Eastern Analytical Symposium
Virtual Student Symposium

May 20, 2022

Sessions

Opening Remarks – 10:00 am

Chromatography & Mass Spectrometry – 10:05 am

Spectroscopy, Chemometrics, & Computation – 1:00 pm

Networking Hour – 2:15 pm

Chromatography & Mass Spectrometry
10:05 am

"Determination of Major Anti-cancer Drug Gemcitabine by High-performance Liquid Chromatography and its Stability in the Artificial Bodily Fluid"

Xavier Welch & Dr. Alexander Samokhvalov

Morgan State University

Gemcitabine is an FDA-approved antimetabolite drug most commonly utilized in chemotherapy to treat aggressive cancers such as pancreatic cancer. Recently Gemcitabine has been extensively investigated in clinical and fundamental studies due to its drug of simple pyrimidine structure, which can exist in two forms: free base (Gem) and hydrochloride (commercial name Gemzar). It is of interest to understand the temporal stability of Gemcitabine in physiological fluids under ambient conditions and to develop facile yet robust quantitative methods of its determination in artificial bodily fluids. Most reports use high-performance liquid chromatography (HPLC) in gradient regimes or sophisticated methods such as HPLC with mass spectrometry (HPLC-MS). Finally, the majority of HPLC reports describe the determination of gemcitabine hydrochloride.

First, we developed novel qualitative and quantitative determination of Gem by facile HPLC-UV method in isocratic mode using reversed-phase (RP) column and a mixture of aqueous electrolyte with small amount of polar organic solvent. This mobile phase resembles the composition of phosphate-buffered saline (PBS) as a model physiological fluid commonly used in biomedical research. Second, we applied this method to assess the temporal stability of Gem in PBS at pH 7.4, 37 °C, and 25 °C in ambient air; it is stable for up to 2.5 days without forming byproducts. Herein, we utilized commercial drug dissolution apparatus with automatic periodic sampling. Research is in progress to study the kinetics of delayed Gem release from complexes with advanced coordination polymers by the HPLC-UV method.

10:30 am

“Quantitative Determination of Selected Urolithin Metabolites in Human Urine by Simple Sample Preparation and UPLC/MS-MS Analysis”

Tracy Ann Lacson

University of Connecticut

We report a simple, reliable, and validated method for the rapid screening and quantification of nine urolithin (UL) metabolites in human urine. Ultra-performance liquid chromatograph coupled with a tandem mass spectrometer (UPLC-MS/MS) was utilized for UL analysis following a simple sample preparation. Optimization of chromatographic and mass spectrometric conditions was performed to maximize the sensitivity and selectivity of the targeted analytes. A validation of the methodology was conducted to account for matrix interferences, linearity, method detection limits (MDLs), UL chemical stability, precision and accuracy for the ULs of interest. MDLs were achieved for the selected ULs ranging form 9.2-18.2 $ng \cdot mL^{-1}$. Excellent linear coefficients of determination were obtained for the range of calibration standards of 5.0-5,000 $ng \cdot mL^{-1}$, with R2 values between 0.9991 and 0.9998. The surrogate compound, 6,7-dihydroxycoumarin, was used to monitor the extraction efficacy and chrysin as the quantitative internal standard. The recoveries of the analytes were 88-99% with surrogate recoveries greater than 82%. This analytical method was developed and validated for processing samples associated with a human study, where it is hypothesized that walnut supplementation improves colonic health and lowers colorectal cancer risk, in part through enhancing UL formation.

10:55 am

“LC-HRMS Characterization of Sulphate Metabolites of 17 Common Mycotoxins Using Microsomal Incubations”

Elissa Mariani, Irina Slobodchikova, Calin Zainea, & Dajana Vuckovic

Concordia University

Mycotoxins are toxic secondary metabolites produced by filamentous fungi that pose adverse health effects to humans and animals. Currently, most analytical methods used for monitoring of human exposure to mycotoxins focus on the detection of the parent compound using LC-MS leading to significant underestimation of mycotoxin exposure. The goal of this study is to perform in vitro microsomal incubation, using PAPS and liver S9 fraction, of 17 mycotoxins from the trichothecene, zearelanone, and aflatoxin classes and to characterize phase II sulphate metabolites. Both 1 hour and 24-hour incubations were performed using a high concentration of mycotoxin (1 µg/ml) to allow detection of the minor sulphate metabolites. Samples were analyzed using a previously validated reversed-phase pentafluorophenyl LC-HRMS method, but a longer elution gradient was used to separate co-eluting isomeric metabolites. Metabolites were characterized using accurate mass, MS/MS, and MS3 fragmentation. 1-hour incubations allowed for the detection of the major metabolite while 24 hour incubations enabled characterization of the minor metabolites. In total, 19 sulphate metabolites were characterized. Furthermore, we examined the optimum workflow to integrate phase I and sulphate microsomal incubations to generate sulphate metabolites of phase I products. These reactions resulted in 25 additional sulphate metabolites for the zearelanone class that to the best of our knowledge have not been previously reported. The characterized metabolites were added to an in-house spectral library which will be used in future biomonitoring studies of mycotoxin exposure.

11:20 am

“Determination of Mycotoxins in Dried Fruits Using LC-MS/MS— A Homogeneity, Troubleshooting and Confirmation of Identity Study”

Steven Tan, Kai Zhang, & David Xu

University of Maryland College Park

As a commonly consumed food type in the US, dried fruits are susceptible to mold development and mycotoxin contamination, depending on processing and storage conditions. To monitor various toxic mycotoxins in dried fruits, it is efficient to simultaneously determine multiple mycotoxins using a single extraction and liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. In this study, we applied a stable isotope dilution and LC-MS/MS method to multi-mycotoxin analysis in dried fruits, selecting raisins, plums, figs, and cranberries for matrix extension. Samples were prepared using cryogenic grinding, followed by the fortification of carbon-13 (^{13}C) uniformly labeled internal standards for 12 target mycotoxins, and extraction using 50% acetonitrile. Homogeneity of prepared samples, defined as particle size Dv90 <850 µm for the tested matrices, was characterized using a laser diffraction particle size analyzer, and reached using cryogenic grinding procedures. The majority of recoveries in the four matrices for aflatoxins and ochratoxin A spiked at 1, 10, and 100 ng/g; fumonisins, T-2 toxin, HT-2 toxin, and zearalenone spiked at 10, 100, and 1,000 ng/g, ranged from 80 to 120% with relative standard deviations (RSDs) of <20%. Deoxynivalenol was not detected at 10 and 100 ng/g in plums, and additional troubleshooting procedures using liquid-liquid extraction (LLE), solid phase extraction (SPE), and elution gradient were evaluated to improve the detectability for this mycotoxin. Furthermore, we confirmed the identity of detected mycotoxins, ochratoxin A and deoxynivalenol, in incurred samples using enhanced product ion scans and spectral library matching.

11:45 am

“Analysis of Chemical Formula of Glycerolipids and Comparison Between Plant-based Meat and Beef/Pork by Using ASAP Method”

Hyunji Yu & Gene Hall

Rutgers University

The research aims to determine the chemical formula of glycerolipids and compare the differences between plant-based meat substitutes and beef and pork by using the Atmospheric Sample Analysis Probe (ASAP) method connected to a Xevo G2-Si Q-TOF-MS instrument. The method allows sensitive and fast analysis of volatile compounds in solids, liquids, and polymers. The data collected from the mass spectrometer instrument displays mass and intensity, and differences in intensity show that each sample has different amounts of lipid compounds. The specific, evident high intensity helps find the particular chemical compound. Popularized three fast-food restaurants were selected for the analysis: Dunkin Donuts, White Castle, and Burger King. The analysis was performed using the lipid maps software. It has been shown that plant-based substitutes and beef/pork contain different chemical compounds, especially triacylglycerols (TAGs) and Diacylglycerols (DAGs). Analyses to find the differences between raw and cooked meat were also performed for both plant-based meat and beef/pork, but the results did not show big differences.

12:10 pm

“Extraction of CBD and Other Cannabinoids From Hard/Soft Candy Edibles”

Kenneth Klarer

University of Connecticut

In recent years, as an increasing number of states have legalized cannabis and other cannabinoids such as cannabidiol (CBD), the number of manufacturers for cannabis and other cannabinoid products has greatly increased. Thus, the necessity for potency testing is of high importance for not only medical use and research, but for recreational use. This study describes the experiment and analysis conducted for the potency testing of a locally manufactured product marketed as a chewable CBD tablet for recreational use using a reproducible and accurate analytical method.

12:35 pm

“Analysis of Short-Chain Per-fluoroalkyl Substances (PFASs) in Connecticut Surface Water”

Isabella McGrath, Sarah Ayers, Anthony Provatas, James Stuart

University of Connecticut

Short-chain PFAS are defined by having a chain of six or fewer carbon molecules. Long-chain perfluoroalkyl substances have been extensively studied and proven to persist in the environment, as well as to cause a range of health effects- including high risk for cancer, birth defects, high cholesterol and blood pressure, and many more. Short-chain PFAS, on the other hand, have not been studied nearly as much, in terms of their persistence in nature. Environmentalists, scientists, and other professionals are becoming increasingly aware of these molecules as companies are beginning to switch over to short-chain PFAS in their products as their long-chain counterparts are beginning to be regulated by the government. The objective of this research is to determine whether short-chain PFAS persist in Connecticut surface water, and if they pose a problem to the relative ecosystem. Analysis of the samples was performed using a UPLC-MS/MS.

Spectroscopy, Chemometrics, & Computation
1:00 pm

"Raman Spectroscopy to Tackle the Analysis of Bloodstains in Crime Scene Conditions"

Alexis Weber, Alexis Barber, & Igor Lednev

University at Albany, SUNY

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred and individuals involved. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed.

Vibrational spectroscopy paired with chemometrics has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, research conducted with these techniques so far have analyzed the aging of bloodstains, specifically the degradation of hemoglobin, in ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that is capable of estimating the TSD of bloodstains in different environments.

There are infinite varieties of potential environmental conditions. Our goal is to determine how potentially "extreme" conditions affect the aging mechanism of bloodstains, high temperature in particular. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the ex vivo aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post deposition. The reproducibility of heated blood analysis and TSD determination model will be discussed.

1:25 pm

“Effect of pH and Lewis Acidity on the Catalytic Activity of Metal Oxyhydroxides for the Hydrolysis of Organophosphorus Nerve Agent Simulants”

Tyler-Rayne Nero, Venkata Swaroopa Datta Devulapalli, Eric Borguet

Temple University

Organophosphorus nerve agents pose physiological threats when released into the atmosphere by severely impacting the nervous symptom, with possible fatal consequences. Hence it is necessary to develop materials that can degrade nerve agents. We hypothesize that metal oxyhydroxides could serve this purpose given the similarity in structure to the secondary binding units (SBUs) of metal-organic frameworks (MOFs) that have already shown catalytic activity.

In this study, we test several metal oxyhydroxides as catalysts for the hydrolysis of dimethyl 4-nitrophenyl phosphate (DMNP), a less toxic and reactive simulant of Sarin. We hypothesize that the catalytic hydrolysis of DMNP is proportional to the Lewis acidity of the metal and the concentration of the metal oxyhydroxides, determined by pH. Hence, we screened several Lewis acidic metals such as, Nb5+, Ti^{4+}, Ce^{3+}, Sn^{5+}, Cu^{2+}, Zn^{2+}, Sc^{3+}, and Fe^{2+} in neutral (pH 7) and basic (pH 10) media. Using UV-vis spectroscopy, we observed that Nb^{5+}, Ti^{4+}, and Ce^{3+} hydrolyzed DMNP with greater activity at pH 10 compared to pH 7, whereas Sn^{5+}, Fe^{2+}, Cu^{2+}, Sc^{3+}, and Zn^{2+} displayed little activity. A release of protons occurs upon dissolution of metal precursor salts in water and as a result, the pH of the reaction mixture dropped, influencing the activity of the resulting metal species. In future work, we will isolate metal oxyhydroxides, deposit them on supports, and test their catalytic properties.

1:50 pm

“Spectroscopic and Computational Analysis of the Dimeric Chlorophyll Acceptor in the M688HPsaA Genetic Variant of Photosystem I”

Elijah Gruszecki, Michael Gorka, Philip Charles, Vidmantas Kalendra, John Golbeck, K. V. Lakshmi

Rensselear Polytechnic Institute

Studies of the photosynthetic reaction center, Photosystem I (PSI), have shown that its polypeptide core contains highly coupled chlorophyll molecules that serve as the primary electron donor and acceptor. Notably, a recent study found that the primary acceptor, A0, is a dimer of chlorophyll a molecules, Chl2 and Chl3, where the electron spin density on the reduced acceptor, A0-, is distributed on both molecules.[1] Previous biochemical studies have shown that the replacement of the soft base sulfur axial ligand of Chl3A from a methionine residue to a hard base nitrogen ligand of a histidine in the M688HPsaA variant of PSI severely impacts forward electron transfer from the A0A cofactor.[2] In this study, we determine the electronic structure of the A0- state of M688HPsaA PSI using a combination of experimental hyperfine sublevel correlation (HYSCORE) spectroscopy and computational analysis including molecular dynamics and density functional theory (DFT).[3] Understanding the electronic structure of the dimeric A0 acceptor in the wild-type and M688HPsaA variant of PSI has widespread implications ranging from the evolution of naturally occurring reaction centers to the development of a new generation of highly efficient artificial photosynthetic systems.

1. Gorka et al. (2021) iScience (Cell Press), 24, 102719.
2. Sun et al. (2014) Biochim. Biophys. Acta Bioenerg., 1837, 1362.
3. Gorka et al. (2021) Biochim. Biophys. Acta Bioenerg., 1862, 148424