. Pandemia, a potem wojna w Ukrainie wywołały dramatyczne zmiany w globalnym przestępczym półświatku. Kartele narkotykowe i zorganizowane grupy przestępcze zajmujące się nielegalnym wytwarzaniem i handlem produktami medycznymi, substancjami psychoaktywnymi i narkotykami stanęły w obliczu zerwanych łańcuchów dostaw, malejących przychodów, zmieniających się rynków i konieczności zmiany modus operandi. Niestety przestępcy bardzo szybko przystosowali się do nowych warunków i dostosowali swoją „ofertę” do zmieniających się zapotrzebowań potencjalnych klientów, jednym z których było wzrastające pozamedyczne stosowanie leków.

Jest to szeroka kategoria, która odnosi się do stosowania leku psychoaktywnego do samoleczenia, w celach rekreacyjnych lub wzmacniających, z receptą lekarską lub bez niej, ale poza przyjętymi wskazaniami medycznymi. W państwach UE są to przede wszystkim: środki uspokajające i nasenne, w tym barbiturany, benzodiazepiny i leki benzodiazepinopodobne, takie jak leki typu „Z” (zopiklon, zolpidem, zaleplon, eszopiklon); opioidy, w tym leki przeciwbólowe i leki stosowane w leczeniu agonistów opioidów; stymulanty przepisywane w leczeniu zespołu nadpobudliwości psychoruchowej z deficytem uwagi (ADHD).

Problem ten dotyczy również leków dostępnych bez recepty, które są stosowane w celach rekreacyjnych, takich jak syropy na kaszel zawierające kodeinę lub dekstrometorfan, niektóre leki zmniejszające przekrwienie (np. pseudoefedrynę), leki przeciwbiegunkowe (np. loperamid) oraz niektóre leki przeciwhistaminowe, takie jak prometazyna, chlorfenamina i difenhydramina. Aktualne dane EMCDDA wykazały, że coraz częściej mamy do czynienia z łączeniem kilku takich substancji w sfałszowanych lekach przeciwbólowych – gdzie opioidy w połączeniu z benzodiazepinami odpowiadają za połowę zgonów związanych z narkotykami we Francji i około jedną trzecią w Portugalii i Irlandii. Według CDC (US Centers for Disease Control and Prevention) w 2022 roku 108 tys. Amerykanów zmarło z powodu zatruć narkotykami, w tym wielu z nich sfałszowanymi lekami (Oxycodone, OxyContin, Percocet, Norco, Vicodin, Adderall, Xanax), przy czym 66% tych zgonów dotyczyło syntetycznych opioidów, takich jak fentanyl i połączenia fentanylu z ksylazyną.

Nowym starym problemem w Europie staje się pozamedyczne/rekreacyjne stosowanie podtlenku azotu, który ma szerokie zastosowania medyczne, przemysłowe, komercyjne i naukowe. Klasyfikowany jest on jako dysocjacyjny środek znieczulający. Jest tani i łatwy w zakupie i użyciu. Kluczem do jego rosnącej popularności jest powszechna dostępność małych, niedrogich wkładów podtlenku azotu używanych do produkcji bitej śmietany. Służą one do napełniania balonów imprezowych, z których następnie wdychany jest gaz, który wywołuje szybkie, ale krótkotrwałe uczucie euforii, relaksu, spokoju i poczucia oderwania.

„Analityka farmaceutyczna jako jedno z narzędzi w zwalczaniu przestępczości farmaceutycznej”

Opoka W.
Uniwersytet Jagielloński, Collegium Medicum

Jedną ze specjalizacji, którą farmaceuci mogą uzyskać to specjalizacja w dziedzinie analityki farmaceutycznej po odbyciu szkolenia specjalizacyjnego oraz po zdaniu egzaminu ustnego i praktycznego przed Państwową Komisją Egzaminacyjną Farmaceutów. Ponadto każdy farmaceuta, któremu zostanie uznany dorobek zawodowy i/lub naukowy za równoważny ze szkoleniem specjalizacyjnym w dziedzinie analityki farmaceutycznej przez Zespół Ekspertów może przystąpić do PESF, co wynika z zapisów Dz.U.2022.0.184 t.j. - Ustawy z dnia 10 grudnia 2020 r. o zawodzie farmaceuty (Art. 73 [Uznanie dorobku za równoważny ze zrealizowaniem programu szkolenia specjalizacyjnego] ustawy o zawodzie farmaceuty). Organem prowadzącym postępowanie w imieniu Ministra właściwego do spraw zdrowia jest Dyrektor CMKP na wniosek farmaceuty legitymującego się dorobkiem naukowym lub zawodowym w dziedzinie analityki farmaceutycznej. Po uznaniu dorobku za równoważny ze zrealizowaniem programu szkolenia specjalizacyjnego w dziedzinie analityki farmaceutycznej i sporządzeniu opinii przez dyrektora CMKP, zawierającej merytoryczne uzasadnienie, wnioskujący farmaceuta może przystąpić do egzaminu teoretycznego i praktycznego, a następnie po spełnieniu wszystkich formalnych wymogów uzyskuje tytuł specjalisty w dziedzinie analityki farmaceutycznej. Dotychczasowy program szkolenia specjalizacyjnego był zawarty w siedmiu modułach, ale ze względu na konieczność aktualizacji programu szkolenia specjalizacyjnego co 5 lat Zespół Ekspertów w składzie: prof. dr hab. n. farm. Włodzimierz Opoka – Konsultant Krajowy w dziedzinie analityki farmaceutycznej; prof. dr hab. n. farm. Marek Wesołowski, prof. dr hab. n. farm. Zbigniew Fijałek, prof. dr hab. n. farm. Anna Jelińska, prof. dr hab. n. farm. Anna Gumieniczek, prof. dr hab. n. farm. Ryszard Kocjan zaproponowali modyfikację dotychczasowego programu i wprowadzenie VIII modułu o nazwie: „Przestępczość farmaceutyczna i analiza farmaceutyczno-kryminalistyczna”.

W module tym ujęto treści teoretyczne i praktyczne, które będą stanowiły podstawowe zagadnienia i techniki stosowane w analizie farmaceutyczno-kryminalistycznej. Szkoleni farmaceuci będą zapoznawać się z podstawowymi zagadnieniami związanymi z przestępczością farmaceutyczną, technikami analitycznymi mającymi zastosowanie w analizie farmaceutyczno-kryminalistycznej, technikami analitycznymi w badaniu leków sfałszowanych, nielegalnych i substandardowych. Ponadto specjaliści będą opracowywać, walidować i stosować instrumentalne metody analizy wykorzystując je w analizie farmaceutyczno-kryminalistycznej, intepretować wyniki badań analitycznych zgodnie z zasadami i regulacjami prawnymi, a także przygotowywać protokoły z badań analitycznych jako elementy opinii biegłego dla organów ścigania oraz będą interpretować dane w zakresie bezpieczeństwa produktów i określać zagrożenia dla pacjentów/konsumentów.

,,Bezpieczne leki dla zwierząt warunkiem bezpiecznej żywności”

Andrzejewska A.
Wiceprezes ds. Produktów Leczniczych Weterynaryjnych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych

„Odpowiedzialność prawna za jakość leku – wybrane zagadnienia”

Jop K.
Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych

„The usefulnes of mass spectrometry for the analysis of drugs of abuse in complex analytical matrices and estimation of the scale of non-medical use of selected drugs”

Plewa S., Klupczyńska-Gabryszak A., Matysiak J.
Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland

Mass spectrometry is a modern analytical technique that, thanks to its high sensitivity, selectivity, and low detection limit, has numerous applications in analyzing complex biological materials. These advantages translate into attempts to use spectrometry as an effective tool for estimating the scale of use of selected substances by a given population. One of the types of research currently used to monitor trends in the consumption of selected intoxicating substances is wastewater analysis. It involves using a sample of sewage water to measure the concentration of selected analytes or metabolites of active substances in order to estimate the amount of a given substance per area from which the sewage sample is obtained and then per population inhabiting a given area. This type of research provides a reliable picture of the relative scale of drug use by a given population, constituting an objective source of information. This is a great advantage over conventional sources of knowledge such as questionnaires, surveys, and interventions. It can be a complementary source of knowledge to law enforcement agencies and state administration activities. Additionally, the analysis of daily differences in concentrations allows you to reflect prevailing trends and examine variability, e.g. between days of the week or between weekdays and the weekend. Thanks to its selectivity, mass spectrometry can be used to analyze not only substances classified as drugs but also to study the scale of drug consumption. Medicines are increasingly becoming substances used in the so-called ‘recreational use’. It is often used to induce a state of intoxication, euphoria, excitement or sedation. The presented examples proved the validity of using modern methods based on mass spectrometry to estimate the scale of consumption of intoxicating substances and the benefits that result from it.

„Przestępczość w środowisku farmaceutycznym – studium przypadku”

Woźniak P.
Centralne Biuro Śledcze Policji

„The use of high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS) to identify pharmacologically active substances in falsified medicinal products and dietary supplements”

Kalicka A.[1], Stępień K.[1]*

[1]Department of Drug Chemistry, Pharmaceutical and Biomedical Analysis, Faculty of Pharmacy, Medical University of Warsaw, Poland.

Counterfeiting of medicinal products is a serious public health problem around the world. The scale of this phenomenon increases every year. The World Health Organization warns that as many as 50% of medicines offered on the Internet are fake, and in developing countries, they constitute about 10% of all medicines. Currently, the most frequently counterfeited group of drugs includes, among others, anabolic-androgenic steroids, preparations used for erectile dysfunction, and weight loss aids. Falsified medicinal products may contain the incorrect dose of the active substance and various impurities. Very often, the composition declared on the packaging differs significantly from the actual one. Therefore, they may pose a threat to the health and even life of people using this type of preparation.

A patient taking a medicinal product is informed that it contains legally regulated substances that affect his body and may cause side effects. There is no such information in the case of the sale and advertising of dietary supplements, which, according to the legal definition, are only intended to supplement our diet. The addition of strong substances to falsified dietary supplements poses a high risk to consumers. It may cause not only strong side effects but also interactions with other medications or supplements. In turn, the use of counterfeit dietary supplements by athletes carries not only health consequences but also the risk of being accused of doping.

Medicinal products and dietary supplements confiscated by law enforcement authorities should be subjected to a detailed pharmaceutical and forensic analysis. To identify the composition of the tested sample, it is necessary to use advanced and expensive analytical methods. High-performance liquid chromatography coupled with mass spectrometry is often used for this purpose.

„Spektroskopia i mikrospektroskopia UV-VIS-NIR / FTIR / Raman w badaniu środków leczniczych oraz szybkiej identyfikacji mobilnej, narkotyków, suplementów diety oraz substancji sfałszowanych”

Kowalski K.
Medson, Poznań

„Comparison of PCA, ASCA, and PLS-DA in experimentally designed lipidomic studies”

Kupcewicz B.[1], Bogusiewicz J.[2], Burlikowska K.[2], Bojko B.[2]*

[1]Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz

[2]Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz

Chemometrics offers many techniques necessary to extract analytically relevant information from multidimensional data and is widely used in omics, especially untargeted analysis. However, in multivariate experimental designs, these methods cannot answer all the questions posed in this type of research, requiring the consideration of relationships between different groups. The solution is to use multidimensional data analysis methods considering the induced and correlated data structure obtained during the designed experiment. The work aims to show a holistic approach to the analysis of multidimensional lipidomic data provided using both exploratory, un-supervised method principal factor analysis (PCA), supervised discriminant analysis PLS-DA, and method combining the statistical advantages of analysis of variance (ANOVA) and simultaneous factor analysis (SCA) – ASCA method. The ASCA method is particularly applicable when the interest is in observing the effects of several interacting experimental factors in multi-factor "omics" projects. Examples of data from two experiments with different groups and factors were used. Experiment 1: analysis of lipidomic profiles of four organs (brain, liver, kidneys, and thigh muscles) collected from 15 adult male mice (12 weeks old) - five each of three strains: BALB/c, C57BL/6, and CD1. Animals were housed in a controlled environment: temperature 22 ± 2 ◦C, 12-hour light-dark cycle, humidity 55 ± 10%, with standard mouse food and water available ad libitum. Samples for chromatographic analysis were collected using the solid phase microextraction (SPME) technique. Experiment 2: analysis of lipidomic profiles of two types of histologically different brain tumors (seven meningiomas and seven gliomas). Sampling with SPME fiber was done twice, the first immediately after tumor removal and the second after 12 months of storing the tumors at -30°C. Lipidomic profiles were then analyzed using PCA and PLS-DA techniques and statistical and chemometric analysis using the ASCA method. Calculations were performed using PLS-Toolbox 7.5 and Matlab R2022b.

„Recombinant human prolidase induces wound healing in experimental models”

Miltyk W.
Department of Pharmaceutical and Biopharmaceutical Analysis, Medical University of Bialystok

"Exercise-induced concentration of 42 plasma free amino acids in endurance- and sprint-trained athletes"

Kusy K.[1], Matysiak J.[2], Kokot ZJ[3], Ciekot-Sołtysiak M[1], Klupczyńska-Gabryszak A[2], Zarębska E.A.[1], Plewa S.[2], Dereziński P.[2], Zieliński J[1]*

[1]Department of Athletics Strength and Conditioning, Poznan University of Physical Education, 61-871 Poznań, Poland

[2]Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, 60-806 Poznań, Poland

[3]Faculty of Health Sciences, Calisia University - Kalisz, 62-800 Kalisz, Poland

Circulating blood is an important plasma free amino acids (PFAAs) reservoir and a pivotal link between metabolic pathways. No comparisons are available between athletes with opposite training adaptations that include a broader spectrum of both proteinogenic and non-proteinogenic amino acids, and that include skeletal muscle mass. We hypothesized that the levels of the exercise-induced PFAAs are related to the type of training-related metabolic adaptation. We compared highly trained endurance athletes (n=11) and sprinters (n=10) aged 20–35 years who performed incremental exercise until exhaustion. Venous blood was collected before and during the test and 30-min recovery (12 samples). Forty-two PFAAs were assayed using LC-ESI-MS/MS technique. Skeletal muscle mass (SMM) was estimated using dual X-ray absorptiometry method. Glutamine and alanine were dominant PFAAs throughout exercise and recovery (~350–650 µmol/l). Total, combined proteinogenic, non-essential, and non-proteinogenic SMM-adjusted PFAAs levels were significantly higher in endurance than sprint athletes (ANOVA group effects: $p=0.007$, $\eta2=0.321$; $p=0.011$, $\eta2=0.294$; $p=0.003$, $\eta2=0.376$; $p=0.001$, $\eta2=0.471$, respectively). The response was more pronounced in endurance athletes, especially for non-proteinogenic PFAAs (ANOVA interaction: $p=0.038$, $\eta2=0.123$). Significant between-group differences were observed for 19 of 33 PFAAs detected, including 4 essential, 7 non-essential, and 8 non-proteinogenic ones. In conclusion, PFAAs response to incremental exercise is associated with the type of training-related metabolic adaptation. A greater turnover and availability of circulating PFAAs for skeletal muscles and other body tissues is observed in endurance- than in sprint-trained individuals. Non-proteinogenic PFAAs, despite low concentrations, also strongly respond to exercise loads, indicating their important, though less understood role in exercise metabolism. Our study provides additional insight into the exercise-induced physiological response of PFAAs and may provide a rationale in discussions regarding dietary amino acid requirements in high-performance athletes. (The project was funded by the National Science Centre Poland, grant OPUS 14 No. 2017/27/B/NZ7/02828. Manuscript under review in PLOS ONE.)

"Single-cell image analysis in ovarian cancer patients"

Zaborowski M.
Gynecologic Oncology Department, Poznan University of Medical Sciences, 33 Polna Street, 60-535, Poznan, Poland

„CardioCarePack as a modern solution for therapy monitored by drug concentration”

Krupczyńska – Stopa K.[1], Szewczyk R.[1], Lenartowicz A.[1], Mironenka J.[1], Soboń A.[1], Stopa M.[1], Kalinowski L.[2], Radulska A.[2], Borkowski T., Marciniak E.[2]*

[1]LabExperts Sp. z o.o. Gdansk,

[2]Gdansk Medical University, Department of Clinical Analytics

Before the active substance goes into production and starts its career as a medicinal product, many years of work pass. It is difficult to count the number of qualitative and quantitative analyses that accompany the development of a medicinal product. Both solvent solutions and biological samples are analyzed – there is no shortage of challenges. Long and thoughtful clinical trials are the basis for planning future therapies. After the introduction of the drug to the pharmaceutical market, it is enough to maintain the correct production regimen, quality control and therapeutic success is guaranteed. Nothing could be more illusory; it is necessary to support the process of effective treatment of the patient to reduce the side effects by implementing Therapeutic Drug Monitoring (TDM). For this, an analysis of the patient's blood or urine is necessary. Liquid chromatography coupled with mass spectrometry (LC-MS/MS) is one of the techniques used to determine the concentration of a drug and its metabolites in biological samples. There are more and more literature reports that can be used in TDM, but as we know, most drug therapies are carried out according to the usual drug administration schemes, and not based on actual concentrations of medicinal substances in the patient's body.

The success of the test of the substances' concentration in the patient's body consists not only of the analytical part, but also the procedure for collecting biological material and the interpretation of the result, medical measures taken. We will present a solution that includes quantitative collection of capillary blood for testing using the Mitra® sampler, its analysis for the presence of selected antiarrhythmic drugs using the LC-MS/MS technique and an IT solution fastening with a brace. Other analytical procedures that have already been used in drug-monitored therapy for the health and safety of patients will be presented.

„Paradoksy analityki surowca konopnego”

Cielecka-Piontek .J [1,2]

[1]Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

[2]Department of Pharmacology and Phytochemistry, Institute of Natural Fibres and Medicinal Plants, Wojska Polskiego 71b, 60-630 Poznan, Poland

Hemp raw material contains numerous secondary metabolites characterized by specific pharmacological activities. The most important ones include cannabinoids, terpenes and flavonoids. According to current pharmacopoeial guidelines (based on the monograph for hemp flower in the German Pharmacopoeia), hemp female inflorescence should have controlled content of cannabinoids: cannabidiolic acid, cannabidiol, cannabinol, Δ8-tetrahydrocannabinol, Δ9-tetrahydrocannabinol and Δ9-tetrahydrocannabinolic acid. The recommended analytical method for the separation and determination of these cannabinoids is the HPLC-DAD method.

However, it should be borne in mind that the bioavailability of cannabinoids and the strength of their pharmacological action depend on their co-presence and the presence of terpenes. However, terpenes, as volatile compounds, require the use of the GC-FID method for their analysis. Considering the above, it is important to recommend the analysis of hemp raw material based on the combined use of various chromatographic techniques. An approach based on the use of various analytical techniques in assessing the quality of hemp raw materials will allow obtaining the closest approximations between the results of analytical tests and the biological activity profiles of the tested hemp raw materials.

Current guidelines for the analysis of hemp raw material also require the definition and clarification of procedures for preparing hemp raw material for analysis, as well as the stability conditions and the impact of changes in the terpene profile on the quality of hemp raw material. It is also important to define procedures for preparing products from hemp raw material and obtaining specific pharmaceutical forms with them, ensuring appropriate bioavailability of individual cannabinoids.

„The root locked in the past – the fruit that opens the future? Pharmacological perspective on the use of Eleutherococcus senticosus fruits”

Załuski D.
Department of Pharmaceutical Botany and Pharmacognosy, Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University, 9 Marie Curie-Skłodowska Street, 85-094 Bydgoszcz, Poland
e-mail: daniel.zaluski@cm.umk.pl
phone: (+48) 512224920

As a major part of Western medicine has been developed from traditional knowledge, it makes sense to take a closer look at our ancestors' knowledge and study that with all the novel concepts and source of the plant material.

In the ethnomedicine of Russia and China, the Eleutherococcus senticosus (Rupr. et Maxim.) Maxim. fruits and roots are used to treat immune-related diseases. Because of the overexploitation of the roots, the species is considered to be endangered and was put on the Red List in some countries (i.e. the Republic of Korea). In this case, the aerial parts of E. senticosus might be considered as a new sustainable source of compounds with an immunostimulative activity.

The current research are aimed to evaluate the safety profile and adaptogenic activity of the E. senticosus fruits in order to support the use of the fruits in folk medicine and in a modern phytotherapy in the EU’s countries. It should give a preliminary answer the question, whether there are possibilities to cultivate this plant in the EU to obtain the raw material on a larger scale?

Our observations justify the traditional use of the fruits, and the fruits are rather safe. The fruits have a potential as a renewable plant-based adaptogen, and the species possibly of an interest for a cultivation in Western Europe. However, further studies in in vivo model regarding a bioavailability are needed to meet all the EU criteria about the plant-based drugs and medicines used in traditional healing systems.

„Współczesne wyzwania w analizie związków pochodzenia naturalnego działających w kierunku centralnego układu nerwowego”

Skalicka – Woźniak K.
Department of Pharmacognosy with Medicinal Plant Unit, Faculty of Pharmacy, Medical University of Lublin

„Cyclodextrins as versatile scaffolds in pharmacy”

Béni Sz.
Department of Analytical Chemistry, Institute of Chemistry, ELTE - Eötvös Loránd University
Address: H-1117 Pázmány Péter stny. 1/A., Budapest, Hungary
Email: szabolcs.beni@ttk.elte.hu

Cyclodextrins (CDs) emerge as dynamic scaffolds in pharmacy, showcasing their pivotal role in analytical chemistry through cyclodextrin modified capillary zone electrophoresis. This presentation will underscore a validated enantioseparation method for tapentadol enantiomers and the chiral separation of alogliptin enantiomers. The spotlight on single isomer CD derivatives accentuates their superiority in achieving enantioselectivity.

We also explore the acid-base properties of persubstituted cyclodextrins, exemplified and presented by the semisynthetic derivative Sugammadex (Suγ-CD). Through 1H NMR-pH titrations, we unveil pKa values and delve into charge-dependent molecular recognition of polyanionic and polycationic CDs. The polycationic heptakis(6-amino-6-deoxy)-beta-CD demonstrates complex formation with the anionic synthetic pentasaccharide drug fondaparinux, presenting exciting possibilities in charge-dependent molecular recognition and offering a potential stabilizer for heparin oligosaccharides.

Expanding the versatility of cyclodextrins, Suγ-CD proves its efficacy as a selective relaxant binding agent for aminosteroid-type anesthetics. Pushing boundaries, Suγ-CD showcases its ability to form stable host-guest complexes with toxic glycoalkaloids from the Solanaceae family. This opens up a novel application as an antidote for plant intoxication.

In conclusion, this presentation will underscore the indispensable role of CDs in pharmacy, providing not only precise characterization and chiral separation but also paving the way for potential therapeutic applications across diverse pharmaceutical domains.

„Methods for optimisation and analysis of lipid nanoparticles”

„Metody optymalizacji I analizy nanocząstek lipidowych”

Zielińska A.
Institute of Natural Fibres and Medicinal Plants - National Research Institute, Poznań (Poland)

Introduction: Optimization and analysis of lipid nanoparticles are crucial steps in developing drug delivery systems and other applications. 'The first generation' consists of Solid Lipid Nanoparticles (SLN), and 'the second generation' includes Nanostructured Lipid Carriers (NLC), which can be applied for targeted drug delivery to specific sites in the body [1-3]. They are also successfully used as pharmaceutical vehicles for poorly water-soluble drugs. Lipid nanocarriers are of great interest as effective carriers to encapsulate natural extracts to increase the physicochemical stability of bioactive. Thus, cannabidiol (CBD) derived from cannabis extract exhibits a range of potent therapeutic properties, including anti-inflammatory, antioxidant, and neuroprotective properties [1,2].
Aim: After producing Compritol-based nanoparticles loaded (cSLN, cNLC) with CBD, this study aimed to characterize the physicochemical properties, performing methods for optimization and analysis. Therefore, the determination of particle size (Z-Ave), polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE), and loading capacity (LC) were calculated. The viscoelastic profiles, differential scanning calorimetry (DSC) patterns, and scanning electron microscopy (SEM) were also performed [1].
Results: Based on statistical analysis, the most optimal composition was selected for synthesizing CBD-cSLN and CBD-cNLC. As a result, CBD-loaded SLN showed a Z-Ave of 217.2 ± 6.5 nm, PDI of 0.273 ± 0.023, and EE of about 74%, while CBD-loaded NLC showed the Z-Ave of 158.3 ± 6.6 nm, PDI of 0.325 ± 0.016 and EE of about 70%. Furthermore, the crystallinity profiles of CBD-cSLN (90.41%) and CBD-cNLC (40.18%) were determined. This effect can be explained by the encapsulation parameters obtained while confirming the liquid nature shown by the rheological analysis. On the other hand, SEM confirmed the morphology and shape of the developed nanoparticles. The agglomeration process of the nanoparticles was excluded, indicating the sample's stability. In the present project, the solid nature and morphology of cSLN/cNLC have been shown to enhance the potential of these nanoparticles to modify the delivery profile of CBD for various biomedical applications.
Conclusions: Optimization of lipid nanoparticles can include changes in lipid composition, manufacturing process, emulsion conditions, and other factors to achieve desired properties such as stability, bioavailability, or ability to deliver the active ingredient to a specific site in the body. In turn, analysis of lipid nanoparticles allows the effects of these changes to be assessed and whether they meet the desired criteria. Both approaches are crucial in many fields, such as pharmaceuticals, cosmetics, and biotechnology.
Funding: This research was funded by the MINIATURA-4 grant (2020/04/X/ST5/00789) of the National Science Centre, within the START 2021 Programme of the Foundation for Polish Science (FNP), and by IOI Oleo GmbH, Germany, which provided all the lipids used in the study.
References: [1] Zielińska A, et al. (2023). Phytocannabinoids: Chromatographic Screening of Cannabinoids and Loading into Lipid Nanoparticles. Molecules, 28(6), 2875. [2] Zielińska A, et al. (2023). Tocilizumab-coated solid lipid nanoparticles loaded with cannabidiol as a novel drug delivery strategy for treating COVID-19: A review. Frontiers in Immunology, 14, 1315. [3] Eder P+, Zielińska A+, et al. (2021). How could nanobiotechnology improve treatment outcomes of anti-TNF-α therapy in inflammatory bowel disease? Current knowledge, future directions. Journal of Nanobiotechnology, 19(1), 1-14.

„Extractions of aerial parts of Hippomarathrum scabrum with conventional and green methodologies: Chemical profiling, antioxidant, enzyme inhibition and anti-cancer effects"

*Nilofar N.[1,2], Duran T.[3], Uba A.I.[4], Cvetanović Kljakić A.[5], Božunović J.[6], Gašić U.[6], Bouyahya A.[7], Yıldiztugay E.[8], Ferrante C.[2], Zengin G.[1]**

[1] Department of Biology, Science Faculty, Selcuk University, Campus, Konya, Turkey
[2] Department of Pharmacy, Botanic Garden "Giardino dei Semplici", Università degli Studi "Gabriele d'Annunzio", via dei Vestini 31, 66100 Chieti, Italy
[3] KTO Karatay University, Faculty of Medicine, Department of Medical Genetics, Karatay, Konya, Turkey, ORCID: 0000-0002-7353-4527
[4] Department of Molecular Biology and Genetics, Istanbul AREL University, Istanbul 34537, Turkey
[5] Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
[6] Department of Plant Physiology, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia
[7] Department of Biotechnology, Science Faculty, Selcuk University, 42079 Konya, Turkey
[8] Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat 10106, Morocco

Hippomarathrum scabrum L. is an endemic medicinal plant in Turkey; however, there have been few studies investigating the phytochemistry and biological properties of these plant has not been investigated. The aim of this work is to determine the chemical composition of different extracts (extracts obtained by using supercritical carbon dioxide extraction, accelerated solvent extraction, homogenizer-assisted extraction, microwave-assisted extraction, and ultrasound-assisted extraction from Hippomarathrum scabrum L., and evaluate their biological properties. The analysis revealed that 5-O-caffeoylquinic acid, rutin and isorhamnetin 3-O-rutinoside were main bioactive compounds. Extract obtained by accelerated extraction contains the highest concentration of 5-O-Caffeoylquinic acid (7616.74±63.09 mg/kg dry extract) followed by the extract obtained by homogenizer-assisted extraction (6682.53±13.04 mg/kg dry extract). In antioxidant tests, all extracts expressed significant antioxidant activity. Also, cytotoxic and anticancer effects of these plant extracts were detected on the human prostate cancer cell line. Intrinsic apoptotic genes were up-regulated and anti-apoptotic genes were down-regulated in human prostate cancer cells after inhibition concentration dose treatment. The findings are promising, suggest the use of these plant extracts could be used as natural sources with different biological activities, as well as anticancer agents.

„Analysis of honeys from Malta"

Attard E.
Rural Sciences & Food Systems
Institute of Earth Systems
Rural Sciences Farmhouse
University of Malta

Honey is generally implicated as a food supplement and it is generally used extensively for its culinary properties. However, honey possesses significant antimicrobial and antioxidant properties and thus has been used commercially in medicines and cosmetics. The island of Malta has been renowned for millennia for its honey production. The name 'Malta' derives from the Greek word 'Melite' which is 'honey-sweet'. In spite of its general composition, in terms of sugars, bee enzymes, nectar and pollen, the general composition of honey and specific marker substances attribute specificity to different honey types. Malta is gifted with three honey seasons; the multi-floral spring honey, thyme summer honey, and carob and eucalyptus autumn honey. For these honey types, we investigated the parameters as set in Council Directive 2001/110/EC, the polyphenolic content and antioxidant activity. Autumn honey was distinctive from the other honey types, by its lower sugar and bee enzyme contents, and higher pH and conductivity values. Although it also possesses higher polyphenolic content, the three seasonal honey types predominate in specific polyphenols. Principal Component Analysis reveals the clustering of seasonal honey types by their physicochemical characteristics, polyphenolic content, and antioxidant activity. This study provides background information to local beekeepers and authorities for the maintenance of good practices in the hive and the potential certification of Maltese honey.

„Investigations of chemical composition and pharmacological activity of nine taxa of the genus Heracleum L. (Apiaceae) from Southeastern Europe"

Ušjak L.
University of Belgrade – Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11221 Belgrade, Serbia

This research included all nine taxa of genus Heracleum (Apiaceae) (three of which are endemic) from Serbia, Montenegro, North Macedonia and Slovenia (60 samples of various plant organs collected from 14 localities in period 2009-2016): H. sphondylium, H. sibiricum, H. montanum, H. ternatum, H. pyrenaicum subsp. pollinianum, H. pyrenaicum subsp. orsinii and H. verticillatum, belonging to group H. sphondylium, and H. orphanidis, all from sect. Heracleum, as well as H. austriacum subsp. siifolium from sect. Wendia.

GC-FID-MS analysis of essential oils, isolated by hydrodistillation, revealed that underground parts oils were dominated by monoterpenes and/or phenylpropanoids (H. sphondylium group), or (Z)-falcarinol (H. orphanidis and H. austriacum); fruit oils by aliphatic esters octyl acetate (sect. Heracleum) or octyl hexanoate (H. austriacum), and leaf and flower oils by aliphatic esters (H. orphanidis) or sesquiterpenes and/or phenylpropanoids. Since potentially phototoxic furanocoumarins were detected in some of these oils, their safety profiles (maximum daily intakes) were estimated. Using LC-MS, and 1H and ROESY NMR spectroscopy, in dichloromethane extracts of underground parts and fruits, 12 furanocoumarins were identified; dominant were pimpinellin, bergapten, byakangelicol, heraclenin and/or imperatorin. In fruit fatty oils, fatty acids (petroselinic acid was dominant), sterols (β-sitosterol was prevalent) and triterpenes were investigated using GC-FID-MS. Results were analyzed using multivariate statistical methods (PCA, nMDS and UPGMA).

For 30 selected essential oils, broad antimicrobial spectra, i.e., activity against 8 bacteria and 8 fungi (by microdilution method), for 23 oils, cytotoxic effect on cancer HeLa, LS174 and/or A549 cells (by MTT test; selectivity was shown on normal MRC-5 cells), and for 8 oils, anti-DPPH activity, were demonstrated. Most notably, against as many as 14 tested microorganisms, at least one investigated essential oil exhibited pronounced activity (MIC<100 μg/mL). Also, 13 essential oils satisfied National Cancer Institute-NCI criterion for cytotoxicity (IC50<30 μg/mL) against at least one cancer cell line.

Acknowledgements
Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant numbers: 173021, 451-03-68/2020-14/200161, 451-03-9/2021-14/200161, 451-03-68/2022-14/200161 and 451-03-47/2023-01/200161).

"Incidental Nanoparticles: The Functional Units of Traditional Chinese Medicine"

Ke L.
School of Food Science and Nutrition, University of Leeds, Woodhouse, Leeds, LS2 9JT, U.K.

Of many exciting fields of future scientific research, nanomedicine attracts a great interest with its transformative potential in solving major problems in disease diagnosis and treatment, and curing the incurable. The fabrication of nanoparticles with multiple functions usually requires sophisticated processes controlled at high precision. On the other hand, traditional Chinese medicine (TCM) has been developed and practised over hundreds of years and shown convincing clinical efficacy in treating infectious and non-communicable diseases, while its therapeutic components and mechanism still remains largely unrecognised. The sheer number of the constituent molecules together with the matrix effects have made even the simplest TCM system enigmatic. Over 90% of TCM are used in the form of decoction, which is a system of multiphase dispersions. Large number of nanoparticles are found in the decoction. They could be small vesicles like exosomes, or complexes of proteins, polysaccharides and small molecules. These nanoparticles were either released from the cellular structure of medicinal materials (e.g., exosome) or self-assembled by the amphiphilic compounds when they are migrating from the cellular structure to the aqueous phase of decoction during the boiling water extraction. One example is the nanoparticles of polyphenol oxidase (PPO) which are negatively charged, stable in aqueous solution and carry caffeine, gallic acid and catechins. The PPO nanoparticles derived from Chinese black tea elevated both extracellular and intracellular antioxidant activities, restored macrophages' cell membrane potential and mitochondrial oxygen respiration, fortified the efficiency of polyphenols and caffeine in restoring the intercellular connection of intestinal epithelium, inhibiting mucosal inflammation, and alleviating ulcerative colitis. The nanoparticles of glycated PPO from Alisma orientale exhibits potential of the organ-targeting delivery vehicle for bioactive phytochemicals. These nanostructures offer not only a novel perspective to understanding the complicated system of traditional medicine, but also the prototypes of precise and multifunctional nanomedicine.

„Analiza biomasy odpadowej wykorzystywanej w syntezie adsorbentów węglowych”

Bazan-Woźniak A., Pietrzak R.
Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemii, Zakład Chemii Stosowanej, ul. Uniwersytetu Poznańskiego 8, 61-614 Poznań

Nieustanny rozwój przemysłu, a także wzrost liczby ludności powodują rosnącą emisję zanieczyszczeń uwalnianych do środowiska. Substancje organiczne, produkty farmaceutyczne, związki takie jak tlenki azotu i siarki, siarkowodór to tylko część zanieczyszczeń, które przyczyniają się do degradacji ekosystemów wodnych i zanieczyszczeń powietrza. Z tego punktu patrzenia ważne jest nie tylko, ograniczenie ilości emisji zanieczyszczeń, ale również skuteczne i efektywne usuwanie ich ze środowiska. Jedną z możliwości jest wykorzystanie różnego rodzaju adsorbentów, które skutecznie będą usuwały zarówno gazowe i jak i ciekłe zanieczyszczenia. Z technologicznego punktu widzenia ważnym aspektem jest koszt produkcji wytworzonego materiału porowatego. Najczęściej wykorzystywanymi materiałami są węgle aktywne, dodatkowo można je otrzymywać z bardzo szerokiej gamy różnych surowców, a także z bioodpadów. Podczas wykładu zostaną przedstawione metody analizy technicznej i elementarnej biomasy odpadowej, która jest wykorzystywana w syntezie węgli aktywnych. Poruszona zostanie kwestia oznaczania wilgoci i substancji mineralnej w strukturze bioodpadów. Ponadto zostanie zaprezentowana metoda analizy elementarnej, która pozwala określić skład procentowy poszczególnych pierwiastków wchodzących w skład surowca naturalnego. Przedstawiona zostanie również metoda miareczkowania Boehma, która pozwala na określenie właściwości kwasowo zasadowych bioodpadów, co jest niezwykle istotne podczas usuwania zanieczyszczeń ciekłych i gazowych. Na koniec zostanie zaprezentowana i omówiona norma pozwalająca na określenie liczby jodowej bioodpadów i węgli aktywnych. Parametr ten pozwala określić przydatność tego typu materiałów do usuwania zanieczyszczeń o rozmiarach zbliżonych do 1 nm.

„Influence of smoking status on metabolic profiles of lung cancer patients - from cancer marker discovery perspective”

Klupczynska-Gabryszak A [1], Daskalaki E.[2], Wheelock C. E.[2,3], Kasprzyk M.[4], Dyszkiewicz W.[4], Grabicki M.[5], Batura-Gabryel H.[5], Kokot Z. J.[6], Matysiak J.[1]*

[1]Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poznan, Poland
[2]Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[3]Departmentof Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden
[4]Department of Thoracic Surgery, Poznan University of Medical Sciences, Poznan, Poland
[5]Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences, Poznan, Poland
[6]Faculty of Health Sciences, Calisia University, Kalisz, Poland

The study aimed to perform serum metabolomics analysis of newly diagnosed lung cancer (LC) patients and patients with chronic obstructive pulmonary disease (COPD), which were selected as a control, non-cancer group. A novelty of the study is patient categorization according to their smoking status, which allowed us to distinguish molecular markers associated with lung cancer from alterations in the metabolic profile resulting from chronic tobacco smoking. We applied a multi-step biomarker selection workflow. First, a high-resolution quadrupole time-of-flight (Q-TOF) mass spectrometry-based metabolomics was used for untargeted profiling of a broad spectrum of metabolites in serum samples taken from LC patients and a non-cancer group. The putative LC markers were then verified using targeted triple-quadrupole mass spectrometry-based methodologies and then validated using a new set of samples. A total of 155 LC patients were included in our study. Patients were divided based on their smoking status and belonged to either the discovery set or the validation set. Each LC subgroup had the highest percentage of patients in stage I cancer, which allowed us to study changes in metabolic profiles occurring in the early stage of the disease. Patients' stratification based on smoking status impacted the discriminating ability of the identified metabolite LC marker candidates. Our research showed that studying the influence of strong environmental factors, such as exposure to smoking, should be considered in cancer marker research since it allows us to eliminate false positives and better understand the shifts in metabolite profiles in cancer patients.

The project received support from the National Science Centre, Poland (grant number: 2017/01/X/NZ7/02064).

„Quantification of prohibited substances in saliva using traditional and alternative microextraction-based sample-preparation methods coupled with LC-MS”

Goryński K.[1], Sobczak Ł.[2], Kołodziej D.[2], Jędrzejewska B.[1]*

[1]Bydgoszcz University of Science and Technology, Faculty of Chemical Technology and Engineering, Seminaryjna 3, 85-326 Bydgoszcz, Poland (gorynski@pbs.edu.pl)

[2]Nicolaus Copernicus University in Toruń, Faculty of Pharmacy, Jurasza 2, 85-089 Bydgoszcz, Poland

Oral fluid has gained significant interest as an alternative matrix for drug testing due to its easy and non-invasive collection. Despite these advantages, achieving suitably low limits of detection remains a clear challenge in the use of oral fluids for drug screening. Therefore, we demonstrate that the application of commercially available SPME fibers followed by liquid chromatography tandem mass spectrometry can enable the comprehensive detection and confirmation of drugs in oral fluid samples. To this end, we develop and test a sample-preparation protocol for a panel of 46 drugs covering the most popular drugs of abuse and doping agents available worldwide. Human saliva samples were collected using a Salivette® device (CE IVD certified) and sampled using SPME devices coated with a C18 extraction phase. The proposed protocol was validated with respect to its lower limits of quantification (LLOQ), linearity, matrix effects, precision, and extraction recovery. After analytical validation, saliva samples from volunteers were analyzed to determine free concentrations of cortisol at different times after awaking. Finally, a 3D-printed prototype device was designed and successfully applied to extract small molecules, thus demonstrating a new modern low-cost approach for bioanalysis.

The project received support from the National Centre for Research and Development under the Lider IX programme (grant LIDER/44/0164/L-9/17/NCBR/2018).

References:

Goryński K., Sobczak Ł. Analytica Chimica Acta, 2024, 1291, 342236.

"Critical issues in quantitative bioanalysis of Dried Blood Spot samples"

Malenović A.[1], Rmandić M.[1], Dotsikas Y.[2]

[1]University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450, Belgrade, Serbia

[2]Laboratory of Pharmaceutical Analysis, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zografou GR - 157 71, Athens, Greece

When using DBS as sample collection tool, several specific factors can contribute to assay bias and compromise regulatory-based acceptance criteria. Certain factors like hematocrit (Hct) level, pipettes, sample volume and laboratory personnel have a potential to contribute bioanalytical method bias inherently. The proper understanding of Hct effect and the efficient resolving of the related concerns may determine the future of DBS sampling practice in regulated bioanalysis. Therefore, we aimed at reaching a procedure that enables accurate and precise blood spotting onto the filter paper by simultaneous investigation of factors that were suggested to be scientifically relevant in this context. The effects of five qualitative factors - temperature of blood samples, type of pipettes, pipetting technique, age of blood samples and analyst - were investigated using a multilevel categorical D-optimal design. Five responses were observed in the study ($RSD_{22\%Hct}$, $RSD_{30\%Hct}$, $RSD_{39\%Hct}$, $RSD_{51\%Hct}$, $RSD_{62\%Hct}$) as they can provide information on the influence of factor settings on the consistency of DBS areas. DBS cards with four spot replicates, corresponding to particular combination of investigated factors defined by experimental plan, were scanned and the area of blood spots was determined by image processing. The principle of backward elimination was applied in computation of the adequate qualitative linear mathematical models with added appropriate two-factor interactions to relate selected responses with studied factors. It was concluded that %RSD value of DBS, regardless Hct levels, is completely independent of type of pipettes and age of blood samples, but can significantly be affected by blood sample temperature, pipetting technique and level of training of analyst. Consequently, the procedure for precise and accurate formation of DBS of uniform area, regardless the Hct value, implies samples at body/room temperature, reversed pipetting technique for rigorous delivery of a sample volume onto the card and a properly trained analyst for handling blood samples. The adequacy of the suggested procedure was confirmed by a verification experiment. Identification of the factors affecting the consistency of DBS formation provided the evidence that this contribution to total assay bias can be successfully controlled and reduced.

„Taurine activates p53-dependent and independent mechanisms to control cancer cell growth”

Iwanicki M.
Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, NJ, USA.

„Lipidomics and metabolomics with Machine Learning for biomarker identification in Meningioma”

Garrett T.
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA

Wystąpienia Młodych Naukowców

„Changes in Serum Protein–Peptide Patterns in Atopic Children Allergic to Plant Storage Proteins”

Kacper Packi [1,2,] Joanna Matysiak [3], Eliza Matuszewska [1], Anna Bręborowicz [4] and Jan Matysiak[1]

[1] Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, 60-780 Poznan, Poland

[2] AllerGen, Center of Personalized Medicine, 97-300 Piotrkow Trybunalski, Poland

[3] Faculty of Health Sciences, Calisia University—Kalisz, 62-800 Kalisz, Poland

[4] Department of Pulmonology, Pediatric Allergy and Clinical Immunology, Poznan University of Medical Sciences, 60-572 Poznan, Poland

Next to cow’s milk and eggs, plant foods, i.e., legumes, tree nuts and cereal grains, most often sensitise atopic children. Storage proteins constitutes the most relevant protein fraction of plant foods, causing primary sensitisation. They exhibit strong allergenic properties and immunogenicity. Our goal was to analyse sensitisation to 26 plant storage proteins in a group of 76 children aged 0–5 years with chronic symptoms of atopic dermatitis using Allergy Explorer ALEX2 and to discover changes in serum protein–peptide patterns in allergic patients with the use of MALDI-TOF-MS. We reported that 25% of children were allergic to 2S albumins, 19.7% to 7S globulins, 13.2% to 11S globulins and 1.3% to cereal prolamins. The most common allergenic molecules were Ara h 1 (18.4%), Ara h 2 (17.1%), Ara h 6 (15.8%) and Ara h 3 (11.8%) from peanuts, and the mean serum sIgE concentrations in allergic patients were 10.93 kUA/L, 15.353 kUA/L, 15.359 kUA/L and 9.038 kUA/L, respectively. In children allergic to storage proteins compared to the other patients (both allergic and non-allergic), the cell cycle control protein 50A, testis-expressed sequence 13B, DENN domain-containing protein 5A and SKI family transcriptional corepressor 2 were altered. Our results indicate that the IgE-mediated allergy to storage proteins is a huge problem in a group of young, atopic children, and show the potential of proteomic analysis in the prediction of primary sensitisation to plant foods. It is the next crucial step for understanding the molecular consequences of allergy to storage proteins.

„Apoptotic agents from the Endolichenic fungi against chemoresistant cancers”

Makhloufi H., Pinon A., Gibot-Leclerc L., Millot M., Champavier Y., Pinault E., Chemin G., Mambu L.*

Univ. Limoges, LABC*i*S, UR 22722; BISCEm, UAR 2015 US42, F-87000 Limoges, France.

Worldwide, cancer is a major cause of death, with breast, lung and colon cancers being the most common. Chemoresistance and the side effects of current treatment are a major obstacle to cancer therapy. The focus today is therefore on developing adjuvant therapies. The LABCiS laboratory is interested in chemodiversity of natural products isolated from endolichenic fungi, non-pathogenic fungi found in lichens. They offer a promising solution in the search for new bioactive compounds with anti-cancer potential.

The Endolichenic fungi (ELF) were previously isolated from lichens collected in Nouvelle Aquitaine Region (France). The objective is to highlight the antiproliferative potential of the Endolichenic extracts and their metabolites against colorectal cancer (HT-29) and triple-negative breast cancer (MDA-MB- 231). Extracts were obtained after the cultivation of ELF strains on 3 media on a small scale.

Screening of 20 Endolichenic extracts against chemoresistant cell line HT-29 highlighted their antiproliferative potential with IC50 values ranging from 2 to 60 µg/mL (MTT Test). EtOAc extracts of PA08S and XC04P were the most active with IC50 values lower than 6 µg/mL.

Solid state fermentation (10 L, SAB) of PA08 produced 5 g of EtOAc extract. Its Liquid-liquid extraction offered two fractions. The evaluation of their antiproliferative activity showed that methanol fraction is active and the hexane fraction has moderate activity with an IC50 Value of 3µg/mL and 16µg/mL respectively. The LC-MS/MS analysis of extract and fractions reveals very few matches in the MS database.

The isolation of the active compounds is in progress and their bioactivity will be tested through apoptosis studies on MDA-MB231 and HT-29 cells.

Conflict of interest: the authors declare no conflict of interest.

„Synthesis and vectorization of novel chalcones and derivatives with anticancer activity"

Letulle C.,[1] Sol V.,[2] Allais F.,[3] Othman M.,[1] Daïch A.,[1] Pouget Ch.,[2] Ata Lawson M.[1]*

[1]Normandie Univ, UNILEHAVRE, URCOM, UR 3221, INC3M, FR 3038 CNRS, F-76600 Le Havre, France

[2]Université de Limoges, Laboratoire LABCiS, UR 22722, Université de Limoges, F-87060 Limoges, France

[3]URD Agro-Biotechnologies Industrielles (ABI), CEBB (Centre Européen de Biotechnologie et de Bioéconomie), AgroParisTech, F-51110 Pomacle 22722, France

cecile.letulle@etu.univ-lehavre.fr

This work describes novel potent anticancer trimethoxylated chalcones and α-methyl chalcones synthesis and vectorization. Chalcones are privileged structures in medicinal chemistry that are accessible by several methodologies such as Claisen-Schmidt condensation. For a long time, the trimethoxylated ring A was thought to be important for anticancer activity by comparison to combretastatin A4 and colchicine which are two powerful antimitotic agents.[1] It was also shown that the introduction of a methyl group in the α position to the chalcone carbonyl group, in most cases led to an improved biological activity.[2] In this context, this study is devoted to the synthesis of new trimethoxylated chalcones and α-methyl chalcones by replacing the B-ring with other heterocycles.

The series of synthesized chalcones presented here would allow us to carry out the structure-activity relationship (SAR) studies. Moreover, it was envisioned, in order to increase the selectivity of synthesized chalcones towards cancer cells, a vectorization through a polyamine arm insertion on B-ring for active targeting and by including in nano-objects constituted of β-cyclodextrins and cellulose nanocrystals (β-CD/CNCx) for passive targeting. [3]

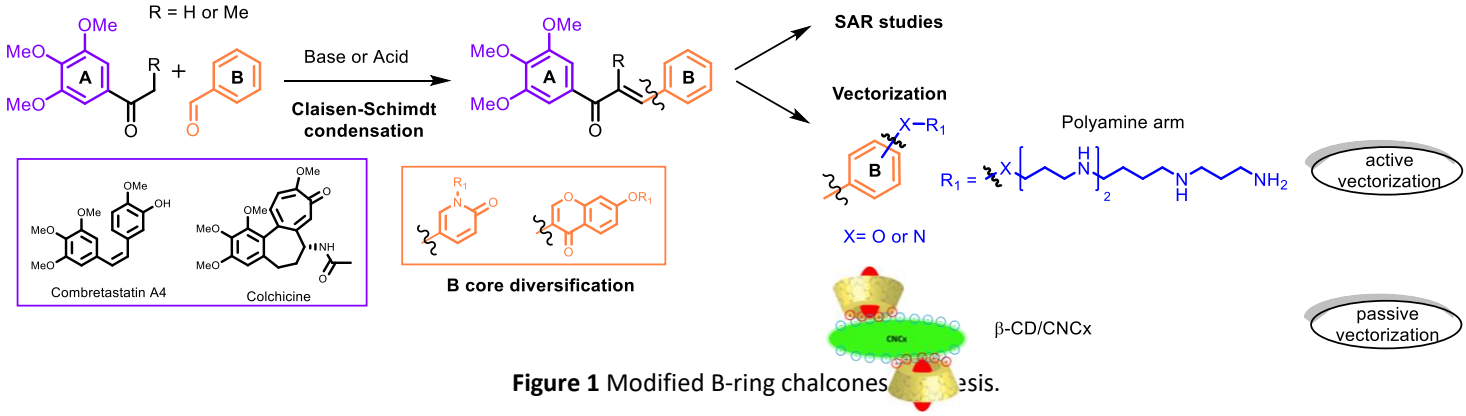


Figure 1 Modified B-ring chalcones synthesis.

References

[1] C. Zhuang, W. Zhang, C. Sheng, C. Xing, Z. Miao, *Chem. Rev.* **2017**, *117,* 7762.

[2] T-H. Do, D-M. Nguyen, V-D. Truong, T-H-T. Do, M-T. Le, T-Q. Pham, K-M. Thai, T-D. Tran, *Molecules.* **2016**, *21*, 329.

[3] B. Rioux, Synthèse et vectorisation de biomolécules type chalcone en vue d'une application anticancéreuse. Thèse d'Université de Limoges, **2016**.

„Analysis of amorphous dispersions of natural compounds obtained bySFC”

Sip Sz.
Department of Pharmacognosy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland.

"Development of procedure for the determination of β-estradiol and its metabolites in human plasma samples by LC-MS/MS technique"

Kaliszewska A.[1], Bastian P.[2], Bączek T.[1], Bukato K.[3], Górska-Ponikowska M.[2], Jakubczyk-Słabicka A.[2], Musiał C.[2], Winczewska Z.[2], Konieczna L.[1]*

[1]Medical University of Gdańsk. Departament of Pharmaceutical Chemistry, Hallera 107, 80-416

[2]Medical University of Gdańsk, Department of Medicinal Chemistry, Dębinki 1, 80-211

[3]Medical Uniwersity of Gdańsk, Division of Gynecology and Obstetrics, Smoluchowskiego 17, 80-214

Introduction: β-Estradiol and its metabolites play various physiological roles in human body, mainly they regulate female sex characteristics and menstrual cycle. Additionaly, estrogens can influence the development of various diseases. These compounds could be potentially used as biomarkers for conditions like different types of cancers or Parkinson's disease [1-3]. The aim of this study was the quantification of βE2 metabolites in plasma samples in order to asses the differences in their levels in healthy and people with different diseases.Methods: In this study we present a LC-MS/MS method for the determination of β-estradiol and its metabolites in biological samples. Analytes were isolated from the samples via solid phase extraction using C18 columns and methanol with 0.1% formic acid as the desorption solvent. The chromatographic separations was performed using a Poroshell C18 column (3.0 x 100 mm; 2.7 µm), thermostated at 40°C. Mobile phase A was water with 0.1% FA, phase B was methanol with 0.1% FA. Flow rate of the mobile phase was 0.5 mL/min. Results: Good linearity for the target analytes was obtained in the range of 0.1 – 50 ng/mL with R2 ranging from 0.9779 to 0.9939. Statistically significant difference (p-value < 0,05) in βE2 and 2-methoxyestradiol levels between two groups, healthy people and depression patients were observed. Some differences were noted between estrogen profiles in control group and patients with heart failure/after heart transplantation. Moreover, some divergences in estrogen levels were also identified between healthy volunteers and individuals with acne. Conclusion: Estrogens could act as biomarkers of lung cancer and Parkinson's diseases. The results obtained in this study suggest that these compounds could also play a part in the diagnosis of various diseases, such as acne, depression or heart failure.

"Development and validation of UPLC-MS/MS method for determination of rivaroxaban in dried blood spot samples"

Pawlak K.[1], Kruszyna Ł.[2], Karaźniewicz-Łada M.[1]*

[1] Department of Physical Pharmacy and Pharmacokinetics, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland;

[2] Department of Vascular and Endovascular Surgery, Angiology and Phlebology, Poznan University of Medical Sciences, Długa ½ St., Poznań, Poland.

Dried blood spot method (DBS) is alternative for blood sample collection. It is safer and more convenient for patients because requires a small amount of blood and can be conducted at home. Rivaroxaban is novel non-vitamin-K oral anticoagulant drug (NOAC) used to treat deep vein thrombosis and prevent blood clots in atrial fibrillation and following hip or knee surgery. Determination of rivaroxaban in DBS may be useful to provide safe and effective pharmacotherapy, due to interindividual variability in response to rivaroxaban treatment and the lack of specific analytical method for evaluation its pharmacological effect. The aim of this study was to develop a simple and fast UPLC-MS/MS method for the analysis of rivaroxaban in DBS, intended for therapeutic monitoring of rivaroxaban concentrations in patients with deep vein thrombosis. Isolation of rivaroxaban from DBS was performed with acetonitrile and 5% formic acid, using ultrasounds. Rivaroxaban and rivaroxaban-d4 (internal standard, IS) were separated on Zorbax Plus C18 column with mobile phase containing 0.1% formic acid both in acetonitrile and water (1:1, v/v). Detection of analytes was performed using a triple quadrupole mass spectrometer with electrospray ionization. The method was validated according to the ICH guideline. The overall run time was about 4 min, with elution of rivaroxaban and IS at around 2 min. Calibration curve of the drug was linear in the concentration range of 2-500 ng/ml with a correlation coefficient ≥ 0.993. Intra-day precision, expressed as coefficient of variation, was ≤7.66%. Intra-day accuracy, expressed as relative error, was ≤16,55%. Extraction recovery of rivaroxaban from DBS was approximately 52,18%. Carry-over and matrix effect were also evaluated. The UPLC-MS/MS method meets validation requirements for quantitative analysis of drugs in biological matrices. The application of the method was confirmed in the determination of rivaroxaban in DBS collected from patients treated with the drug.

"Selected methods of analysis of triterpene compounds"

*Wiśniewska D.[1]**
[1]Department of Organic Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, Grunwaldzka Str. 6, 60-780 Poznan
s89387@student.ump.edu.pl

Compounds of natural origin and their semi-synthetic derivatives are one of the most promising sources of new pharmacologically active substances, among which triterpenes, widespread in the plant world, are used. Since cancer causes an increasing number of deaths, it has been proven that they can become potential cytostatic agents. For this reason, in addition to the known methods of isolation from plant material to obtain new derivatives, attention should also be paid to analytical methods for characterizing them. NMR techniques (1H NMR, 13C NMR, DEPT, 1H-1H COSY, 1H-1H-TOCSY, HMBC), thin liquid chromatography techniques (TLC, HP TLC, RP HP TLC), mass spectrometry techniques (MS, HR MS with various methods of ionizations, such as EI, CI, ESI, FAB, APCI, DESI, MALDI), infrared spectroscopy (IR), GC techniques with different types of detectors (e.g. FID, TCD, FPD, ECD), liquid chromatography techniques (NP LC, RP LC, NP HPLC, RP HPLC, RP UFLC, CC, RP CC) and electrophoresis techniques (CE, HP CE, ZCE, NACE) are applied for isolation of triterpenes from plant material and from mixtures after chemical reactions in analytical / laboratory scale. Most often, the isolation of newly obtained triterpene derivatives from post-reaction mixtures on a laboratory scale is carried out using classical column chromatography, flash chromatography, preparative thin-layer chromatography. The most popular methods for assessing the degree of purity of obtained / isolated triterpenes and confirming their structure are NMR, TLC and MS techniques.

This subject is related to the main research trend that I implement in the Scientific Community of the Department of Organic Chemistry at the Faculty of Pharmacy of the Medical University of Poznan. In this unit, under the supervision of dr hab. n. farm. Barbara Bednarczyk-Cwynar, I carry out chemical modifications of triterpenes, leading to obtaining new derivatives with expected high cytostatic activity against cancer cells.

„Development and characterization of polymeric micelles with magnolol”

Dominiak K., Stawny M.*
Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Magnolol is a lignan found in the bark of Magnoliae officinalis. The compound exhibits anti-inflammatory, antioxidant, anti-cancer and neuroprotective effects by affecting many pathways and enzymes in the human body. However, poor solubility and bioavailability limit its potential use. This study aimed to develop a method to obtain polymeric micelles with magnolol and optimize the process's parameters.

Magnolol and an amphiphilic copolymer, Soluplus, were dissolved in ethanol to obtain appropriate concentrations and then combined with each other in predetermined ratios. The solvent was evaporated, and the resulting film was hydrated in distilled water. Particle size, zeta potential, pH, and osmolarity were measured for the prepared samples. The incorporation process was evaluated by FT-IR and HPLC methods. Using the Box-Behnken model, optimization of the process was carried out.

Solvent evaporation proved a suitable method for obtaining polymeric micelles with magnolol. The physicochemical properties were found to be dependent on the concentration of the incorporated compound, the Soluplus concentration, and the ethanol used for dissolution. Optimization of the process was possible in terms of three dependent variables: osmolarity, zeta potential, and encapsulation efficiency.

In conclusion, polymeric micelles with magnolol can be obtained by a solvent evaporation method, and the Box-Behnken model is a suitable tool for optimizing the parameters of this process.

Founding: This research was funded by the grant OPUS no. 2022/45/B/NZ7/01056 from the National Science Centre, Poland.

„Acute poisonings with antipsychotic and psychotropic drugs – clinical observation and analytical tools”

Jiers W. [1], Sommerfeld-Klatta K. [1], Łukasik-Głębocka M. [2], Zielińska-Psuja B. [1]*
[1] Department of Toxicology, Poznan University of Medical Sciences
[2] Department of Emergency Medicine, Poznan University of Medical Sciences

Antipsychotic (AP) and psychotropic (PS) drugs are used in the treatment of psychiatric disorders. Both groups (typical AP: phenothiazine derivatives, and atypical: quetiapine, QT, as well as PS: tricyclic antidepressants, TCA) exhibit significant differences in their mechanisms of action and impact on various neurotransmitter systems. As the results, different side effects are observed, and intoxications are life-threatening.

The aim of this study was to analyze the clinical observations and toxicological results in a group of patients suspected of poisoning with AP and PS, treated at the Toxicology Department (TD) at the Municipal Hospital in Poznań. Over two months, patients suspected of abusing AP and/or PS were monitored. Medical records revealed that in 23 cases, poisoning resulted from a suicide attempt. During the analyzed period, TCA and QT poisonings accounted for 14% of all admissions to the TD.

30 patients were included in the study, with the majority being women (20). The average age of the patients was 36 years. The analysis was based on medical documentations and toxicological test results.

Ethanol was detected in the blood of 11 patients (average concentration 1.54 g/L). To confirm the presence of AP and PS poisoning tests for QT (11 patients), TCA (16 patients), and olanzapine (1 patient) were performed. At the time of admission, 15 patients were unconscious. 50% experienced disturbances in consciousness, speech, or balance. Medium-sized pupils were observed in 21 cases. Six patients required intubation and mechanical ventilation. The average blood concentrations of TCA and QT were 478 ng/mL and 2.22 µg/mL, respectively.

Poisonings with AP and PS were most commonly the result of suicide attempts. Regardless of the measured drug concentrations, characteristic symptoms included drowsiness, tachycardia, balance, and consciousness disturbances. The conducted analysis highlighted the importance of toxicological studies, allowing for the confirmation of poisoning and accurate diagnosis.

„Analysis of poisonings with selected drugs monitored by blood concentration”

Ruciński P.[1], Murias M.[1], Łukasik-Głębocka M.[2,3], Sommerfeld-Klatta K.[1]*

[1]Department of Toxicology, Faculty of Pharmacy, Poznań University of Medical Sciences, Dojazd Str. 30, Poznań 61-631

[2]Department of Emergency Medicine, Faculty of Health Sciences, Poznań University of Medical Sciences, Rokietnicka Str. 7, Poznań 60-806

[3]Department of Toxicology, Raszeja Hospital, Mickiewicza str. 2, Poznań 60-834

Introduction:

Toxicological tests, including quantitative determination of drugs in blood, play an important role in the diagnosis of patients hospitalized due to acute poisonings. The results obtained support assess the severity of poisoning and implement appropriate therapy. Overdose drug monitoring used in severely poisoned patients enable the analysis of effectiveness treatment procedures.

Aim of the study:

The aim of the study was to analyze cases of poisonings with antiepileptic drugs (valproic acid - VPA, carbamazepine - CBZ), antidepressants (tricyclic antidepressants - TCA) and acetaminophen (ACET) in the years 2015 - 2018. Toxicological tests were carried out at the request of the Toxicological Department at the Municipal Hospital in Poznań. The structure of poisonings was presented (gender, age, number of poisoned patients with selected drugs, and results of toxicological tests).

Materials and methods:

In the period from 2015 to 2018, 47,109 assays were performed, of which tests to confirm the presence of VPA, CBZ, TCA and ACET were ordered 1,648 times. Poisoning with a specific drug was determined based on the blood concentrations: >150 µg/ml for VPA, >20 µg/ml for CBZ, >250 ng/ml for TCA and >20 µg/ml for ACET.

Results:

During the analyzed period, 65 cases of VPA poisonings were confirmed with the average blood drug level 385±236 µg/ml. Of the 437 assays performed, CBZ poisoning was identified in 111 people and the average blood level was 35.9±12.4 µg/ml. 66 patients, most of whom were women (45 cases) were poisoned with TCA (1555±1246 ng/ml). ACET was determined 314 times, and poisoning was identified in 179 patients (90.53±75.02 µg/ml).

Conclusions:

In the period from 2015 to 2018, the largest number of analyzes were performed for TCA, but the highest percentage of confirmed poisonings in relation to the number of tests performed, amounting to 60.2%, was found for ACET.

Sesja e-posterowa

„Analysis of the stability of terbinafine hydrochloride in release solutions"

Dwiecki P.M. [1,2], Muszalska-Kolos I.[1]*

[1]Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

[2]Pharmaceutical Company "Ziołolek" Sp. z o.o., Starołęcka 189, 61-341 Poznań, Poland

Terbinafine as an antifungal drug was introduced to the world market in 1991, and in Poland in 1992. It is an allylamine with broad antifungal activity. It has a fungicidal effect on skin fungi, molds and some dimorphic fungi. It is usually used to treat dermatophytosis, tinea versicolor and onychomycosis, both orally and topically.

Formulations used orally must have adequate pharmaceutical availability, which is assessed by a dissolution test. The analysis of pharmaceutical availability is carried out in vitro, and the test conditions are determined by the relevant regulations and recommendations contained in the Pharmacopoeias. The test is usually performed at a constant temperature of 37oC and in an environment of 0.1 M hydrochloric acid and/or buffers of various pH.

The stability of terbinafine hydrochloride in solutions at pH 1.2 (0.1 M HCl), 3.0 (citrate buffer), 4.5 (acetate buffer), and 5.8 and 6.8 (phosphate buffer) was assessed. The tests were performed in aqueous solutions and 1% Tween 80. Changes in the concentration of the test substance were recorded by HPLC-RP method. In aqueous solutions, losses of approximately 5.9% (pH 1.2), 19.3% (pH 3.0) and 24.3% (pH 4.5) were observed during 42 days. However, in solutions with 1% Tween 80 approx. 7% (pH 1.2), 5.1% (pH 3.0), 49.6% (pH 4.5), 26.9 (pH 5.5) and 37% (pH 6.8). The greatest degradation was observed in citrate buffer pH 4.5. The kinetic parameters for the first-order reaction were determined for solutions with pH 4.5, 5.8 and 6.8 in 1% Tweenie 80. Calculated values of rate constants were (1.87±0.39)·107, (1.02±0.47)·107 and (1.20±0.33)·107; s-1 indicate that the stability of terbinafine is not significantly affected by pH in this range. Thus, in acidic, weakly acidic and neutral environments, solutions of terbinafine hydrochloride are so stable that a possible degradation process does not affect its pharmaceutical availability.

„Adsorption of pharmaceuticals by novel carbonaceous materials from the leaves of Ailanthus altissima (Mill.) Swingle - Case study on the adsorption of tetracycline"

Stojanović J.[1], Zalewski P. [2], Otašević B.[1], Zečević M.[1], Malenović A.[1], Janošević Ležaić A.[3], Ranđelović D.[4], Protić A.[1]*

[1] University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450 Belgrade, Serbia

[2] Poznan University of Medical Sciences, Faculty of Pharmacy, Department of Pharmacognosy, 4 Święcickiego St., 60-780 Poznan, Poland

[3] University of Belgrade – Faculty of Pharmacy, Department of Physical Chemistry and Instrumental Methods, Vojvode Stepe 450 Belgrade, Serbia

[4] Institute for Technology of Nuclear and Other Mineral Raw Materials, Boulevard Franchet d`Esperey 86, Belgrade, Serbia

In the last two decades, there has been a growing awareness of the presence of pharmaceuticals in the aquatic environment. Antibiotics are particularly alarming because their occurrence may result in increased antibiotic resistance. Difficulties in sample preparation and removal of low concentrations of pharmaceuticals from environmental water could be overcome by their adsorption onto novel, non-polluting, and inexpensive materials. In this study, biochar prepared by pirolysis of biomass at 500°C (BC500) and 800°C (BC800) and activated carbon prepared upon treatment with ZnCl2 at 800°C (AC800) were evaluated as potential adsorbents. Ailanthus altissima was selected as a source of raw material, leaf, because it is a widespread invasive tree that negatively affects biodiversity. Tetracycline hydrochloride was selected as a model substance, since it is an antibiotic widely present in environmental water. Central composite design was employed to simultaneously investigate the effects of adsorbate solution pH, ionic strength (KCl concentration), and adsorbent mass on removal efficiency of all three adsorbents, and to find optimal conditions for studying adsorption kinetics and equilibrium on the most promising adsorbent. The removal efficiency and adsorbed mass were calculated from the HPLC-UV determined concentration of tetracycline post-adsorption. Under optimal conditions (10.18 mg of adsorbent, pH 4.42, and ionic strength 165mM), AC800 showed the highest affinity for tetracycline, i.e. 38.22% removal and adsorbed mass of 56.32 mg g-1 compared to 14.57% and 21.48 mg g-1 (BC500) and 18.82% and 27.73 mg g-1 (BC800). Removal efficiency of AC800 was strongly influenced by the adsorbent mass and solution pH. The kinetics study showed a rapid adsorption process (equilibrium attained in 120 minutes), while equilibrium studies revealed a high adsorption capacity for tetracycline (131.55 mg g-1). AC800 has been shown to be a promising novel drug adsorbent and should be further tested for its suitability in water treatment and sample preparation.

„Two curcumin derivatives in lipid emulsions - assessment of incorporation possibilities”

Dettlaff K.1, Konieczka M.1, Gośliński T.[2]*

[1]Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland

[2]Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland

Two difluoroboryloxy curcumin derivatives with potential anticancer activity, containing a hydroxyl group (compound H: (1E,4Z,6E)-5-((difluoroboryl)oxy)-1,7-bis(3-fluoro-4-hydroxyphenyl)hepta-1,4,6-trien-3-one) or methoxy group in the phenyl rings (compound M: (1E,4Z,6E)-5-((difluoroboryl)oxy)-1,7-bis(3-fluoro-4-methoxyphenyl)hepta-1,4,6-trien-3-one) were selected for the study. These compounds reveal poor solubility in water and, thus, low bioavailability, making it difficult to use them in therapy.
The aim of the study was to optimize the process of incorporation of selected curcuminoids into five commercially available intravenous lipid emulsions: Intralipid, Lipofundin, Clinoleic, Lipidem, SMOFlipid. To optimize the method of incorporating curcuminoids into lipid emulsions, the parameters that could potentially increase the degree of incorporation were modified. The following optimal conditions were selected: homogenization time 10 min; pulse time ratio 30:30 s, amplitude 50%, rotator working time 2 h, and rotation speed 15 rpm. The degree of incorporation of curcuminoid derivatives was determined using the UV-Vis spectrophotometric method.

Based on the obtained results, it was found that the parameters that determine the efficiency of the incorporation process are the physicochemical properties of the incorporated compound and to a lesser extent, the composition of the emulsion used, as well as modification of the conditions of incorporation of the compounds. The highest degree of incorporation of compound H (66%) was obtained using Intralipid, and the lowest was noted for samples containing Lipofundin (56%). Compound M, without hydroxyl groups in its structure, was incorporated only in selected lipid emulsions and only to a small extent (5-8%). The resulting compound H formulations revealed an average particle size ranging from 226 nm to 285 nm and a zeta potential from -50 mV to -39 mV.

The results indicate that the developed incorporation method of selected curcuminoids into commercial lipid emulsions is worth further consideration for preparing formulations for parenteral administration.

This research was funded by the National Science Centre, Poland, grant number 2019/35/B/NZ7/01165.

„Impact of ramipril nitrosation on its mutagenic potential – in silico and in vitro safety evaluation”

*Regulska K. [1, 2,3], Kolenda T. [3,4], Michalak M. [5], Stanisz B.[6]**

[1]Pharmacy, Greater Poland Cancer Centre, Garbary 15 Street, 61-866 Poznan, Poland, e-mail: katarzyna.regulska@wco.pl, phone: 48618850704

[2]Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Poznan, Poland, Collegium Pharmaceuticum, Rokietnicka 3 Street, 60-806 Poznan, Poland

[3]Research and Implementation Unit, Greater Poland Cancer Center, Garbary 15 Street, 61-866 Poznan, Poland

[4]Laboratory of Cancer Genetics, Greater Poland Cancer Centre, Poznan, Poland

[5]Surgical, Oncological and Endoscopic Gynaecology Department, Greater Poland Cancer Center Poznan 61-866, Poland.

[6]Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6 Street, 60-780 Poznan, Poland

Introduction: Nitrosamines are potent human mutagens that can be formed ex vivo and in vivo from nitrosatable drug precursors leading to cancer initiation. Angiotensin-converting enzyme inhibitors (ACE-I) have been associated with an increased cancer incidence in several clinical observations. Hence, we investigated whether a commonly used ACE-I, ramipril (RAM) exerts any susceptibility to in vivo interaction with nitrite, potentially resulting in the generation of mutagenic N-nitrosamines.

Materials and Methods: Firstly, a mutagenic potential of RAM nitroso-derivatives was checked by in silico simulation using VEGA-GUI software. The structures of the studied compounds were based on RAM fragmentation pattern. Then, the Nitrosation Assay Procedure by WHO was conducted, which served as a model of endogenous reaction. The resulting post-nitrosation mixtures were subjected to a bacterial reverse mutation test employing Salmonella typhimurium strains TA98 and TA100 with and without metabolic activation. We used a commercial Ames MPF 98/100 microplate format mutagenicity assay kit from Xenometrix (Switzerland).

Results: In silico studies showed that small-molecule RAM derivatives with low lipophilicity could be mutagenic unlike large-molecule ones (N-nitroso-ramipri, N-nitroso-decarboxy-ramiprilat, N-nitroso-ramiprilat). In Ames test, the nitrosation mixture of RAM induced no point mutations in the test bacteria, regardless of the catalytic cytochrome activity. This means that nitrosation of RAM is not associated with severe fragmentation of substrate.

Conclusion: We concluded that endogenous nitrosation of RAM is not the reason for an increased cancer incidence among ACE-I users. However, these results cannot be extrapolated to other ACE-I.

„The influence of excipients on the physicochemical and biological properties of tomato extract containing lycopene”

Kulawik A.[1,2], Rosiak N.[1], Cielecka-Piontek J.[1], Zalewski P.[1]*

[1]Poznan University of Medical Sciences, Faculty of Pharmacy, Department of Pharmacognosy and Biomaterials, 3 Rokietnicka St., 60-806 Poznan, Poland

[2]Phytopharm Klęka S.A., Klęka 1, 63-040 Nowe Miasto nad Wartą, Poland

The most common source of lycopene in diets are tomatoes and products containing tomatoes. More than 85% of this ingredient in our diet comes from these sources. Tomatoes are also the cheapest source for lycopene production. The tomato-based products are a better source of this compound than raw tomatoes. Lycopene is a lipophilic compound insoluble in water. There are various ways to increase the bioavailability of lycopene and one of them is to create binary systems with cyclodextrins. X-ray diffraction (XRD) and Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy techniques were used to confirm the structure of the samples tested and the formation of possible interactions between the tomato extract and the excipient. XRPD analysis confirmed the formation of interactions in the resulting binary system. One of the main observations in the FT-IR-ATR analysis of a binary system is the shifts of the bands characteristic of the auxiliary substance. These shifts occur as a result of the resulting intermolecular interactions (hydrogen bonds). Markers confirming the formation of interactions are the bands corresponding to the hydroxyl (OH) groups of the excipient. Studies of apparent solubility of lycopene-excipients mixtures in 0.1 M HCl showed that excipients influence on their solubility. In vitro antioxidant activity tests showed, that binary systems tomato extract - excipients had enhanced antioxidant activities compared to the native lycopene.

FUNDING

This research was funded by Ministry of Science and Higher Education, grant number DWD/6/0002/2022.

„Investigating the effect of co-surfactant on the properties of intravenous lipid emulsion”

Czerniel J., Przybylski T., Gostyńska A., Stawny M.*
Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, Poznan, Poland

Parenteral nutrition (PN) is a life-saving procedure. However, the long-term provision of such therapy can lead to intestinal failure-associated liver disease (IFALD), manifested by inflammation and cholestasis. Since the lipid emulsion globule size has already been known to affect the liver state, we hypothesized that decreasing the lipid droplets may improve liver function. The reduction of droplet size may decrease their hepatic uptake and prevent the development of liver disease. This work aimed to develop intravenous lipid emulsions (ILEs) and to investigate the effect of selected co-surfactants on their physicochemical properties, stability, and compatibility with the other ingredients of PN admixture. Four ILEs containing MCT, fish, sunflower, hemp, and rapeseed oils as the oil phase and egg yolk lecithin as the main surfactant were developed. The ILE-HS, ILE-ELP, and ILE-T were enriched in 0.25% (w/w) of Kolliphor HS15, Kolliphor ELP, and Tween 80, respectively. The coarse emulsion was obtained using ultrasonic sonication, followed by high-pressure homogenization, and finally, thermal sterilization, resulting in the final emulsion. The developed ILE were characterized by the mean droplet diameter oscillating below 200 nm, with the lowest results for ILE-HS and ILE-T of 174.7±1.0 nm and 173.9±1.0 nm, respectively. All formulations possessed a negative zeta potential and pH in the range of 7.6–8.1. Long-term stability studies confirmed that the 60 days storage of developed ILEs at 4°C ±1°C and 25°C ±1°C without light exposure did not affect their physicochemical properties. The ILE-HS and ILE-T showed compatibility with the aqueous phase of Omegaflex without electrolytes within 24 hours. In conclusion, the addition of a co-surfactant significantly affects the physicochemical properties of the ILE. The ILE-HS and ILE-T were characterized by the most promising parameters for further investigation.

Acknowledgments: The National Centre for Research and Development, grant LIDER/17/0092/L-12/20/NCBR/2021

„The influence of 25 kGy electron beam radiation on the physicochemical and biological properties of curcumin”

*Rosiak N.[1], Garbiec E.[1], Skibiński R. [2], Lewandowska K.[3], Bednarski W. [3], Cielecka-Piontek J.[1], Zalewski P. [1]**

[1]Department of Pharmacognosy, Faculty of Pharmacy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland;
[2]Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland;
[3]Institute of Molecular Physics, Polish Academy of Sciences, Smoluchowskiego 17, 60-179, Poznan, Poland

Turmeric (Curcuma longa L.) contains a substantial amount of curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin. Among them, curcumin is the most abundant and significant compound, with notable antioxidant and anti-inflammatory properties. However, a major challenge in using curcumin is the potential contamination of the plant source with aflatoxins [3], which are known carcinogens, produced by certain molds, particularly Aspergillus flavus. To eliminate aflatoxins from plant material, electron beam sterilization is an effective method, but it's essential to assess its impact on the stability of curcumin due to the chemical instability of some compounds when exposed to ionizing radiation.The aim of the study was to assess the impact of ionizing radiation on the physicochemical properties of curcumin. Using the electron beam sterilization method, curcumin was irradiated with the standard recommended dose of irradiation (25 kGy). As shown by the method, in addition to curcumin, bisdemethoxycurcumin and demethoxycurcumin are present in the sample. HPLC and HPLC-MS/MS methods showed no degradation products, supporting the notion that ionizing radiation did not alter the physicochemical properties of curcumin. Observed changes in IR spectra may suggest that irradiation lead to the creation of free radicals in curcumin, which was also confirmed by EPR research. Antioxidant activity exhibited a decrease when assessed through ABTS, CUPRAC, and DPPH methods. A contrasting trend was observed in the FRAP method, indicating an increase in antioxidant potential after irradiation.It is suggested, that irradiation is a suitable process for curcumin-containing products. The research provides evidence supporting the radiostability of curcumin, which is crucial when considering the use of electron beam sterilization in various applications, especially in the pharmaceutical and food industries, where the preservation of active compounds is essential.
FUNDING This research was funded by the Polish National Agency for Academic Exchange (BPN/BIL/2021/1/00020/U/00001).

„Process optimization of PLGA formulation with fluorocurcumin derivative using Box-Behnken model”

Kuźmińska J.[1], Andrzejak T.[1], Szkudlarek J.[1], Gośliński T.[2], Jelińska A.[1], Muszalska-Kolos I.[1]*

[1]Chair and Department of Pharmaceutical Chemistry,
Poznan University of Medical Sciences, 3 Rokietnicka St., Poznan, Poland;
[2]Chair and Department of Chemical Technology of Drugs,
Poznan University of Medical Sciences, 3 Rokietnicka St., Poznan, Poland

Bladder cancer is responsible for approximately 200,000 deaths annually worldwide, accounting for 2.1% of all cancer deaths. The epidemiology of this disease is a growing problem, despite the development of new anti-cancer therapies. Curcumin clinical use is limited due to poor bioavailability and solubility. To improve bioactivity of curcumin new fluoro derivatives were synthesized, but they revealed low solubility. The way to modify the bioavailability of curcumins is a strategy based on the use of carriers, such as PLGA, which can contribute to improving pharmacokinetic and pharmacodynamic parameters.The research aimed to optimize the process of obtaining a formulation of fluorocurcumin analogue which shows biological activity against bladder cancer lines (5637 and SCaBER) from PLGA to improve the physicochemical parameters of the curcumin derivative. The optimization of the formulation manufacturing process was planned based on the Box-Behnken model, using the response surface method for three independent variables: concentration of polyvinyl alcohol (PVA), sonification power and amount of encapsulated substance. The effects of the independent variables on mean particle size (MDD), polydispersity index (PdI) and zeta potential (ZP) were evaluated. MDD and PdI of the obtained formulations were determined using the dynamic light scattering (DLS) method, while ZP using the laser Doppler electrophoresis (LDE) method. Parameters such as yield and incorporation rate of curcumin derivative into PLGA were determined using a validated HPLC method.The formulations obtained by process optimization were characterized by mean MDD in the range of 238-539 nm; PdI values in the range of 0.10-0.47; and ZP values from -11.13 to -17.77. The regression model used for MDD and PdI, determined by the dynamic light scattering method for the formulation containing fluorocurcumin analogue, was statistically significant and can be applied for the optimization of the process.

The National Science Center financially supported this research (grant no. 2019/35/B/NZ7/01165).

„A Stability-Indicating HPLC Method for the Estimation of Alverine citrate and Ibuprofen Impurities in Oral Solid Dosage Form”

Janczura M.[*1,2], Cielecka-Piontek J.*[1]

[1]Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań

[2]Pharmaceutical Company „Synteza” Sp. z o.o., św. Michała 67/71, 61-005 Poznań, Poland

It is necessary to develop a stability-indicating method for alverine citrate and ibuprofen-related impurities in API and solid oral dosage forms which are available in fixed-dose combinations drugs (FDC). To date, there is no single method that has been reported for the determination of alverine citrate and ibuprofen combination impurities in either bulk drugs or in pharmaceutical formulations of alverine along with ibuprofen. A novel, stability-indicating, HPLC method was developed for the determination of alverine citrate and ibuprofen in the presence of their impurities and degradation products. The method was developed using a Poroshell column 120EC-C18 4,6 mm x 250 mm x 4 µm with a flow rate of 0.7 mL/min and detector wavelength at 206 nm. The mobile phase A : 0,01 M lauryl sulfate in a mixture of acetonitrile:water (55:45) (V:V) adjusted to pH=3,0 with ortophosporic acid 85%. Mobile phase B:acetonitrile. Proportion Phase A:Phase B (80:20) (V:V). Alverine citrate and ibuprofen were subjected to the stress conditions of oxidative, acid, base, photolytic, and thermal degradation. Impurities resulting from the stress studies were well-resolved, thus confirming the test method as stability-indicating. Validation of the method was carried out as per International Conference on Harmonization guidelines. The method is selective, precise, linear and accurate in the range of 80% to 120%. The presision was confirmed by repetability (RSD≤3,7%). The recovery value characterizing the accuracy of the method is in the range 98%-102%.

„Accelerated degradation and in vitro tests for estimating photostability and phototoxicity of timolol"

Lejwoda K.[1], Gumieniczek A.[1], Filip A.[2], Berecka-Rycerz A.[1]*

[1]Department of Medicinal Chemistry, Medical University of Lublin, Poland
[2]Department of Cancer Genetics with Cytogenetics Laboratory, Medical University of Lublin, Poland

Many drugs can absorb radiation in the UV-Vis range that may induce photodegradation and generation of toxic photoproducts. As the awareness of the drug safety increases, the combined effects of light and chemicals draw attention from general public which supports the initiative to study the mechanism of potential phototoxicity of different drugs, including timolol that is frequentely used in ocular formulations.

Timolol as a pure substance was exposed to irradiation in the range 300-800 nm (2.699, 18.902, 37.804, 56.7006, 75.608 and 94.510 kJ/m2) under different pH values (1-13). The stressed samples were quantified with a selective validated HPLC method to obtain percentage degradation and respective kinetic parameters. Generation of ROS, i.e. singlet oxygen (SO) and superoxide anion (SA) was also examined to estimate the phototoxic risks according to ICH guidelines. Timolol (20-200 µM) were irradiated with respective energies of 4.9, 23.4, 68.7, 136.0 and 268.4 J/cm2. Next, selective chemical tests with N,N-dimethyl-4-nitrosoaniline reacting with SO, and nitrotetrazolium blue reacting with SA, were performed. In addition, in vitro phototoxicity tests (MTT and 3T3-NRU) were involved in this project.

Photodegradation of timolol followed the first-order kinetics. Stability of the drug was the lowest at pH 1-4 and 13 (> 90% degradation), and the highest at pH 7-10 (32.15-45.61% of degradation). The results of ROS assays showed that TIM may be potentially phototoxic (SO is ≥ 25 and the amount of SA is ≥ 70 according to ICH guidelines). In addition, the MTT and 3T3-NRU tests results suggest some phototoxicity risk. Also, some new structures for degradation products of timolol based on HR-LC/MS results were proposed.

Summarizing, the presented data could be helpful for the rational design of new formulations of timolol or even some modifications in its chemical structure.

„Photo-stability of dapagliflozin and empagliflozin in solutions: kinetics of degradation and products of degradation”

*Berecka-Rycerz A., Biernacka A., Gumieniczek A.**

Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

The photo-stability test consisted in exposing the both dapagliflozin and empagliflozin to UV/VIS irradiation, using a Suntest CPS+ xenon lamp. The study was performed in the presence of additional stress factors: acetonitrile, methanol and the buffers of pH 4-10. Sensitivity of these two gliflozins to light was assessed, and the order and rate constants of the photo-decomposition reactions were determined.

After accelerated degradation, the LC-UV method was used to quantify the both gliflozins in the presence of degradation products and to evaluate the kinetics of degradation. A Purospher® RP18 end-capped column (125 mm×4 mm, 5 µm) as stationary phase and a mobile phase with a flow rate of 1 ml/min, consisting of a mixture of acetonitrile and water: for dapagliflozin (37:63) and empagliflozin (33:67) were applied. The determination was carried out at two detection wavelengths: 225 nm and 276 nm.

When acetonitrile or methanol were used as a stress factor, the peaks of the photo-products of dapagliflozin and empagliflozin appeared after 42 h of irradiation/37804 kJ/m2 (approximately 2 ICH cycles). After 84 h of irradiation/75608 kJ/m2 percentage degradation of dapagliflozin was 27.50% and 32.60% while empagliflozin showed degradation of 32.76% and 36.08%. In the buffer of pH 10, the appearance of photo-decomposition product peaks were observed after 21 h of irradiation/18902 kJ/m2 (approximately 1 ICH cycle). In turn, percentage degradation of 36.79% for dapagliflozin and 27.54% for empagliflozin was obtained after 42 h of stressing. From the analysis of the above results, photo-degradation reactions of dapagliflozin and empagliflozin were shown as the first order reactions.
More tests are planned for the future to explain the structures as well as potent toxicity of the obtained photo-degradation products, using HR-LC/MS and in silico toxicity tests.

„Design of experiments-based optimization of asiaticoside ultrasound-assisted extraction from Centella asiatica”

Witkowska K, Paczkowska-Walendowska M, Cielecka-Piontek J*

Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Poznan, Poland

Introduction: Centella asiatica is a valued plant material with known anti-inflammatory and antimicrobiological properties. The aim of the research was to optimize, for the first time, ultrasound-assisted extraction (UAE) of Centella asiatica.

Materials & Methods: Using the Design of Experiments (DoE) approach, a Box-Behnken plan was developed. As independent factors were selected the content of extraction mixture, its temperature and the time of extraction process. As the parameters used to assess extraction efficiency were choose: sum of content of active components (asiaticoside, asiatic acid and madecassic acid) measured by validated HPLC method, total content of phenolic compounds and antioxidant (DPPH scavenging assay) as well as anti-inflammation activities (inhibition of hyaluronidase activity).

Results: On the basis of the experimental studies and statistical analyses, it was possible to predict the model and indicate the optimal parameters of the extraction process. The optimal UAE parameters for C. asiatica extraction were 70% methanol as the solvent at a temperature of 70 °C for 3 cycles per 60 min.

Conclusion: DoE is a useful approach to optimize extraction of plant materials with increased extraction efficiency and a limited number of experiments.

Acknowledgments: This research was funded by National Science Center (Poland), under Sonata grant (number 2020/39/D/NZ7/01824).

„Ergot - pilot studies of biological potential”

Studzińska-Sroka E.[1], Zapór M.[1], Szulc P.[2], Cielecka-Piontek J.[1]*

[1]Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[2]Department of Agronomy, Poznan University of Life Sciences, Dojazd 11, 60-632 Poznan, Poland

Ergot is a spore form of Claviceps purpurea, a fungus-parasite. This fungus attacks plant inflorescences and grains, mainly cereals and grasses, manifesting in purple-black structures – sclerotia [1]. Currently, due to the toxic effects of the alkaloids contained in it, ergot is not used in medicine, while ergot is not used in medicine, while ergot alkaloid derivatives are used to treat temporarily migraine pain or some neurological diseases [2]. Our study aims to evaluate the biological activity of ergot extracts with obtained different polarities solvents (dichloromethane, acetone, methanol, acetone 60%, methanol 60%, water). For this purpose, the activity using various mechanisms of antioxidant activity using DPPH and CUPRAC methods was determined. The impact of ergot extracts on generating the reactive dopaquinone was also tested by assessing the ability to inhibit tyrosinase. The analysis of the ability to inhibit acetylcholinesterase and butyrylcholinesterase estimated the probable impact on the acetylcholine level in the brain tissue. Moreover, the ability to chelate copper and iron ions, cofactors of important enzymes, catalysts of some reactions creating free radicals or the ions accumulating in nervous tissue during neurodegenerative diseases has been tested. The obtained results showed the high dose-dependent biological activities of the polar extracts: 60% acetone extract, 60% methanol extract, and aqueous extract. The next interesting extract was the methanol extract, whose biological potential was different regarding tested mechanisms. The demonstrated biological activity indicates an interesting potential of ergot extracts, including their importance in supporting the treatment of nervous system diseases. Due to the described harmfulness of the raw material, further research is necessary, including detailed phytochemical characterization of the obtained extracts and tests of their toxicity and extended biological activity.

References

1. Orlando, B. et al. World Mycotoxin J. 2017, 10, 327–338
2. Chen, J.-J. et al. RSC Adv. 2017, 7, 27384–27396

„Albumin-based nanoparticles of lutein”

Żółnowska I.[1], Gostyńska A.[1], Stawny M.[1]*

Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Lutein is a macular pigment often reduced in people with liver disease. It is known to prevent degenerative liver conditions by reducing the accumulation of free cholesterol, attenuating lipid peroxidation that lowers malondialdehyde levels, and inhibiting the production of pro-inflammatory cytokines. Due to the physicochemical properties of lutein, low solubility in water, and high oxidation potential, the research aimed to develop protein-based carriers of lutein. Such nanoformulations are characterized by high solubility in water and a safety profile after intravenous administration. Nanoparticles were prepared by a desolvation technique. The glutaraldehyde was used as a hardening agent. The effect of the lutein-to-albumin ratio on particle size, zeta potential, and polydispersity index was evaluated. The particle size of lutein-loaded albumin-based nanoparticles ranged from 214.9 ± 5.0 nm to 373.1 ± 14.5 nm. Storing the developed nanoparticles over one month at 4 ± 2 °C affected their size (of maximum ± 90.9 nm). The changes in zeta potential values were also observed. The lowest zeta potential (21.7 ± 0.5 mV) was recorded for nanoparticles characterized by a lutein-to-albumin ratio of 1:15 (w/w). However, nanoparticles with a 1:5 (w/w) lutein-to-albumin ratio had the highest zeta potential (-17.9 ± 0.2). All tested samples showed lower zeta potential after one month of storage. The developed lutein-loaded nanoparticles are promising for further investigation of intravenous delivery systems for lutein and studies of their safety profile and biological effects after parenteral administration.

Funding: This research was funded by the National Center of Science, Poland, through Grant No. 2021/43/O/NZ7/00690

„Anti-aging properties of chitosan-based hydrogels rich in blueberry fruit extract”

Erdem C.[1,2], Studzińska-Sroka E.[2], Paczkowska-Walendowska M.[2], Zalewski P.[2], Cielecka-Piontek J.[2]*

[1]Ege Üniversitesi, İzmir, Turkey
[2]Department of Pharmacognosy and Biometrials, Poznan University of Medical Sciences, Poznań, Poland

Introduction: Photoaging is a process related to the unfavorable influence of environmental factors, including UV radiation, which leads to an increase in the number of reactive oxygen species (ROS), the presence of which may initiate enzymes, including hyaluronidase, which causes the destruction of hyaluronic acid, which is a factor responsible for maintaining skin elasticity and hydration, and tyrosinase, the increased activity of which will accelerate skin pigmentation. Scientific research has shown that plant extracts rich in polyphenols (including *Vaccinium myrtillus* L.; blueberry) can reduce free radicals in the body and inhibit hyaluronidase and tyrosinase. Therefore, the modern cosmetics industry uses plant products to produce cosmetics that delay the effects of skin aging. Therefore, our work aimed to prepare hydrogels for topical application by adding blueberry fruit extracts, assessing their biological potential, and characterizing their pharmaceutical properties.
Method: Using the Design of Experiments (DoE) approach, chitosan-based hydrogels were prepared with evaporated acetone-water (1:1 *v*/*v*) extracts from dried blueberry fruits. The content of extract and chitosan (medium molecular weight) were selected as independent factors. The parameters used to assess hydrogel properties were chosen: antioxidant activity (using DPPH scavenging assay), hyaluronidase and tyrosinase inhibitions, dissolution profiles of standards from hydrogels and their viscosity.
Results: The activity of hydrogels statistically depends on the extract content; however, the enzyme-inhibiting activity results from the presence of the extract and chitosan itself. An increase in the concentration of chitosan in the hydrogel base causes an increase in the viscosity of the hydrogel and, consequently, a slower release of active compounds. Based on the experimental work carried out, as well as statistical analyses, it was possible to determine the optimal composition of the chitosan-based hydrogel with blueberry fruit extract.
Conclusions: The conducted research suggests the validity of using blueberry fruit extracts to prepare topical preparations with anti-aging properties.

„Evaluation of the biological potential of Galega officinalis as support to treat metabolic diseases”

Barchuk O.[1], Studzińska-Sroka E.[2], Cielecka-Piontek J.[2]*

[1]Department of Management and Economy of Pharmacy, Drug Technology and Pharmacoeconomics of Postgraduate Faculty, Danylo Halytsky Lviv National Medical University, Pekarska, 69, 79010 Lviv, Ukraine
[2]Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland

Metabolic diseases, including diabetes, dyslipidemia, or metabolic syndrome, result from biochemical disorders of the body. In medicine, plant extracts are often used to support their treatment. The herb of Galega officinalis is used in Eastern European medicine to treat prediabetes and mild hyperglycemia. However, despite the long tradition of G. officinalis herb products application, knowledge about the biological activity of alcohol-water extracts in supporting the treatment of metabolic diseases is limited. The aim of our research was to analyze the biological potential of ethanol-water extract of G. officinalis, which would be important in the prevention of metabolic disorders and protection against the complications resulting from their occurrence.
For this purpose, based on methods determining the ability to scavenge free radicals and reduce the oxidation state of transition metal ions, we determined the antioxidant activity of G. officinalis herb extract. We also tested their potential to inhibit enzymes regulating carbohydrate metabolism (alpha-glucosidase, alpha-amylase) and lipid metabolism (lipase), as well as enzymes important in inhibiting inflammation and tissue integrity (hyaluronidase, collagenase). The tested extract was also subjected to HPLC analysis.
The obtained results indicate that alcohol extract from G. officinalis herb was characterized by moderate antioxidant activity and a dose-dependent ability to inhibit the enzymes selected for testing. The analysis of the extract using the HPLC method proved that a significant percentage of the active compounds present in the extract is rutin.
In conclusion, ethanol-water extracts from G. officinalis herb have interesting biological potential and, after conducting more extensive research, may prove helpful in treating and preventing various metabolize diseases.

Funding: This research was funded by Poznan University of Medical Sciences.

„PLGA nanoparticles as a carrier for diphencyprone”

Przybylski T., Czerniel J., Stawny M.

Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Diphenylcyclopropenone (diphencyprone, DPCP) is a sensitizing agent used in dermatology in topical therapy of alopecia areata, viral warts, and melanoma. Due to its low solubility, it has been applied as an acetone solution, which has to be protected from light and presents a short shelf-life. The nanotechnology-based carriers such as polymer nanoparticles are an interesting solution to overcome these shortages. Such formulations may allow for the drug substances in vitro and in vivo protection, targeted delivery, increased bioavailability, and controlled release rate. Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable polymer whose properties depend on the synthesis method and the monomers' ratio. The aim of the research was to develop a preparation methodology for DPCP-loaded PLGA-based nanoparticles. This study assessed the impact of the following factors: the process conditions, the amounts of DPCP and PLGA, and the type of polymer used on the physicochemical properties of nanoparticles. The developed carriers were characterized by morphology, size, and entrapment efficiency. Nanoparticles containing the DPCP and different PLGA Resomers in the 1:10 ratio were developed to compare the entrapment efficiency and the purity of samples prepared using different matrices. The highest agreement with the polymer FT-IR spectrum was recorded in the case of lyophilized nanoparticles based on Resomer RG502H, where the purity index was above 0.9854. The smallest particle size (229 ± 1 nm) and the lowest polydispersity index value (0.0463 ± 0.0137) were obtained for DPCP-loaded Resomer RG752H-based nanoparticles. The entrapment efficiency differed slightly depending on the polymer used, reaching 72.21%, 70.99%, and 72.08% for RG502, RG502H, and RG752H, respectively. The obtained results allowed us to conclude that the properties of the prepared nanoparticles depend on the process parameters, the amount of DPC and PLGA, and the polymer used.

„Cannabidiol – lornoxicam PLGA-based carriers”

Bącler A., Przybylski T., Czerniel J., Stawny M.

Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

This research aimed to develop a methodology for preparing PLGA-based carriers for a physical mixture of cannabidiol (CBD) and lornoxicam (LOR). The solvent evaporation method was used with a variable polymer type and different CBD and LOR to PLGA ratios to obtain nanoparticles. The particle size, zeta potential, and polydispersity index were determined. The assessment of the incorporation process was performed by FT-IR and UV-Vis methods. In the case of Resomer RG502 used as a polymer matrix, the influence of the process conditions and the ratio between active substances and polymer on the physicochemical properties of the structures was observed. Nanocarriers were characterized by an average particle size ranging from 202 to 246 nm, zeta potential ranging from -10.6 to +1.3 mV, and polydispersity index below 0.197. Entrapment efficiency of CBD and LOR into polymer matrices were 56.39% and 60.61%, 37.10% and 26.02%, 45.33% and 55.87% for Resomer RG 502, Resomer RG 502H, and Resomer RG 752H, respectively. Qualitative evaluation showed that the highest purity index of the FT-IR spectra of lyophilized carriers compared to the PLGA FT-IR spectrum was observed in the case of Resomer RG502-based carrier characterized by the 1:10 LOR and CBD to PLGA ratio. Our results showed that changing the parameters of the preparation process of PLGA-based nanoparticles loaded with LOR and CBD affects their size and zeta potential.

„Interpopulational and intrapopulational variation of essential oil of Teucrium montanum L. ”

Marčetić M.[1], Zbiljić M.[2], Lakušić D.[3], Lakušić B.[2]*

[1]Department of Pharmacognosy, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

[2]Department of Botany, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

[3]Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade – Faculty of Biology, Takovska 43, Belgrade, Serbia

Corresponding author’s e-mail: mirjana.marcetic@pharmacy.bg.ac.rs

Teucrium montanum L. (Lamiaceae) is widely distributed in the Balkan Peninsula, extending from the sea coast to altitudes of over 2100 m a. s. l. with the highest number of occurrences in the zone between 500 and 1000 m. The aim of the study was to investigate the chemical composition of essential oil of individually sampled aerial parts of T. montanum from nine different populations from Balkan Peninsula, eight from Serbia and one from Albania. The plant material was collected and analysed as individual plants (total of 72 samples). The essential oils were isolated by hydrodistillation and qualitative and quantitative analysis was performed by GC-FID/MS. The obtained results were evaluated by analysis of variance (ANOVA), principal components analysis (PCA) and canonical discriminant analysis (CDA). The composition of essential oils was variable among populations (interpopulational) and among individuals within population (intrapopulational variability). The main compounds were germacrene D, shyobunol, germacrene D-4-ol, cis-sesquisabinene hydrate, limonene, γ-cadinene, α-bisabolol, sabinene and epi-α-cadinol. The ANOVA showed that the most statistically significant compounds were trans-verbenol, myrtenal and trans-pinocarveol, but also the main constituents ($p<0.05$), except epi-α-cadinol. The PCA analysis revealed the separation of four populations, while others were overlapped. Three populations were clearly separated in the CDA analysis: the population from western Serbia (Tara mountain) characterised by higher trans-verbenol (13.7-19.6%) content, the population from Albania (Skadar) with higher content of germacrene D (15.6-49.9%) and hydrocarbons like nonacosane (2.0-13.1%) and the population from eastern Serbia (Gornjak gorge) with higher limonene (1.6-51.7%) and (E)-caryophyllene (4.4-12.4%) content. Obtained understanding of the nature of variability of essential oil chemical composition enables the potential production of quality and chemically defined plant material.

„The use of high-performance liquid chromatography and spectroscopic methods in the analysis of dietary supplements and drugs containing valerian root ”

Piekuś-Słomka N., Krajczewska M., Kupcewicz B.*
Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Nicolaus Copernicus University, 85-089 Toruń, Poland

Chronic sleep disorders and anxiety are an increasing challenge for health care worldwide. The use of synthetic drugs to treat insomnia is often associated with numerous side effects and for this reason, interest in plant products, including dietary supplements, is growing. Due to the simplified marketing authorization procedure for dietary supplements, there is a great need to control their quality.

Valerian (Valeriana officinalis) is one of the most frequently chosen product of plant origin because of its proven effectiveness in alleviating sleep and anxiety disorders. The main component responsible for its anxiolytic effect is valerenic acid (VA). It was shown that acetoxyvalerenic acid (AVA) also present in valerian root does not have a desired pharmacological effect. What is more, it can even eliminate the effect of VA by competitive antagonism. Therefore, dietary supplements should contain not only a high amount of VA but also the ratio of VA to AVA content ought to be high. Additionally, reporting the composition as the sum of valerenic acids (as recommended by the European Pharmacopoeia) may be misleading.

The study aimed to investigate the quality of plant dietary supplements and medicines containing valerian root and their comparative analysis. For this purpose, a quantitative analysis of VA and AVA content was carried out using HPLC. The antioxidant activity was measured using the DPPH assay. Determination of the elemental profile of studied products was carried out by ED-XRF spectrometry. In the comparative analysis of the studied products chemometric techniques were employed.

The results of the study indicate that dietary supplements containing valerian are characterized by very variable quality. Among them, we identified products with similar quality to medicines, as well as those that do not contain valerenic acids in quantities higher than limit of quantification.

„Amino acids analysis in non-invasive exhaled breath condensate samples ”

Konieczna L.[1], Kaliszewska A[1], Bączek T.[1], Niedźwiecki M.[2], Siebert I.[3], Gutknecht P.[3], Skrzypkowska M. [4]*

[1]Medical University of Gdańsk, Department of Pharmaceutical Chemistry, Gdańsk, Poland
[2]Medical University of Gdańsk, Department and Clinic of Pediatrics, Hematology and Oncology, Gdańsk, Poland
[3]Medical University of Gdańsk, Department of Family Medicine, Gdańsk, Poland
[4]Medicinal University of Gdańsk, Department of Immunology, Gdańsk, Poland

Introduction: Exhaled breath condensate (EBC) is a non-invasive method of collecting airway lining fluid that can be used to diagnose various diseases. EBC contains a variety of substances, including cytokines, chemokines and amino acids. One of the main advantages of EBC is its non-invasive nature, which makes it particularly useful for monitoring diseases in children and other population. In addition, EBC can be collected repeatedly over time which allows for longitudinal monitoring of disease progression and response to treatment EBC has been studied in children with leukemia and obesity patients.
Methods: In this study we present LC-MS/MS method for the determination of panel of amino acids. The chromatographic separation was carried out on C18 column. The mobile phase A consisted of water with addition of 10 mM ammonium formate and 0.1% formic acid, while B consisted of methanol.
Results: Panel of 29 amino acids was determined in EBC samples. Significant differences in the levels of amino acids in patients with leukemia and the control group were assessed. Arginine, asparagine, glutamine, histidine, homoarginine, methionine, proline, hydroxyproline, threonine, tyrosine, and valine – were found to be specific to leukemia. The method was also applied to determine amino acids in EBC patients qualified for surgical bariatric treatment. Some differences between amino acids profile in bariatric patients before and after bariatric surgery was observed.
Conclusion: Our method offers a distinct, straightforward, and environmental friendly analytical platform for the simultaneous analysis of amino acid profiles. The presented study confirms the possibility of using amino acid profiles as biomarkers of leukemia and obesity patients before and after bariatric surgery.

References

1. Konieczna L, Pyszka M, Okońska M, Niedźwiecki M, Bączek T, J Chromatogr. A, vol. 1542, 2018, 72-81.
2. Patent EP3559675, A method for monitoring of amino acids in biological material, 16.08.2023.

„Novel BODIPY – based photosensitizers possessing benzoxadiazole substituents as effective anticancer agents”

Porolnik W.[1], Karpinska N. [1], Kucinska M.[2], Piskorz J.[1]*

[1]Chair and Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan;
[2]Chair and Department of Toxicology, Poznan University of Medical Sciences, Dojazd 30, 60-631 Poznan.

Photodynamic therapy (PDT) is successfully applied for treating localized cancers and other premalignant or non-malignant dermal lesions and is a perspective modality to treat microbial-induced pathologies. PDT involves the use of a photosensitizer and light of appropriate wavelength to induce oxidative stress, leading to the eradication of targeted cells [1]. Boron dipyrromethene (BODIPY) derivatives are organic chromophores that have found a wide range of applications in various fields, such as diagnostic imaging, DNA and protein labeling, chemical sensing, and photovoltaics. BODIPYs exhibit many properties suitable for PDT agents, including high absorption coefficient, good stability, chemical robustness, and high structural tunability, which allows modifying their photophysical properties such as absorption and emission wavelengths, solubility, and the rate of singlet oxygen generation [2]. Novel BODIPYs with benzoxadiazole substituent and their brominated or iodinated derivatives were synthesized and characterized using mass spectrometry, UV-Vis spectrophotometry, and various NMR techniques. Subsequent photochemical studies allowed to evaluate the absorption and emission properties as well as the singlet oxygen generation ability of obtained compounds. In vitro photodynamic activity studies were performed on human ovarian cancer cells (A2780) and triple-negative breast cancer cell line (MDA-MB-231). It was found that the introduction of bromine or iodine atoms into the BODIPY core caused significant enhancement of singlet oxygen production, which is considered the main cytotoxic agent in PDT. BODIPY derivatives possessing bromine or iodine atoms revealed high activity towards both studied cells. Thus, these compounds are very promising photosensitizers for application in photodynamic therapy.

References

[1] G. Gunaydin, M. E. Gedik, S. Ayan, Front. Chem. 2021.
[2] M. C. Malacarne , M. B. Gariboldi, E. Caruso, Int. J. Mol. Sci. 2022,

This work was supported by the National Science Centre, Poland, under grant no 2021/41/N/NZ7/00371

„Phytochemical and preformulation studies of pomegranate peel”

Ignacyk M., Paczkowska-Walendowska M., Cielecka-Piontek J.

Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Poznan, Poland

Introduction: The aim of this study was to obtain series of extracts from pomegranate peel using a Design of Experiments model and to evaluate their anti-inflammatory and antioxidative properties. Additionally, a selected extract was subjected to preformulation studies for the development of a pharmaceutical dosage form for oral cavity application.

Materials & Methods: The study was conducted by determining the total polyphenol content (TPC), performing DPPH radical scavenging assay, CUPRAC and FRAP tests and evaluating the inhibition of hyaluronidase activity. The obtained results were subjected to statistical analysis to determine the most optimal process conditions. The optimized extract was subjected to the electrospinning process using PCL and PVP polymers. The obtained nanofibers were analyzed for their biological and mucoadhesive activities and their morphology was evaluated along with the identification of the formed fibers.

Results: The most optimal extraction conditions were a methanol concentration of 70% in the extraction solvent, a process temperature of 70°C and a process time of 90 minutes. The electrospinning process was most efficiently performed using PVP in combination with the extract, although the resulting product showed limited mucoadhesive properties. It exhibited the highest biological activity among the obtained fibers and had an amorphous structure. On the other hand, the fiber produced from a blend of PCL and PVP in a ratio of 1:3 w/w demonstrated the highest structural resistance to the addition of the lyophilized plant extract.

Conclusion: Pomegranate peel was evaluated as a raw material rich in polyphenols with anti-inflammatory and antioxidative activities, making it potentially suitable for use in mucoadhesive formulations within the oral cavity for the prevention and treatment of periodontal diseases.

Acknowledgments: This research was funded by National Science Center (Poland), under Sonata grant (number 2020/39/D/NZ7/01824).

„Development of an Analytical Method for the Quantification of Withanolides in Ashwagandha (Withania somnifera) Using High-Performance Liquid Chromatography (HPLC)”

Nagalska M.[1], Trzaskoma P.[1], Piątek A.[1], Nowacka E.[1], Gościniak A.[1], Cielecka-Piontek J.[1]

[1]Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

Introduction: The purpose of this study was to develop an analytical method using high-performance liquid chromatography (HPLC) for the quantification of six specific withanolides (withanolide IV, withaferin A, withanolide V, 12-deoxywithastramonolide, withanolide A and withanolide B) in Ashwagandha (Withania somnifera). Ashwagandha is known for its potential therapeutic benefits, and accurate identification of these bioactive compounds is crucial for price evaluation, as well as for advancing pharmacological and medical research.

Methods: The HPLC methodology was optimized for efficient separation and quantification of the target witanolides. This optimization included the selection of an appropriate chromatographic column, mobile phase and detector settings. In addition, the method underwent a validation process that included determination of limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision.

Results: The developed HPLC technique proved effective in quantifying the six vitanolides found in Ashwagandha. Validation results, including aspects such as linearity, limit of detection (LOD), limit of quantification (LOQ) and precision and accuracy, demonstrated the validity of the method.

Conclusion: This summary highlights the successful establishment of an analytical methodology for the precise determination of six key withanolides in Ashwagandha, using HPLC. The application of this methodology holds promise for future research into the medicinal properties and therapeutic potential of Ashwagandha.

The study was funded by a grant from the Ministry of Education and Science under the program "Student Scientific Circles Create Innovations" (SKN/SP/570882/2023)

„Optimization of ultrasound-assisted Ashwagandha (Withania somnifera) extraction process using Box-Behnken design”

Piątek A.1, Nowacka E.1, Nagalska M.1, Trzaskoma P.1, Gościniak A.1, Cielecka-Piontek J.1
[1]Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

Introduction: The extraction process is a key step to make the best use of the potential of plant material. The goal of this study was to increase the efficiency of the extraction process, through the use of process optimization. The extraction process focused on the use of ultrasound to extract valuable compounds, with key input parameters including alcohol concentration in the extraction mixture, extraction time and raw material to solvent ratio.

Methods: A Box-Behnken Design method was used to optimize the extraction process. Various combinations of input parameters were tested, and the responses were evaluated to determine the optimal conditions. The input parameters were the percentage of alcohol in the extraction mixture, the duration of the extraction process and the ratio of raw material to solvent. The output parameter was the amount of witanoids obtained from 1 gram of raw material.

Results: The Box-Behnken Design methodology yielded a set of optimized conditions for the ultrasonic-assisted extraction process. The Box-Behnken Design methodology yielded optimized conditions for the ultrasound-assisted extraction process. These conditions resulted in improved extraction yields and product quality.

Conclusion: The Box-Behnken Design methodology made it possible to assess how the parameters studied affect the witanoids content of the extract obtained, allowing a better understanding of the process and to select parameters that allow obtaining an extract with high health-promoting potential.

The study was funded by a grant from the Ministry of Education and Science under the program "Student Scientific Circles Create Innovations" (SKN/SP/570882/2023)

„Development and validation of an HPLC-FLD method for the determination of gentamicin in small volumes of blood samples ”

*Dobrzyńska M.[1], Główka A.[2], Karaźniewicz-Łada M.[1]**

[1]Department of Physical Pharmacy and Pharmacokinetics, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland;
[2]Department of Bromatology, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland

Gentamicin is an aminoglycoside antibiotic used in the treatment of several types of bacterial infections. Therapeutic drug monitoring (TDM) is recommended due to the drug nephrotoxicity, ototoxicity, narrow therapeutic range and high inter-individual variability of pharmacokinetics. TDM of gentamicin in neonates is challenging because of the limited volumes of blood samples. The aim of the study was to develop and validate an HPLC-FLD method for the determination of gentamicin in small volumes of blood samples. Chromatographic separation of gentamicin and neomycin, used as internal standard, was performed on the Zorbax Eclipse Plus C18 column and a mobile phase composed of acetonitrile and water (90:10, V/V). The plasma sample (50 µL) was extracted with dichloromethane to remove lipid components and the analytes were derivatized with 9-fluorenylmethoxycarbonyl chloride. The method was validated according to the FDA requirements. The lowest limit of quantification was 0.1 µg/ml and the linearity has been confirmed for the plasma drug concentration range of 0.1-20 µg/ml. The inter-day precision expressed by the coefficient of variation was < 6%, and the accuracy was in the range of 94.2-114.6%, while the intra-day precision and accuracy values were < 18% and < 109%, respectively. The stability of gentamicin was evaluated in the samples stored for 24 h in an autosampler, in plasma samples stored for 3 h at room temperature and after a double freezing and thawing procedure. The utility of the method was confirmed for the analysis of gentamicin in samples obtained from a neonate treated with the drug. The determined concentrations were within the therapeutic range. The HPLC-FLD method meets validation requirements for quantitative analysis of drugs in biological matrices. The method can be used for TDM of gentamycin in small volume volumes of blood.

„Photodynamic and sonodynamic activity of novel porphyrazine/phthalocyanine hybrid complexes”

Wysocki M.[1], Ziental D.[1], Jozkowiak M.[2], Dlugaszewska J.[3], Piotrowska-Kempisty H.[2], Güzel E.[4], Sobotta L.[1]*

[1]Chair and Department of Inorganic and Analytic Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[2]Chair and Department of Toxicology, Poznan University of Medical Sciences, Dojazd 30, 60-631 Poznan, Poland
[3]Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[4]Department of Engineering Fundamental Sciences, Sakarya University of Applied Sciences, 54050 Sakarya, Türkiye

INTRODUCTION

Porphyrazines and phthalocyanines are similar classes of porphyrinoids, known for their excellent photodynamic performance. Due to similarity of their structures, the possibility of creating hybrid compounds also exists. Merging both structures can provide unusual properties, useful in the treatment of bacterial infections or cancer diseases.

MATERIAL AND METHODS

Stability and singlet oxygen generation under light irradiation and ultrasound (1 MHz, 3 W) of hybrids 1 and 2 were measured using spectrophotometric method with 1,3-diphenylisobenofuran (DPBF) as a singlet oxygen quencher. The dark toxicity and activity of both compounds against MRSA and Staphylococcus epiderdmidis were measured by colony forming units (CFU) counting method. The cytotoxicity against SCC-25 and FaDu cell lines and human fibroblast MRC-5 were measured by MTT assay. Inhibitory effect of hybrid 2 on enzymes was measured using commercially available assay kits.

RESULTS AND DISCUSSION

The compounds appeared to be stable during sonication and irradiation. For photodynamic manner, the singlet oxygen quantum yields reached 0.15 in DMF and o.17 in DMSO for hybrid 1, and 0.1 in DMF and 0.18 in DMSO for hybrid 2, whereas for sonodynamic manner the generation of ROS showed 35.9% decrease in DPBF absorbance. Both hybrids reached >5 log reduction against both MRSA and S. epidermidis under photodynamic pathway, while under sonodynamic pathway the reduction was negligible. The hybrids showed photodynamic activity only toward MRC-5 viability, whereas in sonodynamic pathway only FaDu cells were slightly affected. The hybrid 2 revealed up to 51% on enzymes.

CONCLUSION

The porphyrazine/phthalocyanine hybrids revealed moderate yield of singlet oxygen and ROS in both photodynamic and sonodynamic manner. Their photodynamic activity against MRSA and S. epidermidis was high (>5 log), although in sonodynamic manner their performance was negligible. Both photodynamic and sonodynamic activity against cancer cells and fibroblasts were poor. The hybrid 2 showed inhibitory impact on enzymes.

„The molecular corona of magnetic liposomes formed upon incubation with cell culture medium – nanoLC-MALDI-TOF/TOF MS/MS analysis"

Karolina Kustrzyńska [1], Eliza Matuszewska [1], Małgorzata Józkowiak [2], Paulina Skupin-Mrugalska [1]*

[1]Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences
[2]Department of Toxicology, Poznan University of Medical Sciences

The molecular corona (MC) of nanoparticles is the sum of all biomolecules, which are adsorbed by the surface of nanoparticles (NP) when they contact a biological environment. The aim of the presented study was to compare the composition of MC formed on the surface of magnetic liposomes under various conditions. Magnetic liposomes were obtained by thin lipid film hydration with iron oxide nanoparticles encapsulated in the aqueous core, followed by extrusion. MC of magnetic liposomes was formed by incubation in a cell culture medium with or without human fibroblasts. Following incubation, MC-magnetic liposome complexes were separated from the cell culture medium in the magnetic field using the MACS MicroBead (Miltenyi Biotec) system. The proteins comprising MC were then subjected to proteolytic digestion with trypsin, after which the resulting peptides were purified and concentrated using ZipTip C18 micropipette tips (Merck) and separated by nano-liquid chromatography (nanoLC). In the next step, the tryptic fragments were identified using the SwissProt database based on fragmentation spectra obtained with a tandem matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer. As a result of the experiments, over 1,000 proteins taxonomically restricted to Homo sapiens were identified in the samples. The number and type of proteins identified differed between MC formed under various conditions (by incubation with or without human fibroblasts). The differences were most pronounced for proteins involved in biological regulation and cellular, metabolic, and developmental processes, as well as in response to stimulus, localization, and growth. We demonstrated that MC formed either in cell culture medium alone or with human fibroblasts is characterized by different compositions. Meaningfully, we showed that proteins excreted by cells into the medium are adsorbed on the surface of nanoparticles, affecting MC composition.

Acknowledgments: The authors acknowledge financial support from the National Science Center, grant number 2021/42/E/NZ7/00287.

„Biochemical analysis of dandelion (Taraxaci radix) root anticancer metabolites”

Pawelec S., Jędrejek D.*

Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation – State Research Institute, Pulawy

Phytochemical studies on T. officinale demonstrated that the plant contains many bioactive compounds, such as hydroxycinnamic acids, flavonoids, sesquiterpene lactones, triterpenoids, inulin, and coumarins, that have been shown to exert a wide range of biological actions, such as anticarcinogenic and hepatoprotective. It has been described that for antiproliferative and cytotoxic effects on cancer cells of Taraxaci radix, the pentacyclic triterpenoid fraction is responsible. Although the major components such as α- and β-amyrin, lupeol, and taraxasterol of dandelion root have been described, new compounds are still being documented, an example of which are the recently characterized phenylacetate inositol esters and sesquiterpene lactone-proline conjugates. The literature review and our own research show that triterpenoids are present in dandelion roots in lower concentrations compared to other groups of metabolites (such as hydroxycinnamic acids and sesquiterpene lactones). In addition, pentacyclic triterpenoids occur in Taraxaci radix mainly in the form of aglycones, which makes it impossible to be the main active ingredient of aqueous dandelion extracts. Thus, despite many reports investigating dandelion root extracts' broad anticancer effect, the active constituents responsible for the observed activity and its underlying mechanisms remain unknown. Our research aims to isolate fractions, under the control of biological tests and metabolomics analysis, which will allow for assigning the biological effects to a specific group of metabolites.

Literature:
1. Jedrejek, D. et al. 2019. Food and Chem. Toxicol. 126, 233-247.
2. Williams, C.A. et al. 1996. Phytochem. 42, 121–127.
3. You, Y. et al. 2010. Food Chem. Toxicol. 48, 1632–1637.
4. Kenny, O. et al. 2014. Phytochem. 98, 197–203.

This work is funded by the National Science Center – Preludium 2022/45/N/NZ9/03440

„UHPLC-QToF-MS characterization of bioactive metabolites of oak seedlings (Quercus robur L.) as key phytoconstituents responsible for the therapeutic effects of this plant”

*Soluch A.**
Department of Biochemistry, Institute of Soil Science and Plant Cultivation, State Research Institute, ul. Czartoryskich 8, 24-100 Pulawy, Poland

Preparations of Quercus species mainly are used as hemostatic, antidiarrheic, astringent, or antiseptic agents, as well as for the treatment of burns, and inflammation of oral and anal mucosa. Most of the pharmacological effects can be explained by the presence of high amounts of tannins, flavonoids, triterpenoids and other secondary metabolites, present in all parts of the oaks. The biological activities reported for the Quercus genus plant, include antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, gastrointestinal disorder, skin disorder, antiobesity, anticancer and neurogenerative effects. The bioactivity studies so far have mostly been performed in vitro and in vivo with animals and clinical tests are very limited. Therefore, it is necessary to further explore knowledge in the identification of bioactive compounds responsible for the therapeutic effects of these plants.

In connection with the above, liophilized and defatted in a Soxhlet apparatus roots and aerial parts of local oak seedlings were extracted using an automatic extractor Dionex ASE 200. Phytochemicals were then purified from the extract by solid phase extraction SPE. Secondary metabolite fractions were eluted from HLB columns with 85 % methanol. Qualitative determination of the analytes was performed on a high-resolution Q-TOF mass spectrometer (Bruker Daltonics, Germany). The presence of various groups of bioactive metabolites was confirmed, mainly phenolic acids, flavonoids, tannins and triterpene saponins.

References:
1. Şöhretoğlu, D., et al. (2020). Phytochem. Rev., 19(6), 1379-1426.
2. Perez, A. J., et al. (2017). J. Agric. Food Chem., 65(23), 4611-4623.
3. Mady S. M., (2023). Front. Pharmacol., 14, 1120146.
4. Buche, G., et al. (2021). Metabolites, 11(10), 684.

„Phytochemical study of flavonoids of Taraxaci Flos”

Jędrejek D., Pawelec S.*

Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation – State Research Institute, Czartoryskich Str. 8, 24-100 Puławy

Taraxacum officinale L. has a long history as a medicinal plant to treat liver and gallbladder disorders and diverse digestive ailments. Nowadays, dandelion herb is a Pharmacopeial and Generally Recognized As Safe (GRAS) material that is approved for pharmaceutical use by European Medicines Agency and U.S. Food and Drug Administration. The biological activity of a plant preparation is closely correlated with its chemical composition and the presence of bioactive molecules, so an indispensable part of bioactivity studies is phytochemical analysis, for which techniques such as LC-MS and NMR are used. In addition, tools for the identification of specific metabolites and biological markers using embedded databases of natural substances and high-resolution MS data, such as SIRIUS, MSDial and MSFinder, have been growing in popularity for several years.Previous phytochemical studies of dandelion flowers have revealed the presence of numerous flavone type compounds (both glycoside derivatives and aglycones), such as luteolin, chrysoeriol, apigenin and tricin1,2. Recently, the presence of 4 flavonolignans (tricin derivatives) was confirmed3. Our newest phytochemical study of the flavonoid fraction of Taraxaci Flos, based on its chromatographic fractionation and HR-MS analysis, has made it possible to distinguish and tentatively identify flavonoid metabolites not previously described in the genus Taraxacum. The currently ongoing HPLC preparative isolation of the selected signals and NMR analysis of some of them already at this stage allow us to conclude that we are dealing with several new flavonoids of the biflavone (bi-luteolin and bi-tricin) and flavonolignan (tricin-lignan) types. In the next stage of work, the isolated metabolites will be tested for diverse biological activity.

1. Williams, C.A. et al. 1996. Phytochem. 42, 121–127.
2. Jedrejek, D. et al. 2017. Chemico-Biological Interactions 262, 29–37.
3. Choi, J. et al. 2018. Bioorganic Med. Chem. Lett. 28, 476–481.
The research was partially funded by NCN grant Preludium 2022/45/N/NZ9/03440.

„A Novel Method for Fractioning Honeybee Venom (Apis mellifera) ”

Wieliński W. [1,2], Matuszewska E. [1], Klupczyńska-Gabryszak A. [1] , Matysiak J. [1]*

[1] Chair and Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

2 Students' Scientific Society of Proteomics and Metabolomics; Chair and Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

Introduction

Honeybee venom (HBV) is a complex mixture of proteins, peptides, and other bioactive compounds with a wide range of pharmacological and toxicological properties. It is used in apitherapy, a traditional form of medicine, to treat a variety of inflammatory and other conditions. However, HBV can also cause serious side effects, including anaphylaxis and death. Therefore, it is important to fully characterize the proteomic and metabolomic content of HBV and to identify the substances responsible for its side effects. Other bee products, such as honey and royal jelly, have been well-studied, but there is a lack of comprehensive qualitative and quantitative data on HBV.

Methods

Ultra-high performance liquid chromatography (UHPLC) was used to collect new fractions of honeybee venom based on their UV spectrum peaks. UHPLC enables more precise separation of mixtures than traditional HPLC, resulting in improved peak separation and higher resolution. This leads to unprecedented resolution in downstream analysis with MALDI TOF/TOF mass spectrometry. The fractions collected using UHPLC were of higher purity than those collected using traditional HPLC, which allowed for better characterization.

Results:

Ultra-high performance liquid chromatography (UHPLC) was used to collect new fractions of honeybee venom based on their UV spectrum peaks for separation. This method enabled the more precise separation of mixtures than traditional HPLC, resulting in improved peak separation and higher resolution. The purity of the obtained fractions allowed for the characterization of the fractions with unprecedented accuracy. Follow-up studies will be conducted to further investigate the properties and potential applications of these new fractions.

Possible applications:
The new method of honeybee venom fractioning can provide valuable insights into the proteomic and metabolomic composition of the venom. This information can be used to develop new, more precise, and less toxic therapies. That may be beneficial to patients.

„Supercritical fluid extractor as a pioneering tool for increasing the solubility of apigenin – a neuroprotective compound of plant origin ”

Stasiłowicz-Krzemień A., Rosiak N., Cielecka-Piontek J.*
Poznan University of Medical Sciences, Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Rokietnicka 3 Street, 60-806 Poznan, Poland

Apigenin, a prevalent monomeric flavonoid belongs to the flavone subclass of flavonoids with antioxidant, anti-inflammatory, antibacterial, and neuroprotective activity. This bioactive compound is abundantly distributed in various herbs, vegetables, and fruits, making it a significant component of the human diet with potential health benefits. However, its bioavailability is limited due to low solubility.

The aim of the research was to increase the solubility of apigenin with various excipients with the use of co-precipitation with a functional carrier in supercritical carbon dioxide with the use of a supercritical fluid extractor.

Apigenin and excipients were accurately weighed and placed in an extractor vessel, which was then heated to 50°C. Carbon dioxide was introduced into the vessel at a pressure of 5000 PSI. The static process was allowed to proceed for 30 minutes. To determine the solubility, excess amounts of apigenin and the systems were placed in glass vials with distilled water and shaken for 24 hours. After selecting the excipient with the highest potential to increase apigenin solubility, various parameters of amorphization were investigated to achieve the greatest enhancement in solubility. Changes in the crystalline behavior of apigenin were assessed using X-ray powder diffraction. The concentration of apigenin was analyzed using high-performance liquid chromatography with a ReproSil Chiral-JM-R C18 column (150 mm × 4.6 mm; 5 µm) and a mobile phase consisting of 0.1% formic acid/acetonitrile (45:55 v/v). The flow rate was set at 1.0 mL/min, with an injection volume of 10 µL, and the detection wavelength at 269 nm.

Co-precipitation with a functional carrier in supercritical carbon dioxide was found to be an effective method to amorphize apigenin and increase its solubility. The most significant potential for increasing apigenin solubility was found with Soluplus.

Acknowledgments: This research was funded in whole by National Science Centre, Poland, the grant Preludium nr UMO-2021/41/N/NZ7/01125.

„Development and optimization of cannabidiol chromatographic analysis ”

Sobczak A., Zieliński P., Jelińska A.*

Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznań, Poland

In recent years, there has been significant interest among scientists and consumers in cannabidiol (CBD), a main bioactive compound in hemp. The phenomenon is primarily linked to the potential health benefits of CBD consumption, its lack of psychoactivity, a low side effect profile, and the variety of product forms available. Due to the global increase in interest in CBD products, the development and optimization of HPLC methods for CBD analysis have become crucial for the hemp industry and regulatory agencies.

In this study, we aimed to develop and validate an HPLC-DAD method that allows monitoring of the CBD content in the pure raw material and at various stages of research on its new formulation. Hence, it was decided to select the method parameters to ensure chromatographic separation between cannabidiol and potential impurities and decomposition products.

Firstly, the analytical wavelength was selected (220 nm), and the composition of the mobile phase was optimized in terms of quality and quantity (ACN and 10%ACN in gradient elution). The type of stationary phase (LiChrosorb RP18, 125 x 4 mm, 5 µm (Merck, Darmstadt, Germany), optimal temperature of column (40°C) and autosampler (15°C) were determined.

In the next research stage, the analytical method was validated. It was found to be selective, linear in the range from 4.13 µg/ml to 101.10 µg/ml (r = 0.9999), sensitive, and with LOD and LOQ values of 0.60 µg/ml and 1.83 µg/ml, respectively. The method was characterized by the correct precision (for the inter-day precision RSD% = 0.41-1.92, for intra-day 0.82-3.17%) and accuracy (recoveries between 97.8% and 104.1%). The robustness test demonstrated the method's capacity to remain unaffected by minor operational parameter changes (observed CBD content changes were no more than 1%, and the symmetry coefficient ranged from 0.9-1.0). The results obtained confirmed the usefulness of the developed method.

„A novel approach for high-throughput screening of enabling formulations for poorly soluble drug”

Słaba A., Polarczyk A., Czajkowski M., Skupin-Mrugalska P.*
Katedra i Zakład Chemii Nieorganicznej i Analitycznej, Uniwersytet Medyczny w Poznaniu

Enabling formulations are drug delivery technologies specially designed to expedite the release and absorption of poorly soluble drugs. Without an enabling formulation, most drugs in today's pipeline - over 90% of drugs in development are poorly soluble - would be ineffective if given orally, simply because not enough of the dose would be absorbed to achieve a therapeutic response.

MultiScreen® assay (MerckMillipore) is designated for determining a compound's aqueous solubility - an essential early measurement in drug discovery. The presented study aimed to adapt MultiScreen® for early research and development of enabling formulations. Here, the specific objective focused on selecting the polymer component in a ternary solid dispersion composed of polymer and phospholipid.

Amorphous solid dispersions (ASD) in polymer-phospholipid matrix with a model Biopharmaceutics Classification System class 2 drug were used as a model enabling formulations. ASDs with BCS2 drug were obtained by freeze-drying in 96-well plates and analyzed by differential scanning calorimetry to characterize the solid-state form. Freeze-dried dispersions were dispersed in a dissolution medium simulating fasted state intestinal fluid, incubated for 15 min or 1 h in 96-well filter plates, and filtered to the acceptor plates. The concentration of the model BCS2 drug was then analyzed by high-performance liquid chromatography equipped with a diode-array detector.

As a result of the experiments, we observed the supersaturation potential of the studied enabling formulation, which allowed the selection of polymer providing the highest concentration of BCS2 drug in the acceptor compartment.

We demonstrated that the 96-well filter plate setup can be used for early research and development of enabling formulations regarding excipient selection and formulation composition. Furthermore, the presented approach makes formulation screening faster and more effective.

Acknowledgments: The authors acknowledge financial support from the National Centre for Research and Development, grant number LIDER/56/0231/L-11/19/NCBR/2020.

„Preliminary assessment of the biocompatibility and bioactivity of newly synthesized silver(I) coordination compounds with tinidazole”

Żyro D.[1], Łazarenkow A.[1], Szemraj M.[2], Sienkiewicz M.[2], Sikora J.[1]*

[1]Department of Bioinorganic Chemistry, Department of Medicinal Chemistry, Faculty of Pharmacy, Medical University of Lodz, , Muszynskiego 1, 90-151 Lodz, Poland

[2]Department of Pharmaceutical Microbiology and Microbiological Diagnostic, Medical University of Lodz, Muszynskiego 1, 90-151 Lodz, Poland

For years, in medicine and pharmacy, the antimicrobial properties of Ag(+) have been well-known and utilized. Silver exhibits a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. It is often used in the production of medical devices, and in pharmacy, silver nitrate and silver sulfadiazine have found applications. Tinidazole is an antibiotic used in the treatment of various bacterial and parasitic infections. The problem of microbial resistance to commonly used drugs has prompted researchers to seek new compounds with antimicrobial properties. Tinidazole, due to its chemical properties, can form coordination compounds with Ag(+). Previous research conducted in the Department of Bioinorganic Chemistry on coordination compounds of metronidazole and silver ions (I) showed that such structures possess significantly better biological activity than the individual substances that form them. The continuation of these studies involved the synthesis of a complex of an azole derivative – tinidazole with silver methanesulfonate (I) and silver nitrate (I).

The aim of this study was to provide an initial assessment of the biocompatibility and antimicrobial activity of the newly synthesized complexes and their constituent ligands. The influence of the compounds on basic blood coagulation parameters was examined, including extrinsic and intrinsic coagulation pathways by CoagChrom-3003 device from BioKsel. In a spectrophotometric erythrotoxicity test using human blood erythrocytes, the impact of the tested compounds on the protein-lipid membrane was evaluated. A preliminary assessment of the antimicrobial activity of all compounds was conducted.

Biocompatibility studies indicated that the new compounds can be considered as biocompatible over a wide range of concentrations since they did not show statistically significant effects on both PT (Prothrombin Time) and APTT (Activated Partial Thromboplastin Time) parameters, as well as on the erythrocyte membrane. They demonstrated efficacy against some Gram-positive and Gram-negative bacterial cultures, which proved to be better than ligand and salts.

„Metabolomics as a method for evaluation of endotype differences in childhood asthma”

Rzetecka N. [1], Matysiak J. [1], Matysiak J. [2], Sobkowiak P. [3], Wojsyk – Banaszak I. [3], Bręborowicz A. [3], Klupczyńska – Gabryszak A. [1]*

[1]Poznan University of Medical Sciences, Faculty of Pharmacy, Department of Inorganic & Analytical Chemistry Rokietnicka 3, 60-806 Poznan, Poland

[2]Calisia University, Faculty of Health Sciences, 62-800 Kalisz, Poland

[3]Poznan University of Medical Sciences, Department of Pulmonology, Pediatric Allergy and Clinical Immunology, 60-572 Poznan, Poland

Asthma is a common chronic respiratory disease in adults and children. Children with asthma represent a challenging group of patients who are extremely difficult to diagnose. Often there is a lack of cooperation between doctor and patient, so diagnostic methods used in adults are not used in children. Asthma is called a heterogeneous disease that manifests in different phenotypes and endotypes. Phenotype describes the clinical "observable characteristics", as well as response to treatments, so phenotypes are partly dependent on different asthma disease processes. But "endotype" represents subtypes of asthma. Endotypes are a different form of classification than phenotypes and describe distinct disease entities with a specific etiology mechanism. Each endotype can include several phenotypes, just as phenotypes can be present in more than one endotype. Metabolomics is an omics science that studies low-molecular-weight metabolites found in a biological system. Metabolites (including amino acids, sugars, fatty acids, vitamins) have a significant impact on balancing the body's internal environment and managing diseases related to redox balance, oxidative stress, or inflammation. In metabolomic studies, proper patient characterization, selection, and sample collection are crucial. Biospecimens that can be utilized in metabolomics studies to analyze the development of asthma, reaction to environmental exposures, and asthma severity include blood, urine, saliva, induced sputum, exhaled-breath condensate, nasal lavage fluid, and stool. The selection of a biospecimen should be based on its adequacy and suitability for study, as well as on its collection and storage procedures.

The aim of the planned project is to characterize serum metabolome profiles and define endotypes in childhood asthma. The project will contribute to broadening the knowledge about the mechanisms of childhood asthma and to estimating the diagnostic utility of the observed alterations in the metabolome of patients.

Acknowledgment: This study was supported by Grant No. 2021/43/O/NZ5/00480 from the National Science Centre, Poland

„Untargeted Lipidomic Approach for Gynecological Cancers Differentiation"

Pietkiewicz D.[1], Christopher M.[2], Garrett T. J.[2], Matysiak J.[1]*

[1]Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, 3 Rokietnicka Street, 60-806 Poznań
[2]Department of Pathology, Immunology and Laboratory Medicine, University of Florida, 1600 SW Archer Road, Gainesville, FL 32610

Lipids play an important role in signaling pathways and participate in the process of inflammation, immunity, cell proliferation, and differentiation. Recent findings suggest that lipids are necessary elements supporting oncogenic signaling and the energetic needs of rapidly proliferating cancer cells [1]. Thus, they should be one of the main study objects when it comes to gynecological cancers differentiation.
For this study, 57 samples were selected from the University of Florida Biorepository: 9 samples of benign neoplasm of the ovary, 25 samples of primary cancer of the ovary, 10 samples of primary cancer of the endometrium, 13 samples of primary cancer of the uterus. Lipids extraction was carried out by the Folch's method. The analyses were carried out using ulta-high-performance liquid-chromatography high-resolution mass spectrometry (UHPLC-HRMS). For acquiring high-resolution and accurate-mass results, the Q Exactive Hybrid Quadrupol-Orbitrap mass analyzer was utilized for UHPLC-HRMS analyses. The analyses were performed in positive and negative ion modes to discover all the classes of lipids in the analyzed samples.
The study allowed to reveal different classes of lipids present in the analyzed samples of selected gynecological cancers. The results of the performed analyses will be subjected to bioinformatics analyses in order to identify lipids and metabolites, as well as to find connections of molecular pathways for selected gynecological cancers.

The research was a part of the support obtained from the NAWA Agency for financing foreign trips for participants of the Doctoral School run by the Poznan University of Medical Sciences, PPI/STE/2020/1/00014/DEC/02.

1. Butler, L.M.; Perone, Y.; Dehairs, J.; Lupien, L.E.; de Laat, V.; Talebi, A.; Loda, M.; Kinlaw, W.B.; Swinnen, J. V. Lipids and Cancer: Emerging Roles in Pathogenesis, Diagnosis and Therapeutic Intervention. Adv Drug Deliv Rev 2020, 159, 245–293, doi:10.1016/j.addr.2020.07.013.

„Plasma free amino acids quantitation in highly trained athletes during exercise and during post-exercise recovery”

Plewa S.[1], Klupczyńska-Gabryszak A.[1], Ciekot-Sołtysiak M.[2], Zarębska E.A.[2], Zieliński J.[2], Matysiak J.[1], Kusy K.[2]*

[1] Poznan University of Medical Sciences, Department of Inorganic and Analytical Chemistry, Poznań, Poland
[2] Poznan University of Physical Education, Department of Athletics Strength and Conditioning, Poznań, Poland

Alteration in the synthesis and degradation of muscle proteins play an important role during repair, growth and remodeling after physical stress. Proteins are chains of amino acids, thus we decided to verify if plasma amino acid concentrations change during exercise of increasing intensity and during the post-exercise recovery period. The aim of the project implemented in cooperation with Poznan University of Physical Education was to analyze the profile of amino acids in human blood plasma during exercise and during post-exercise recovery. The novelty in our project is tracking changes in plasma free amino acid concentrations over a longer period (annual cycle) in people adapted to completely different types of physical exercise (speed versus endurance). So far, only single efforts, training sessions or sports competitions have been analyzed, and people with different training profiles have not been directly compared. In order to quantify a wide panel of amino acids the tandem mass spectrometry technique with a triple quadrupole analyzer combined with high-performance liquid chromatography (HPLC-MS/MS) was used. Analyzes of plasma samples were performed using the aTRAQ labeling reagent (SCIEX). In the results, the study allowed for the determination of a wide spectrum of amino acids and biogenic amines in the plasma of athletes - 33 analytes in each sample, with an analysis time of less than 18 minutes. The amino acids levels response to incremental aerobic exercise until exhaustion and subsequent recovery was sucesfully quantified and the manuscript is under preparation.

Acknowledgments:
This research project was funded by National Science Centre Poland, grant OPUS 14 No. 2017/27/B/NZ7/02828. We also thank all the excellent athletes and their coaches for participating in the study.

Curcumin-loaded intravenous lipid emulsion - the physicochemical characterization, stability, and compatibility Studies

Julia Bartkiewicz, Joanna Czerniel, Maciej Stawny, Aleksandra Gostyńska*

Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, Poznan, Poland

Curcumin (CUR) is a plant-derived active substance with pleiotropic activity, including anti-inflammatory and antioxidant effects. It is characterized by a high safety profile; however, its therapeutic use is limited due to low bioavailability. The aim of this study was to develop a CUR-loaded intravenous lipid emulsion and assess the possibility of using it as an ingredient of parenteral nutrition. In addition, the influence of chosen independent variables: the excipients to the CUR ratio, the lipid emulsion volume, and the sonication time on the mean droplet diameter (MDD) was determined. The curcumin was introduced into intravenous lipid emulsion (Lipofundin MCT/LCT 20%) by solubilization of curcumin-loaded mixed micelles. The mixed micelles consisting of different ratios of phosphatidylcholine and sodium deoxycholate were prepared by the solvent evaporation method. The developed formulations were characterized by MDD, polydispersity index (PDI), percent of fat residing in globules larger than 5 µm (PFAT5), zeta potential (ZP), pH, and osmolality (Osm). Box-Behnken model was used to determine the influence of chosen independent variables on MDD. The 30-day stability of CUR in developed formulations stored at 4 ± 2ºC was assessed using UV-Vis spectrophotometry. The compatibility studies were performed by adding developed formulations to parenteral nutrition (Lipoflex special). The developed CUR-loaded intravenous lipid emulsion was characterized by MDD ranging from 205.9 to 214.3 nm, low PDI of below 0.08, and PFAT5 in the range of 0,0001 to 0,0047%. The solubilization of CUR-loaded mixed micelles with Lipofundin MCT/LCT 20% affected its ZP, pH, and Osm. All developed formulations were compatible with parenteral nutrition, however, the stability of active substance was variable. The statistical analysis showed a significant impact of the excipients to CUR ratio on MDD. The adopted method allows for developed CUR-loaded intravenous lipid emulsion compatible with parenteral nutrition.

Acknowledgments: The National Centre for Research and Development, grant LIDER/17/0092/L-12/20/NCBR/2021

„Zróżnicowanie Poziomów Kannabinoidów w Wyselekcjonowanych Olejkach Konopnych dostępnych w Polsce”

Duczmal D.[1,2], Niedzielska K.[2], Bazan-Wozniak A.[1], Pietrzak R.[1]*

[1]Uniwersytet im. Adama Mickiewicza w Poznaniu, Wydział Chemii, Zakład Chemii Stosowanej, ul. Uniwersytetu Poznańskiego 8, 61-614 Poznań dominik.duczmal@amu.edu.pl

[2]Polygen sp. z o.o., Górnych Wałów 46/1, 44-100 Gliwice, Polska

Kannabinoidy, będące grupą substancji chemicznych obecnych w roślinach oraz organizmach zwierzęcych, zdobywają coraz szersze uznanie dzięki możliwym korzyściom zdrowotnym. Znajdują one zastosowanie w terapii różnorodnych stanów i dolegliwości, w tym w łagodzeniu napadów epilepsji, redukcji bólu nowotworowego, minimalizacji symptomów lęków i depresji, stabilizacji nastroju oraz jako wsparcie w terapii uzależnień od nikotyny i alkoholu[1], a także w traktowaniu zespołu stresu pourazowego[2].

THC (tetrahydrokannabinol) i THCA (kwas tetrahydrokannabinolowy) stanowią dwa z najbardziej ugruntowanych kannabinoidów, będących substancjami psychoaktywnymi obecnymi w konopi, znanej także jako marihuana. Niemniej jednak, dostęp do medykamentów zawierających kannabinoidy jest często ograniczony, a ich cena może być znacząca. Tym samym, ograniczone możliwości zakupu farmaceutyków, które mogą złagodzić objawy różnych schorzeń, spowodowały wzrost zapotrzebowania na produkty zawierające ekstrakt z konopi włóknistych, w szczególności te bogate w CBD (kannabidiol). Kluczowym zamierzeniem przedmiotowego badania była analiza zawartości kannabinoidów w selekcyjnie wybranych olejkach konopnych dostępnych w Polsce. W tym celu dokonaliśmy ekstrakcji kannabinoidów obecnych w analizowanych produktach, po czym zidentyfikowaliśmy wyizolowane związki za pomocą techniki chromatografii cieczowej, używając do tego aparatury Thermo Scientific Ultimate 3000..

Otrzymane wyniki analizy są niezwykle istotne dla lepszego zrozumienia składu i jakości dostępnych olejów konopnych. Ta wiedza może pomóc osobom podejmować bardziej świadome decyzje przy wyborze terapeutycznych produktów pochodzących z konopi. Dodatkowo, badanie to otwiera nowe perspektywy dla badania kannabinoidów jako potencjalnych leków i suplementów zdrowotnych, co może rewolucjonizować dziedziny medycyny i farmakologii.

LITERATURA

[1] Therapeutic potential of cannabis in pain medicine. R. D. Hosking and J. P. Zajicek British Journal of Anaesthesia 101 (1): 59–68 (2008)

[2] Use of cannabinoids for the treatment of patients with post-traumatic stress disorder. Marika L. Forsythe ORCID logo und Andrew J. Boileau. Journal of Basic and Clinical Physiology and Pharmacology

„Easily influenced patients and "deceptive, curative dietary supplements" - Case report”

Fekete M.[1], Madarász B.[1], Forrai J.[1], Ungvári Z. [1], Varga J.T. [2]
[1]Department of Public Health, Semmelweis University, Faculty of Medicine, Budapest, Hungary
[2]Department of Pulmonology, Semmelweis University, Budapest, Hungary

In the case of individuals with severe and chronic illnesses, they are considerably more vulnerable and susceptible due to their fear of the disease, making them easier to influence than the average person. Typically, lay patients and consumers possess very little knowledge about the characteristics of various products, do not receive accurate information, and are easily misled. If various products are presented as having efficiency similar to pharmaceuticals and false claims are made, such as dietary supplements being suitable for the treatment of cancer and other diseases, the prosecution has the opportunity to initiate consumer protection legal proceedings against the business. According to the prevailing Hungarian and European Union regulations, it is not permissible to declare or imply disease prevention or treatment properties for dietary supplements, even if businesses have conducted some form of testing regarding the actual effects of the product. This occurred with a dietary supplement product containing turmeric, where the company committed an infringement by claiming that turmeric is effective in reducing inflammation in cases of arthritis, osteoarthritis, rheumatoid arthritis, and acute infections. Furthermore, they asserted that it improves circulation, thus reducing the risk of heart attacks and thrombosis, and, according to the latest research, reduces the risk of cancer and Alzheimer's disease. In addition, they claimed it had pain-relieving properties, aids in post-stroke recovery, is a potent antioxidant, neutralizes health-damaging free radicals, and has protective effects on the liver and gallbladder. It acts as a diuretic, supports kidney function, enhances fat metabolism, and helps maintain ideal body weight, and so forth. Similar cases have been reported by patients, which we will discuss in our presentation. In this particular instance, an extended legal procedure transpired, and the imposed fine was of a substantial magnitude.

Funding: Project: no. TKP2021-NKTA-47 was funded bythe National Research, Development and Innovation Fund under the TKP2021-NKTA, with the support from the Ministry of Innovation and Technology of Hungary. MF was supported by the ÚNKP-23-4-I-SE-2 new national excellence program of the ministry for innovation and technology from the source of the national research, development and innovation fund.

Sesja VOD

“Dark side of tattoo”

Beata Bystrowska[1], Agnieszka Smok-Wilisowska[3], Bartłomiej Rospond[2], Joanna Piotrowska[2]

1. Department of Biochemical Toxicology, Faculty of Pharmacy, Jagiellonian University Medical College, Poland
2. Department of Inorganic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Poland
3. Toxicology Research Group, Department of Toxicology, Jagiellonian University Medical College, Poland

Tattoo, like makeup, is one of the oldest forms of body decoration, known since prehistoric times, and its appearance and meaning have evolved over the centuries. The oldest tattoos probably had, in addition to their decorative role, magical and healing functions. Currently, tattoos are a type of fashion and a specific subculture, at least 12% of Europeans have them. At the turn of the 20th and 21st centuries, artistic tattooing flourished. Nowadays, tattoos are no longer clearly associated with prison and criminal subculture, but are becoming an increasingly popular and fashionable way of decorating the body. Artistic tattoos are characterized by individual, original patterns, very often unique, allowing you to assign a specific tattoo to a given person.

The skin is a dynamic organ and a tattoo is a permanent change in its structure. There is the possibility of unforeseen reactions or complications. Currently, the threat results from the fashion for tattooing large surfaces of the body. Tattoos carry the risk of infection and chemical hazards. The inks use about 100 different pigments and a similar number of additives. The most dangerous ones include polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene, primary aromatic amines, heavy metals including nickel, cadmium, chromium, copper and cobalt, and preservatives. Generally, under the right conditions, e.g. when exposed to bacteria or UV light, inks can cause the dye to break down into a nitrogen-based chemical compound, which may have a direct impact on cancer. Another issue is the size of the pigment particles used in inks - they are usually very small particles (so-called nanoparticles). The study found that half of the 18 inks examined using electron microscopy contained particles of this size.

The pharmacokinetic behavior of different types of nanoparticles requires detailed study and a database of health risks associated with different nanoparticles (e.g. target organs, tissues or cells) should be established. The presence of contaminants such as metallic catalysts present in nanotubes and their role in observed health effects should be taken into account, as well as the health impact of nanomaterials. Therefore, it is important to be aware of potential risks and take appropriate precautions.

„Using mass spectrometry to characterise the composition of selected bee products”

Matuszewska E., Klupczyńska-Gabryszak A., Rzetecka N. , Plewa S., Matysiak J.*

Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

Bee products include royal jelly, bee pollen, bee bread, propolis, bee larvae and bee venom. They benefit the human body because they contain nutrients and bioactive compounds. However, because they are of natural origin, they may contain toxic inorganic contaminants, including heavy metals. In addition, bee products, especially bee venom, have pronounced allergenic properties. Therefore, a complete understanding of the composition of bee products is needed to ensure their safe use for health purposes.

The study used advanced sample preparation techniques and modern mass spectrometry methods to characterise the composition and bioactivity of selected bee products. As a result of the analyses, several hundred proteins were identified, including both beneficial and allergenic proteins. Quantitative studies of amino acids shown that lysine is the predominant amino acid in royal jelly, and proline in propolis and bee pollen. Elemental studies of bee pollen, propolis and royal jelly shown that the concentrations of macro- and micro-elements and contaminants in bee products vary depending on the product type.

The conducted mass spectrometry-based studies allowed in-depth characterisation of the composition and bioactivity of beehive products with therapeutic potential. The research led to the identification of new proteins in bee products and the quantification of a broad panel of amino acids. For the first time, such a wide range of chemical elements was determined in bee products collected in Poland. Complete knowledge of the content of biologically active compounds and the levels of mineral contaminants in bee products makes it possible to assess the safety of their oral intake.

Acknowledgments:
This research project was funded by National Science Centre Poland, grant 2016/23/D/NZ7/03949

„Photodegradation of API contained within commercial products for skin ”

Jamrógiewicz M.[*1], Bray A.[1], Gołuński G.[2], Bełdzińska P.[2]*

[1] Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, 80-416 Gdańsk, Hallera 107, Poland, +48 58 349 16 56, marzena.jamrogiewicz@gumed.edu.pl
[2] Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdańsk, Gdańsk, Poland

The first source of direct patient exposure to drug photodegradation is skin medications in the form of creams, gels or liquids. We present photostability testing of popular pharmaceutical products on skin containing naproxen and indomethacin. Using the FTIR spectroscopy and DSC, the changes in the chemical structure and physical form of both pure APIs and these substances in commercially available medicinal products might be assessed with satisfactory result. We also performed Ames tests for evaluation promutagenic and mutagenic potential of samples after photodegradation.

Characteristic ranges of the indomethacin FTIR spectra are significantly changed during photoirradiation its liquid preparation. Interesting is that after isolation Ind from liquid there is observed its polymorphic change. Bands at 3370 cm-1, 2730 and 2624 cm-1, which correspond to O-H stretch and H-bonding become a broad also for spectra of Liq_Ind24h. Further changes approving degradation are noticed as spectral shifts of bands corresponding to O-H, -C-O-, -C-O-C, -C-N-, or C-Cl group. Generally, a lot of spectral changes approve degradation of Ind in Liq_Ind preparation. FTIR spectra as well as DSC results of Npx_gel show only subtle shifts without degradation after photoexposition. In the DSC curves Liq_Ind peak disappears after 24h.

Ames test was performed both with preincubation and with metabolic activation for Ind and Liq/Ind analysing promutagenic and mutagenic potential. Results obtained in the Ames test indicate no promutagenic or mutagenic activity of both tested samples neither on S. typhimurium TA98 nor on S. typhimurium TA100 strains, irrespective of UV irradiation.

The formulation of preparations applied to the skin requires the use of additional methods of isolation of the drug substance in order to isolate it from excipients and test API stability. The FTIR method and DSC are a good, alternative methods of drug stability testing compared to pharmacopoeias’ methods.

„Application of spectrophotometic methods in assessment the antioxidant potential of medicinal mushrooms”

Paterska M.[1,2], Cielecka-Piontek J.[1,2], Krejpcio Z.[3]*

[1] Department of Pharmacognosy and Biomaterials,
Faculty of Pharmacy, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[2] Department of Pharmacology and Phytochemistry,
Institute of Natural Fibres and Medicinal Plants – National Research Institute – Poland, Kolejowa 2, 62-064 Plewiska, Poland
[3]Department of Human Nutrition and Dietetic
Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznan, Poland

Introduction: Mushrooms are a source of numerous bioactive compounds that can be used in disease prevention (e.g., antioxidants) or treatment (e.g., erinacins). Three mushroom species were selected for research: Hericium erinaceus (Bull.) Pers., Ganoderma lucidum (Curtis) P. Karst., and Coprinus comatus (O.F. Müll.) Pers. The antioxidant activities of extracts from the fruiting bodies were analyzed.

Materials and Methods: The fungal fruiting bodies were powdered, 70% methanol was added, and they were extracted using ultrasound-assisted extraction (UAE). The obtained extracts were concentrated using a vacuum evaporator and dissolved in DMSO. Antioxidant properties were determined using three spectrophotometric methods: DPPH and ABTS (according to the method by Studzińska-Sroka et al. 2019), and CUPRAC (Chanaj-Kaczmarek et al. 2015).

Results: In the DPPH method, the IC50 values, representing the amount of antioxidant required to reduce half of the initial DPPH concentration. The results showed that Ganoderma lucidum has the highest antioxidant activity (IC50 = 0.0280 g/mL), followed by Hericium erinaceus IC50 = 0.22 g/mL and Coprinus comatus IC50 = 0.15 g/mL. In the CUPRAC method the result was expressed as IC0.5, which corresponds to the extract concentration needed to reduce the absorbance to a value of 0.5. The highest antioxidant activity was observed in Ganoderma lucidum (IC0.5 = 3 mg/mL), followed by Coprinus comatus (IC0.5 = 47 mg/mL) and Hericium erinaceus (IC0.5 = 31 mg/mL). In ABTS method the highest antioxidant activity was shown in Ganoderma lucidum (97.09% ABTS radical scavenging capacity), followed by Coprinus comatus (83.35%) and Hericium erinaceus (60.53%).

Conclusions: Extract from Ganoderma lucidum showed the highest antioxidant activity in all methods. The results suggest the potential use of mushroom extracts in pharmaceutical products aimed at reducing oxidative stress in human cells.

Fot. Szymon Plewa

ISBN: 978-83-959554-9-5